IJPSR (2024), Volume 15, Issue 3 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 June 2023; received in revised form, 25 November 2023; accepted, 27 November 2023; published 01 March 2024

PROBIOTICS: ANAVANT-GARDESTEP INTHE THERAPY OF RHEUMATOID ARTHRITIS

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Keywords:

Lactobacillus, Inflammation, Dysbiosis, T-reg cells, *L. rhamnosus*, TNF-α

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ABSTRACT: Rheumatoid arthritis (RA) is a chronic progressive inflammatory autoimmune ailment with multifactorial etiology. The human gut is a massive intricate ecosystem where microbiota, nutrients, and host cells interact extensively to uphold gut homeostasis. Gut microbiota acts as a modulator of immunity and dysbiosis has detrimental effects on health by triggering a persistent inflammatory response that may eventually result in disease. Probiotics are live microorganisms that can normalize gut microbiota and boost the host's health when administered in adequate amounts. Recent research has demonstrated that probiotics have important roles in the makeup of the gut microbiota, which can prevent pathogenic bacteria from colonizing the intestine, support the host in developing a strong intestinal mucosa defensive barrier, and reinforce the host immune system. Probiotics have positive effects on health, including immune system regulation and anti-inflammatory activity by preventing the release of pro-inflammatory cytokines like TNF-. Probiotics may affect NF-kB, proteasome activity, Toll-like receptors, as well as their regulators and stimuli, at various sites along the MAPK pathway. Because of the close association between gut microbiota and human immunity, controlling the gut microbiome with probiotics has proven to be a very efficient strategy to boost human immunity in Rheumatoid arthritis. The preclinical and clinical trials demonstrating probiotics' effectiveness in treating Rheumatoid arthritis are summarized in this review together with the recent information on the interaction between immunology, probiotics, and gut microbiota.

INTRODUCTION: Rheumatoid arthritis is an autoimmune disease fundamentally affecting the joints of the body. It is a type of arthropathy characterized by chronic inflammation of synovial joints, bones, and cartilage [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.15(3).699-09

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(3).699-09

The major observatory criteria for RA are inflammation in symmetric joints (proximal interphalangeal and metacarpophalangeal joints), morning stiffness, fatigue, swelling, and redness near joints; which if left untreated, proceeds to extra-articular sites and increases mortality and morbidity.

Untreated RA leads to the development of various complications like rheumatoid nodules, lung disease, atherosclerosis, hematologic complexity, bone erosion, and restricted motion due to joint damage [2]. RA affects 0.5 to 1% of adults

worldwide, with most patients being above 50 years of age [3]. Numerous factors such as genetic factors like HLA-DRB1 and environmental factors like diet, smoking, alcohol, and obesity contribute to the pathogenesis of RA causing chronic inflammation [4]. Synovial vascular injury, immune system activation, and neoangiogenesis induce autoimmunity. This assists in the proliferation of synoviocytes and fibroblasts, activation of residential macrophages, and infiltration of lymphocytes and neutrophils in the synovial membrane. Etiological factors lead to the modification of auto-antigens which causes activation of CD4+ T-cells and B-cells, stimulating the increased release of pro-inflammatory cytokines accompanied by the production of autoantibodies against them. Further osteoclast activity is increased in the synovial membrane due to activation of TNF-alpha, IL-6, RANK-L, and various cartilage degrading enzymes like metalloproteases which are produced by attracted neutrophil granulocytes pain, swelling, and bone erosion are manifested through this cellular event [5, 6].

Pharmacological medications used to treat rheumatoid arthritis are traditional, synthetic, biological DMARDs, corticosteroids, NSAIDs, and COX-2 inhibitors that show a range of side effects on the lungs, heart and liver and changes in lipid profile [2]. Nutritional interventions along with medications, physiotherapy, and regular exercise have been applied in the management of Rheumatoid arthritis. Out of several factors involved in the onset and progression of Rheumatoid arthritis, one such inciting factor is gut dysbiosis. This causes disturbance in the gut microbiome, eliciting an immune response and triggering chronic inflammation. Thus, modulation of immune homeostasis by gut microbiota is a possible alternative in treating autoimmune diseases [7].

GUT Microbiome and Immune System: The word microbiome refers to a collection of all microorganisms present within a biotic community. The majority of constituent microorganisms which include commensal and symbiotic microbes are bacteria [8]. In the human gut, approximately 1000 types of bacteria are present. In the human body, gut microbiota produces metabolites to communicate with the immune system and modulates immune responses. The interaction between gut microbiota and the immune system of humans plays a crucial function in the development of a range of disorders [9]. RA affects approximately 1% of the human population and is characterized by persistent synovial inflammation, ultimately leading to bone damage [10]. Many studies have shown evidence that oral and gut microbiome plays an important role in the pathogenesis of RA [11]. Disbalance in oral and gut microbiome concentration induces immune response as shown in **Table 1**. Analysis of dental, fecal, and salivary samples of RA patients by Zhang *et al.* shows dysbiosis of the gut and oral microbiome [10]. Breban *et al.* performed a study on fecal microbiota which showed evidence of increased *R. gnavu*s leading to dysbiosis in spondylarthritis and rheumatoid arthritis patients [12].

One of the main connections between RA and gut microbiota is considered to be T-cells. Regulatory T cells which are a CD4+ T cell subtype and Th17 cells are in equilibrium in healthy individuals but an elevated level of Th17 cells and a decrease in T reg cells is observed in RA patients. An increase in *Prevotellacopri* and a decrease in Bacteroides in RA patients have shown that *P. copri* may be pathogenic [10]. Studies have highlighted that the immune response generated from *P. copri* could stimulate Th1 cells. Under normal conditions, gut microbiota like *Faecalibacterium prausnitzii*, *Roseburia intestinalis,* and *Anaerostipes butyraticus* break down complex carbohydrates into SCFA [13].

SCFAs consisting of butyrate, acetate, and propionate play a crucial role in inflammatory signaling, protecting against pathogen infiltration and maintaining intestinal barrier integrity as demonstrated in **Fig. 1.** SCFAs act on colonocytes *via* sodium-dependent monocarboxylate transporter-1 (SLC5A8) and increase the function of Treg cells by inhibiting the expression of the HDAC gene. Studies have shown the involvement of HDACs in auto aggressive phenotype of fibroblast-like synoviocytes (FLSs) which is one of the main cell types involved in damage to RA joint tissue. SCFAs activate many G-Protein coupled receptors like GPR109a, GPR43, and GPR41 which regulate cellular turnover and maintain

intestinal epithelium physiology. SCFAs like butyrate stimulate DCs and Macrophages by binding with GPR109a leading to pro-inflammatory responses. Studies have shown that butyrate led to increased production of IL-10 and regulation of pro-inflammatory cytokines like TNF-α, IL-12, and IL-6 [13]. Butyrate also increases the production of TGFβ in intestinal epithelial cells which leads to the accumulation of Treg cells and regulation of inflammatory mechanism. TGF-β inhibits T effector proliferation and suppresses the differentiation of T effector into Th1 and Th17 cells. PPAR γ stimulated by SCFAs is necessary for maintaining a hypoxic environment in the gut that allows the growth of only obligate anaerobes [14]. An increase in the quantity of facultative anaerobes leads to gut dysbiosis that causes disruption of barrier function. Dysbiosis causes elevated inflammatory response leading to inflammation in the mucosal line and disrupts barrier function leading to a leaky gut [15].

TABLE 1: ALTERATION IN THE CONCENTRATION OF ORAL AND GUT MICROBIOME DURING RA

Body Part	Bacteria	Alteration in Concentration
Oral	*Anaerglobus geminatus*	Increases
	Leptotrichia	Increases
	Porphyromonas gingivalis	Increases
	Prevotella intermedia	Increases
	Tannerella forsythia	Increases
Lungs	*Proteobacteria*	Increases
	Pseudocardia	Increases
Gut	*Bacteroidetes*	Decreases
	Bifidobacterium	Decreases
	Clostridia-like bacterium (XIVa–IV)	Increases
	Clostridium coccoides	Decreases
	Eggerthella	Increases
	Eubacterium	Decreases
	Eubacterium rectal	Decreases
	Faecalibacterium prausnitzii	Increases
	Firmicutes	Increases
	Lachnospiraceae	Increases
	Lactobacillaceae	Increases
	Ruminococcaceae	Increases

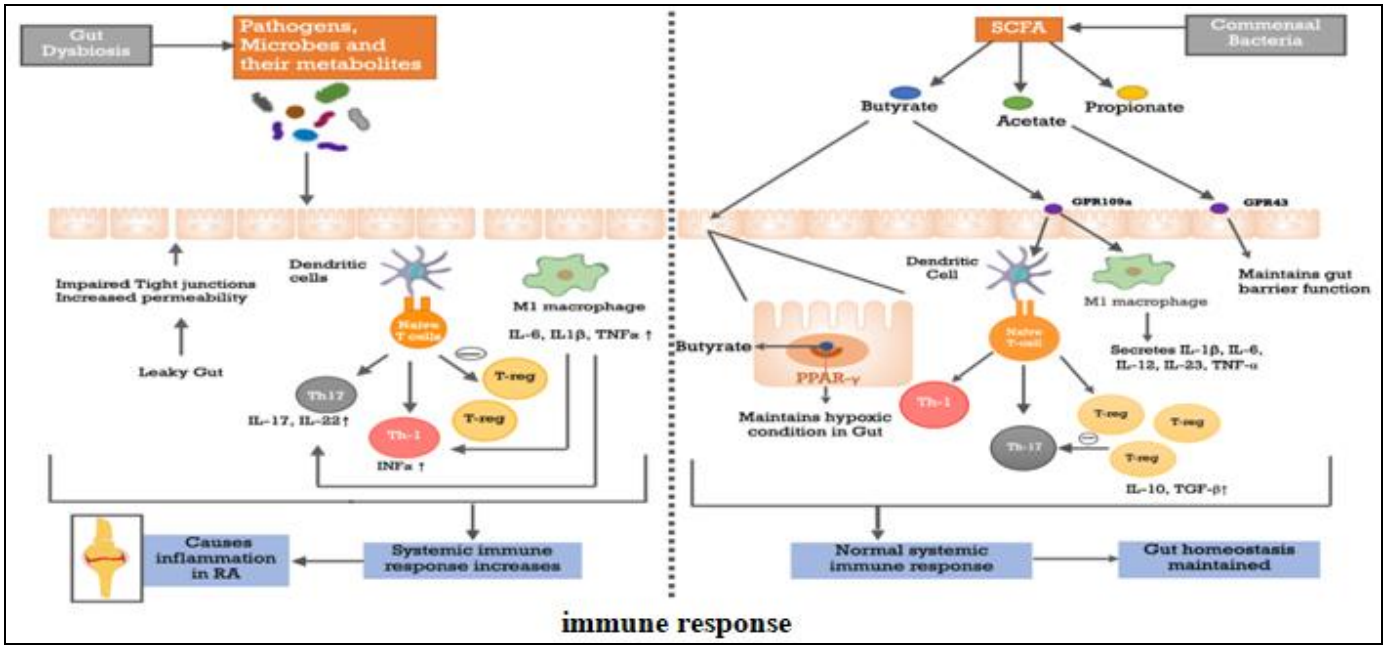

FIG. 1: ROLE OF GUT HOMEOSTASIS DISTURBANCE IN INDUCING RHEUMATOID ARTHRITIS RELATED.
PPAR-γ= Peroxisome proliferator-activated receptor gamma, T-reg cells= T regulatory cells, Th cells= T-helper cells, TNF-α= Tumor necrosis factor-alpha, TGF-β= transforming growth factor.

Probiotics: The word probiotics is derived from the Greek words "pro" and "bios" meaning "for life" and was first used by Lilly and Stillwell in 1965 to represent substances secreted by bacteria that aid in the growth of other microorganisms [16]. Probiotics play an essential role in maintaining health by boosting the immune system. Among the various definitions provided, FDA and WHO defines probiotics as "live microorganisms which confer health benefits to the host when administered in adequate amounts" [17]. In recent years, the use of probiotics has increased drastically. Many probiotic products are present in the market that uplift the immune system, express anti-inflammatory reactions, reduce digestive discomfort, and can also be used in the treatment of many gut-related diseases like IBD, IBS, and gastroenteritis [18].

The last decade has seen extensive research about probiotics in inflammatory responses. Many preclinical and clinical studies conducted so far shown in **Table 2** & **3,** have given evidence of probiotics modulating the innate and adaptive immune system. Alleviated joint inflammation in mice and reduced mucosal inflammation have been observed in the administration of probiotics [19, 20]. Clinical studies to study the effectiveness of probiotics in treating infections were mainly carried out using strains belonging to the genera Lactobacillus and Bifidobacterium [21]. *Lactobacillus* which comprises a variety of species such as *L. acidophilus, L. rhamnosus, L. bulgaricus, L. reuteri, L. casei, L. johnsonii, L. Plantarum, L. crispatus, L. delbruckii, L. gallinarum, L. gasseri, L. paracasei* can bind to intestinal cells and are acid-tolerant in the stomach's acidity [22].

Probiotic Lactobacillus strains have shown enhancement in intestinal barrier integrity followed by maintenance of immune tolerance and decreased movement of bacteria across the intestinal mucosa [23]. *Bifidobacterium* species include *B. animalis, B. adolescentis, B. bifidum, B. breve, B. infantis, B. lactis,* and *B. longum* which inhibit inflammatory responses in intestinal epithelial cells. Apart from these other bacterial strains used as probiotics include *Bacillus subtilis, Streptococcus thermophilus, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Lactococcus lactis, Pediococcus pentosaceus,* as well as the yeasts like *Saccharomyces boulardii* and *Saccharomyces cerevisiae* [22]. Probiotic strains have been tested to study clinical effects on Helicobacter pylori infection, inflammatory bowel disease, antibiotic-associated diarrhea (AAD) in children, and many other gastrointestinal infections. Strains like Lactobacillus GG have shown a positive effect on urinary tract infections and even in AAD in children. Also, strains like Lactobacillus gasseri OLL 2716 (LG21) and Lactobacillus acidophilus (johnsonii) La1 showed reduced gastric inflammation [21].

Inflammation is one of the defense mechanisms of the body that can be triggered by both internal and external aspects. In GIT, an assemblage of lymphoid tissues forms gut-associated lymphoid tissue (GALT) and acts as a checkpoint for inflammatory responses. On the ingress of bacteria, NLRs, and TLRs cooperate to give an inflammatory response. During inflammation caused by either allergy or autoimmune diseases, the bacterial flora of the gut plays a significant role in its mitigation [24]. Probiotics alter the gut microbiome by maintaining an equilibrium of beneficial and harmful bacteria. They mediate intestinal immunity by enhancing the level of good bacteria and lowering the levels of bad bacteria that cause allergies and diseases [9].

Probiotics can withstand physiological stress conditions like stomach acid, low pH, and the presence of bile salts. Properties exhibited by probiotic strains include resistance to pancreatic enzymes and bile acids, the ability to adhere to the intestinal mucosa and epithelia, child development, immune regulation, creation of antimicrobial-like substrates, fitness, and health [25]. Many experiments performed on mice model has shown that joint inflammation may occur due to local and systemic immunity activated by gut microbiota. Probiotics impact systemic immune responses and ensure homeostasis of healthy microbiota leading to increased application of probiotics in autoimmune diseases as adjuvant therapy. The studies performed in EAE mice after probiotic administration showed immunoregulatory effects. Probiotic strains like *Lactobacillus spp., Pediococcus acidolactici, Bifidobacterium bifidum, Bifidobacterium animalis, and Bacteroides fragilis* showed decreased Th1/Th17 levels and elevation of

Treg cells along with the promotion of IL-10 and TGF β secretion [26].

Selection of Probiotics: The selection of probiotic strains depends on various criteria of which safety and efficacy play the most essential role. Basic strain identification and its characterization is crucial as many non-living cells may also showcase properties of probiotics after which investigating with established assays in experimental animals in a controlled manner should be carried out. The selection of probiotics also depends on their ability to cross natural barriers of GIT like acidic environments and degradation by digestive enzymes. Their *in-vivo* function needs to be predicted but their *in-vitro* studies are usually utilized. Scientific parameters like strain's replication capability, stabilization, and integration into the final product must be checked. Its stability till the expiration date and inside the host must also be determined. The viability and activity of probiotics during their movement in GIT and storage should also be determined. Their ability to obstruct pathogens and antibiotic resistance are also determining factors. Lactobacillus and Bifidobacterium are the most commonly used probiotic strains because of their beneficial effects in the human gastrointestinal tract, such as reduction of serum cholesterol, alleviation of lactose intolerance, anti-infection, antimicrobial, antioxidant, anti-allergic, antimutagenic, anticarcinogenic properties, blood lipid reduction, and immune system stimulation. *L. casei* as well as *L. acidophilus* both show their viability in acidic conditions and L. rhamnosus CRL1505 hauls down viral-linked lung damage by modulating immune-coagulative responses and removing respiratory viruses. Bifidobacteria are generally used because of their wide range of mechanisms for bile salt tolerance. Some of its examples are *B. adolescentis*, *B. animalis subsp. animalis*, *B. animalis subsp. lactis*, *B. bifidum*, *B. longum*, *B. breve* and *B. infantis*. The threshold limit of tolerance varies from strain to strain according to the research [9].

Some other criteria include protein and carbohydrate utilization patterns, phenotype and genotype testing for stability, development of compounds with antibacterial properties and immunity-inducing ability, suppression of spoilage-type infection or organisms, and so on. A probiotic strain should neither be pathogenic nor trigger an allergic response to the host and have the ability to proliferate and colonize in GIT. Some of the techniques used for strain identification are: Screening by *In-vitro* testing, Molecular techniques for identification, and Strain-specific identification techniques [9, 27, 28].

Mechanism of Action of Probiotics:

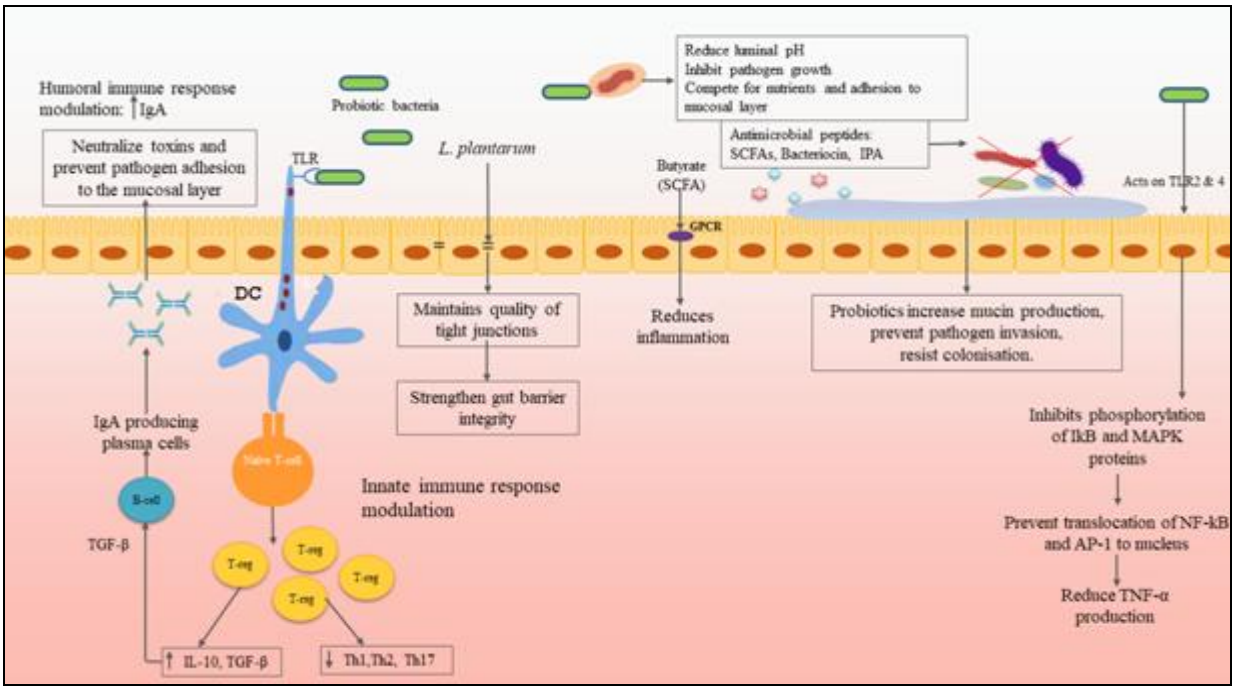

FIG. 2: DIFFERENT MECHANISMS OF ACTION THROUGH WHICH PROBIOTICS ACT

1. Probiotic bacteria bind to immune cells and increase T-reg cell activity, reducing pro-inflammatory response. They increase the release of secretory IgA antibodies mediated by upregulation of TGF-β and increased plasma cell activation.

2. They also release antimicrobial peptides which help inhibit pathogen growth.

3. Maintain gut wall integrity by inducing the release of tight junction proteins and increasing mucus secretion which prevents entry of pathogens inside the lumen.

4. By inhibiting various intracellular pathways directly, reduce immune cell production.

The beneficial outcome of probiotics on inflammatory markers and immunity has led to extensive study of the mechanism of probiotics. Many animal models like the Collagen-Induced Arthritis Animal Model (CIA) and Adjuvant-Induced Arthritis Animal Model (AIA) have been experimented on to study the effect of probiotics on rheumatoid arthritis. Studies showed that treatment with probiotics like Bifidobacterium adolescentis improved the balance between pro and anti-inflammatory markers and abate gut dysbiosis [29].

The combination of lactobacillus and Bifidobacterium species elevates the level of tolerogenic dendritic cells and encourages naive T cells to develop into T reg cells and also control the imbalance between Th1/Th2 inflammatory response. An animal model for rheumatoid arthritis, collagen-induced arthritis (CIA) is used to demonstrate that *L. casei* has a positive effect by reducing immunological responses of the Th1 type [30, 31]. Probiotics shows strain-specific mechanism and their action is based on their capability to reduce the production of pro-inflammatory cytokines, including IL-12, TNF-α, and IFN-γ [32]. Intestinal flora plays a major role in enhancing immunity, treating metabolic disorders, and maintaining the integrity of the mucosal barrier [26]. Imbalance in intestinal flora leads to a change in gut homeostasis causing gut dysbiosis i.e., perturbations in gut microbiota that may trigger autoimmune disease and chronic inflammation [32]. Probiotics show a certain regulatory effect on intestinal microecology and assist in the restoration of balance in gut microbes. Modulation in the immune response can be achieved by either proliferation, inhibition of pathogenic bacteria, or elevating the immune response of commensal bacteria. The method of inhibiting the growth could either be the production of an antimicrobial agent like bacteriocin or through competitive inhibition of binding to gut mucosal receptors. Butyrate is a biologically active molecule essential in regulating gut barrier function and immune response. The production of such secondary metabolites elevates by increasing the quantity of good commensal bacteria like *Faecalibacterium, Eubacterium, Roseburia, and Akkermansia* [31].

Gut-associated lymphoid tissue (GALT) consists of 70% of the host's immune cells and is a crucial protective immune system in GIT. Antigens and pathogens that enter GIT through follicular epithelial cells and microfold cells present on the Peyer's patches get recognized by the pattern recognition receptor (PRR). Pattern recognition receptors such as toll-like receptors (TLR) and nucleotide-binding oligomerization domains (NODs) are expressed in epithelial cells, endothelial cells, dendritic cells, and macrophages. Microorganisms like pathogens, commensal bacteria, and probiotics express microbe-associated molecular patterns (MAMPs) which interact with pattern recognition receptors (PRR) present in intestinal mucosa and give action on the host immune system [33].

The recognition of microbial-associated molecular pattern (MAMPs) plays an important role in innate immunity; a major contributor to acute inflammation caused by microbial infection or tissue damage [34]. On invasion by the pathogen in the body, the lipopolysaccharides present in pathogens form a complex with TLR-4 receptor which is anchored to the cell surface by surface molecule CD14. This further stimulates the migration of transcription factor-nuclear factor kappa B (NFκB) in the nucleus which increases the production of inflammatory cytokines like IL-6 & IL-8 and develops acute inflammation [33, 35]. As shown in **Fig. 2** different mechanisms through which Probiotics work: 1. Reinforcement of intestinal epithelial barrier, 2. Blocking pathogen adhesion to the intestinal mucosa, 3. By secretion

of anti-microbial substances, 4. Secretory IgA antibody and 5. Immune response modulation.

Reinforcement of Intestinal Epithelial Barrier: The epithelial barrier is the semi-permeable membrane regulating the entry of certain nutrients and restricting the entry of pathogens. Epithelial integrity is influenced by tight junctions, mucus production, and antimicrobial peptides. Being the first line of defense, the epithelial barrier protects against harmful compounds, and its disruption can cause entry of toxins and antigens in the lumen and induce an inflammatory response. *Lactobacilli* enhance the quality of tight junctions by regulating the production of tight junction proteins E-cadherin and β-catenin.

E. coli nissle 1917 prevents the destruction of barrier by enteropathogenic bacteria *E. coli* and restores epithelial integrity by changes in tight junction protein expression (zonula occludens (ZO-2), occludins, and claudins) and PKC which helps in the enhancement of tight junction complex. VSL3 and *L. casei* also help in maintaining barrier function by similar actions. Peptides p40 & p75 secreted by *L. rhamnosus* help reduce inflammation by inhibiting epithelial cell apoptosis. They prevent cytokine-induced damage by activating anti-apoptotic PK-B in a phosphatidyl inositol-3-kinase-dependent pathway and by inhibiting pro-apoptotic p-38/mitogen-activated protein kinase (MAPK). Mucin is a major macromolecular glycoprotein constituent of mucus secretions from goblet cells. During pathological conditions, their function is disrupted. *Lactobacillus plantarum* (strain 299v) increases the expression of mucin (MUC2 and MUC3) in cells and prevents direct contact of the pathogen to cell surface and their entry. VSL3 and *L. acidophilus* also induce mucin gene expression in HT29 cells [17, 36].

Blocking Adhesion of Pathogen To Intestinal Mucosa: Interaction of pathogen proteins with mucosal receptors leads to infection. Therefore, probiotics and host cells are necessary for averting interaction. Due to their ability of adhesion, probiotics compete with pathogens for attachment to receptors and prevent them from doing it [36]. Lactic acid bacteria have surface proteins like adhesin protein, or lipoteichoic acids and saccharide moieties which promote attachment of bacteria to the mucous layer. After which they increase the synthesis of mucus by cells. The mucus forms a thick layer over cells and prevents the adhesion of pathogens. This shows the link between a surface protein of probiotic bacteria and the competitive exclusion of pathogens. Without adhesion, colonization cannot take place and inflammation is prevented. *L. plantarum* induces expression of MUC2 & MUC3 mucins and also prevents attachment of enteropathogenic *E. coli* [17]. Adhesion of L. *reuteri* and *L. fermentum* to mucus is mediated by MapA (mucus adhesion-promoting protein) [37].

Antimicrobial Peptides: Probiotics produce metabolites (histamine, butyrate, inosine, GABA) that show anti-inflammatory effects. SCFAs produced by commensal bacteria as well as probiotics play a significant role in maintaining gut homeostasis. They bind to FFAR2, FFAR3, OR GPR109a receptors of intestinal cells and reduce cytokine production by neutrophils and inhibit the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway. They induce tolerogenic DC cells, which stimulate naïve T cells to differentiate into T-reg cells. Butyrate inhibits histone deacetylase and increasesthe accumulation of T-reg cells. Indole derivatives produced by probiotics reduce TNF-α production and increase anti-inflammatory cytokine production by interacting with AhR (Aryl Hydrocarbon Receptor) present on immune cells [38]. Histamine released by L. reuteri 6475 acts on H2 receptors of IECs (Intestinal epithelial cells) and macrophages to reduce pro-inflammatory cytokines [36].

Other antimicrobial substances synthesized by probiotics are LMW molecules like organic acids and bacteriocins. Organic acids such as acetic acid and lactic acid enter the bacterial cell in undissociated form and dissociate inside the cytoplasm. This causes a lowering of intracellular pH and accumulation of ionized organic acids inside the cell leading to the death of the pathogen. Antibacterial substances like bacteriocin are synthesized by Gram-positive bacteria (usually Lactic Acid Bacteria, including lactacin B from *L. acidophilus*, plantaricin from *L. plantarum*, and nisin from *Lactococcus lactis*) [17]. “Small molecules microbe originated (SMOM) homeostasis” in the

host is attributed to the proficiency of bacteriocin in maintaining homeostasis.

They structurally and functionally resemble molecules synthesized by the host cells. They assist in killing pathogens by pore formation or inhibiting cell wall synthesis [39].

Secretory IgA Antibodies: Plasma cells present in the epithelial lining of the gut produce IgA antibodies which exist as dimers, linked by the J chain produced from plasma cells, and form a complex with secretory component assembled by polymeric-Ig receptor (pIgR). sIgA antibodies regulate adaptive immune response by interacting with immunoglobulin receptors, getting secreted in the intestinal lumen, and exhibiting an anti-inflammatory response.

The secretory component ensures firm adherence of IgA to the mucosal surface while the antibody gives action by preventing pathogen adhesion as well as invasion, neutralizing toxins, antigens, and virus replication, and maintaining gut homeostasis. IgA antibody complexes with antigens and when taken up by dendritic cells, it down-regulates T-cell production and expression of pro-inflammatory cytokines [40, 41]. A study suggested that oral administration of L. gasseri SBT 2055 for 5 weeks activated the TLR2 pathway which increases expression of TGF-β in dendritic cells and promotes B-cell differentiation into IgA-producing plasma cells [42].

Immune Response Modulations: The humoral and innate immunity responses are mediated by probiotics through the pathway of bacterial-epithelial-immune cells crosstalk [36]. TLRs present on Intestinal epithelial cells recognize MAMPs of bacteria and activate MAPK and NF-kB pathways. These pathways are important for the transcription and release of TNF-α in case of inflammation. When any environmental factor affects the host, mitogen-activated protein kinases: ERK1, ERK2, JNK, and p38 undergo phosphorylation which increases transcription of the gene coding for pro-inflammatory cytokine, TNF-α. Being bound to inhibitor molecule IkB, NF-kB is present in an inactive form in the cytoplasm.

When external factor affects homeostasis, IkB gets phosphorylated by the IkB kinase complex (IKK) and undergoes degradation via ubiquitation. Thus NF-kB which is a heterodimer molecule with 2 sub-units=p50 & p65 is released free. It translocates to the nucleus and activates TNF-α production. Probiotics reduce inflammation by down regulating these pro-inflammatory cytokine-producing pathways. It will inhibit the phosphorylation of MAPK proteins and IkB, in turn preventing translocation of AP-1 and NF-kB to the nucleus respectively. So, the production of TNF-α will be hampered. *L.casei* Shirota inhibits phosphorylation of ERK1, ERK2 & IkB, acting on both cell-signaling pathways. VSL3 reduces TNF-α by inhibiting the NF-kB pathway [43, 44].

TABLE 2: PRE-CLINICAL STUDIES OF PROBIOTICS

Probiotics	No. of Animals	Duration of Treatment	Route of Administration	Effects
L. casei [45]	20 Wistar Female rats	28 days	Oral	-inhibits COX-2 -pro-inflammatory cytokines
L. casei + *L. acidophilus* [46]	30 Rats (CIA model)	28 days	Oral	-show antioxidant properties -↓ IL-6, TNF-α, IL-12 (↑IL-10, IL-4)
*B. coagulans*and prebiotic insulin [47]	48 Wistar male rats	21 days – fibrinogen (Fn) 28 days – serum amyloid A (SAA) 35 days – TNF-α	Oral	Significant ↓ in anti-inflammatory effects inhibit SAA and Fn production
L. helveticus [48]	8-12 Rats (CIA model)	42 days	Parenteral (intradermal)	-↓ number of immune cells, B cells, CD4 T cells -↓ joint inflammation and cartilage damage
Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium bifidum [49]	Adjuvant-induced arthritis in rats	15 days	Injected into paw	Downregulates the markers of arthritis

Bifidobacterium animalis subsp. Lactis (HN019) [50]	Ligature-induced periodontitis in 32 rats with experimental RA	39 days	Parenteral	-↓ anti-atrullinated protein antibodies level -↓ in inflammatory mediator

TABLE 3: CLINICAL STUDIES OF PROBIOTICS

Probiotics	No. of Patients (Age)	Duration of Treatment	Route of Administration	Concentration	Effects
L. rhamnosus GG [51]	21 Placebo-controlled Patients	12 months	Oral	10^{10} CFU	No significant difference in Rheumatoid arthritis
B. coagulans [52]	45	60 days	Oral (1 caplet)	2×10^9 CFU	-↓ C-reactive Protein and improvement in pain scale
L. rhamnosus, L. reuteri [53]	29 Patients	3 months	Oral	2×10^9 CFU	-Clinically no improvement in RA measured by ACR20. -Functional improvement was observed.
L. casei 01 [54]	46 (20-80 years old)	8 weeks	Oral	10^8 CFU/day	It decreases pro-inflammatory cytokines
L. case 01 [55]	46 (20-80 years old)	8 weeks	Oral	10^8 CFU/day	- ↓ serum high-sensitivity C-reactive protein levels -↓ swollen joint counts
L. casei + L. acidophilus + B. Bifidum [56]	60 (25-70 years old)	8 weeks	Oral	2×10^9 CFU/day (each bacterium)	↓ RA activity, insulin levels, and C-reactive protein
L. casei 01 [57]	46 (20-80 years old)	8 weeks	Oral	10^8 CFU/day	No significant effect on the oxidative status of patients with RA was observed
B. coagulans 58	46 children	12 weeks	Oral	112.5×10^9	↓ in IL-6 levels
L. plantarum 299 v and Prebiotic: Anti-inflammatory Diet rich in fatty acids and fibers [59]	50	10 weeks	Oral		Reduction in Disease Activity Score in 28 joints-Erythrocyte Sedimentation Rate (DAS28-ESR)
Five freeze-dried strains: *Lactobacillus acidophilus* La-14, *Lactobacillus casei* Lc-11, *Lactococcus lactis* Ll-23, *Bifidobacterium lactis* Bl-04 and *B. bifidum* Bb-06 [60]	42	60 days	Oral	10^9 CFU/g of each strain	reduction in white blood cell count ($P = 0.012$) and tumor necrosis factor-α ($P = 0.004$) and interleukin 6 plasma levels

CONCLUSION: The last few decades have observed an emergence in research and studies demonstrating the vital play of nutrients in the management of diseases and disorders while safeguarding health.

The microbiota present in the human body regulates immunity as well as other functions of the body. This has led to an extensive study on probiotics and their positive intervention in mediating inflammatory pathways. Clinical and preclinical studies have observed a reduction in inflammation through the consumption of probiotics. When used as an adjuvant therapy along with prescribed drugs and meal, probiotics has shown drastic positive changes in diseases. Species like Lactobacillus have shown satisfying results in the management of rheumatoid arthritis. However, there is still room for development in the field of probiotics and its effects as nothing is ascertained due to a lack of intricate study.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: The authors have no conflicts of interest.

REFERENCES:

1. Scherer HU, Häupl T and Burmester GR: The etiology of rheumatoid arthritis. J Autoimmun 2020; 110: 102400.
2. Lin YJ, Anzaghe M and Schülke S: Update on the Pathomechanism, Diagnosis, and Treatment Options for Rheumatoid Arthritis. Cells 2020; 9(4): 880.
3. van der Woude D and van der Helm-van Mil AHM: Update on the epidemiology, risk factors, and disease outcomes of rheumatoid arthritis. Best Pract Res Clin Rheumatol 2018; 32(2): 174–87.
4. Wąż KZ, Kucharz EJ, Kopeć-Mędrek M, Pieczyrak R and Kotyla P: Intestinal dysbiosis and increased intestinal permeability as a potential risk factor for the development and progression of rheumatoid arthritis. Rheumatology Forum [Internet]. 2022; 8(4): 169–77. Available from: https://journals.viamedica.pl/rheumatology_forum/article/view/RF.2022.0024
5. Aletaha D and Smolen JS: Diagnosis and Management of Rheumatoid Arthritis. JAMA 2018; 320(13): 1360.
6. Zampeli E, Vlachoyiannopoulos PG and Tzioufas AG: Treatment of rheumatoid arthritis: Unraveling the conundrum. J Autoimmun 2015; 65: 1–18.
7. Bodkhe R, Balakrishnan B and Taneja V: The role of microbiome in rheumatoid arthritis treatment. Ther Adv Musculoskelet Dis 2019; 11: 1759720X1984463.
8. De Luca F and Shoenfeld Y: The microbiome in autoimmune diseases. Clin Exp Immunol 2018; 195(1): 74–85.
9. Bungau SG, Behl T, Singh A, Sehgal A, Singh S and Chigurupati S: Targeting Probiotics in Rheumatoid Arthritis. Nutrients 2021; 13(10).
10. Bergot AS, Giri R and Thomas R: The microbiome and rheumatoid arthritis. Best Pract Res Clin Rheumatol 2019; 33(6): 101497.
11. Ferro M, Charneca S, Dourado E, Guerreiro CS and Fonseca JE: Probiotic supplementation for rheumatoid arthritis: a promising adjuvant therapy in the gut microbiome era. Front Pharmacol 2021; 12: 711788.
12. Breban M, Tap J, Leboime A, Said-Nahal R, Langella P and Chiocchia G: Faecal microbiota study reveals specific dysbiosis in spondyloarthritis. Ann Rheum Dis 2017; 76(9): 1614–22.
13. Yoo JY, Groer M, Dutra SVO, Sarkar A and McSkimming DI: Gut Microbiota and Immune System Interactions. Microorganisms 2020; 8(10).
14. Byndloss MX, Olsan EE, Rivera-Chávez F, Tiffany CR, Cevallos SA and Lokken KL: Microbiota-activated PPAR-γ signaling inhibits dysbiotic Enterobacteriaceae expansion. Science (1979) [Internet]. 2017; 357(6351): 570–5. Available from: https://www.science.org/doi/abs/10.1126/science.aam9949
15. Kinashi Y and Hase K: Partners in Leaky Gut Syndrome: Intestinal Dysbiosis and Autoimmunity. Front Immunol 2021; 12.
16. Fuller R: History and development of probiotics. In: Probiotics. Dordrecht: Springer Netherlands 1992; 1–8.
17. Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C and Gil A: Probiotic Mechanisms of Action. Ann NutrMetab 2012; 61(2): 160–74.
18. Vitetta L, Briskey D, Alford H, Hall S and Coulson S: Probiotics, prebiotics and the gastrointestinal tract in health and disease. Inflammopharma 2014; 22(3): 135–54.
19. Immunomodulatory Effects of Probiotics in the Intestinal Tract. Curr Issues Mol Biol 2008;
20. Kano H, Kaneko T and Kaminogawa S: Oral Intake of Lactobacillus delbrueckii subsp. bulgaricus OLL1073R-1 Prevents Collagen-Induced Arthritis in Mice. J Food Prot 2002; 65(1): 153–60.
21. Sullivan A: Probiotics in human infections. Journal of Antimicrobial Chemotherapy 2002; 50(5): 625–7.
22. Stavropoulou E and Bezirtzoglou E: Probiotics in Medicine: A Long Debate. Front Immunol 2020; 11.
23. Lee BJ and Bak YT: Irritable Bowel Syndrome, Gut Microbiota and Probiotics. J Neurogastroenterol Motil 2011; 17(3): 252–66.
24. Hakansson A and Molin G: Gut Microbiota and Inflammation. Nutrients 2011; 3(6): 637–82.
25. Shukla R, Ruwali M, Sharath Pawar N and Flora SJS: Role of Probiotics in Rheumatoid Arthritis. In: Probiotic Research in Therapeutics. Singapore: Springer Singapore 2021; 273–94.
26. de Oliveira GLV, Leite AZ, Higuchi BS, Gonzaga MI and Mariano VS: Intestinal dysbiosis and probiotic applications in autoimmune diseases. Immunology 2017; 152(1): 1–12.
27. Lye HS, Balakrishnan K, Thiagarajah K, Mohd Ismail NI and Ooi SY: Beneficial Properties of Probiotics. Trop Life Sci Res 2016; 27(2): 73–90.
28. Yadav R and Shukla P: An overview of advanced technologies for selection of probiotics and their expediency: A review. Crit Rev Food Sci Nutr 2017; 57(15): 3233–42.
29. Fan Z, Yang B, Ross RP, Stanton C, Shi G and Zhao J: Protective effects of Bifidobacterium adolescentis on collagen-induced arthritis in rats depend on timing of administration. Food Funct 2020; 11(5): 4499–511.
30. So JS, Kwon HK, Lee CG, Yi HJ, Park JA and Lim SY: Lactobacillus casei suppresses experimental arthritis by down-regulating T helper 1 effector functions. Mol Immunol 2008; 45(9): 2690–9.
31. Balakrishnan B and Taneja V: Microbial modulation of the gut microbiome for treating autoimmune diseases. Expert Rev Gastroenterol Hepatol 2018; 12(10): 985–96.
32. Sales-Campos H, Soares SC and Oliveira CJF: An introduction of the role of probiotics in human infections and autoimmune diseases. Crit Rev Microbiol 2019; 45(4): 413–32.
33. Butel MJ: Probiotics, gut microbiota and health. Med Mal Infect 2014; 44(1): 1–8.
34. Amenyogbe E: Application of probiotics for sustainable and environment-friendly aquaculture management-A review. Cogent Food & Agriculture 2023; 9(1): 2226425.
35. Walker WA: Mechanisms of Action of Probiotics. Clinical Infectious Diseases 2008; 46(2): 87–91.
36. Liu Y, Tran DQ and Rhoads JM: Probiotics in Disease Prevention and Treatment. The Journal of Clinical Pharmacology 2018; 58: 164–79.
37. Van Tassell ML and Miller MJ: Lactobacillus Adhesion to Mucus. Nutrients 2011; 3(5): 613–36.
38. Liu Y, Alookaran J and Rhoads J: Probiotics in Autoimmune and Inflammatory Disorders. Nutrients 2018; 10(10): 1537.
39. Singh A, Vishwakarma V and Singhal B: Metabiotics: The functional metabolic signatures of probiotics: current state-of-art and future research priorities—metabiotics: probiotics effector molecules. Advances in Bioscience and Biotechnology 2018; 09(04): 147–89.
40. Maldonado Galdeano C, Cazorla SI, Lemme Dumit JM, Vélez E and Perdigón G: Beneficial effects of probiotic

consumption on the immune system. Annals of Nutrition and Metabolism 2019; 74(2): 115-24.
41. Maldonado Galdeano C, Cazorla SI, Lemme Dumit JM, Vélez E and Perdigón G: Beneficial Effects of Probiotic Consumption on the Immune System. Ann Nutr Metab 2019; 74(2): 115–24.
42. Sakai F, Hosoya T, Ono-Ohmachi A, Ukibe K, Ogawa A and Moriya T: *Lactobacillus gasseri* SBT2055 Induces TGF-β Expression in Dendritic Cells and Activates TLR2 Signal to Produce IgA in the Small Intestine. PLoS One 2014; 9(8): 105370.
43. Vincenzi A, Goettert MI and Volken de Souza CF: An evaluation of the effects of probiotics on tumoral necrosis factor (TNF-α) signaling and gene expression. Cytokine Growth Factor Rev 2021; 57: 27–38.
44. Thomas CM and Versalovic J: Probiotics-host communication: Modulation of signaling pathways in the intestine. Gut Microbes 2010; 1(3): 148–63.
45. Picchianti-Diamanti A, Panebianco C, Salemi S, Sorgi ML, Di Rosa R, Tropea A, Sgrulletti M, Salerno G, Terracciano F, D'Amelio R and Laganà B: Analysis of gut microbiota in rheumatoid arthritis patients: disease-related dysbiosis and modifications induced by etanercept. International J of Molecular Sciences 2018; 19(10): 2938.
46. Amdekar S, Singh V, Kumar A, Sharma P and Singh R: Lactobacillus casei and Lactobacillus acidophilus Regulate Inflammatory Pathway and Improve Antioxidant Status in Collagen-Induced Arthritic Rats. Journal of Interferon & Cytokine Research 2013; 33(1): 1–8.
47. Abhari K, Shekarforoush SS, Hosseinzadeh S, Nazifi S, Sajedianfard J and Eskandari MH: The effects of orally administered *Bacillus coagulans* and inulin on prevention and progression of rheumatoid arthritis in rats. Food Nutr Res 2016; 60(1): 30876.
48. Yamashita M, Matsumoto K, Endo T, Ukibe K, Hosoya T and Matsubara Y: Preventive Effect of Lactobacillus helveticus SBT2171 on Collagen-Induced Arthritis in Mice. Front Microbiol 2017; 8.
49. Achi SC, Talahalli RR and Halami PM: Prophylactic effects of probiotic Bifidobacterium spp. in the resolution of inflammation in arthritic rats. Appl Microbiol Biotechnol 2019; 103(15): 6287–96.
50. Cardoso RS, Messora MR, Silva PHF, Oliveira LF, Leite-Panissi C and Salvador S: Effects of *Bifidobacterium animalis* subsp. lactis HN019 on ligature-induced periodontitis in rats with experimental rheumatoid arthritis. Benef Microbes 2020; 11(1): 33–46.
51. Horta-Baas G, Romero-Figueroa MD, Montiel-Jarquín AJ, Pizano-Zárate ML, García-Mena J and Ramírez-Durán N: Intestinal dysbiosis and rheumatoid arthritis: a link between gut microbiota and the pathogenesis of rheumatoid arthritis. Journal of Immunology Research 2017; 2017.
52. Lee NK, Kim WS and Paik HD: Bacillus strains as human probiotics: characterization, safety, microbiome, and probiotic carrier. Food Science and Biotechnology 2019; 28: 1297-305.
53. Pineda M de LA, Thompson SF, Summers K, de Leon F, Pope J and Reid G: A randomized, double-blinded, placebo-controlled pilot study of probiotics in active rheumatoid arthritis. Med Sci Monit 2011; 17(6): CR347-54.
54. Vaghef-Mehrabany E, Alipour B, Homayouni-Rad A, Sharif SK, Asghari-Jafarabadi M and Zavvari S: Probiotic supplementation improves inflammatory status in patients with rheumatoid arthritis. Nutrition 2014; 30(4): 430–5.
55. Alipour B, Homayouni-Rad A, Vaghef-Mehrabany E, Sharif SK, Vaghef-Mehrabany L and Asghari-Jafarabadi M: Effects of *Lactobacillus casei* supplementation on disease activity and inflammatory cytokines in rheumatoid arthritis patients: a randomized double-blind clinical trial. Int J Rheum Dis 2014; n/a-n/a.
56. Zamani B, Golkar HR, Farshbaf S, Emadi-Baygi M, Tajabadi-Ebrahimi M and Jafari P: Clinical and metabolic response to probiotic supplementation in patients with rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. Int J Rheum Dis 2016; 19(9): 869–79.
57. Vaghef-Mehrabany E, Homayouni-Rad A, Alipour B, Sharif SK, Vaghef-Mehrabany L and Alipour-Ajiry S: Effects of Probiotic Supplementation on Oxidative Stress Indices in Women with Rheumatoid Arthritis: A Randomized Double-Blind Clinical Trial. J Am Coll Nutr 2016; 35(4): 291–9.
58. Shukla A, Gaur P and Aggarwal A: Effect of probiotics on clinical and immune parameters in enthesitis-related arthritis category of juvenile idiopathic arthritis. Clin Exp Immunol 2016; 185(3): 301–8.
59. Vadell AK, Bärebring L, Hulander E, Gjertsson I, Lindqvist HM and Winkvist A: Anti-inflammatory Diet In Rheumatoid Arthritis (ADIRA)—a randomized, controlled crossover trial indicating effects on disease activity. Am J Clin Nutr 2020; 111(6): 1203–13.
60. Cannarella LAT, Mari NL, Alcântara CC, Iryioda TMV, Costa NT and Oliveira SR: Mixture of probiotics reduces inflammatory biomarkers and improves the oxidative/nitrosative profile in people with rheumatoid arthritis. Nutrition 2021; 89: 111282.

How to cite this article:
Shah JM, Sutar BV, Pradhan VS, Patel PP and Goswami PD: Probiotics: anavant-gardestep in the therapy of rheumatoid arthritis. Int J Pharm Sci & Res 2024; 15(3): 699-09. doi: 10.13040/IJPSR.0975-8232.15(3).699-09.