Chapter

Immunomodulatory Potential of *Lactobacillus acidophilus*: Implications in Bone Health

Asha Bhardwaj, Leena Sapra, Bhupendra Verma and Rupesh K. Srivastava

Abstract

Lactobacillus acidophilus is homofermentative anaerobic rod-shaped gram-positive bacteria. *L. acidophilous* is one of the most common probiotics and is used for the treatment of various gastrointestinal, metabolic and inflammatory disorders. *L. acidophilous* produces antimicrobial compounds, maintains gut permeability and prevents dysbiosis. *L. acidophilus* also shows various other properties such as: it is anticarcinogenic, lowers serum cholesterol level and improves lactase metabolism of host. One of the most significant property of *L. acidophilous* is that it modulates the immune system and can prevent various inflammatory disorders. *L. acidophilous* influences several immune cells such as Th17 cells and Tregs. Various studies reported that inflammation induces bone loss and leads to several bone pathologies such as osteoporosis, rheumatoid arthritis and periodontitis. Recent studies have shown the potential of probiotics in preventing inflammation mediated bone loss. *L. acidophilous* is one of these probiotics and is found capable in inhibition of various bone disorders. *L. acidophilous* restores the dysregulated immune homeostasis and prevents inflammatory bone loss. Thus, *L. acidophilous* can be a potential therapeutic for the management of various bone pathologies. In this book chapter we reviewed various immunomodulatory properties of *L. acidophilous* along with its efficacy in preventing dysbiosis and maintaining gut permeability. We also discussed the potential role of *L. acidophilous* as a therapeutic for the management of inflammation induced bone disorders.

Keywords: probiotics, *Lactobacillus acidophilus*, immune cells, dysbiosis, gut permeability, bone

1. Introduction

The word "Probiotics" is derived from Latin language meaning life [1] and came into attention in 1953 by the German scientist Werner Kollath who defined them as "active substances that are essential for healthy development of life". Later on, in 1992 Fuller defined them as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" [2]. Currently probiotics are defined as "live organisms that when administered in adequate amounts confer health benefits on the host" and are specified by the Food and Agriculture Organization of the United Nations and the World Health Organization

(FAO/WHO, 2001). Probiotics are present mainly in fermented foods like cheese, bread, wine, kefir and kumis and are commercially available in the market as powders, tablets and packets [1]. Probiotics are used from centuries for the treatment of various diseases but it was not known until the 20th century that probiotics are healthy bacteria that replace harmful microbes in the gut and regulate gut flora [3]. The most extensively used probiotics are *Lactobacillus* and *Bifidobacterium*. Other common probiotics are *Bacillus*, *Streptococcus*, *Enterococcus* and the fungus *Saccharomyces* [4]. Probiotics are used for the treatment of various gastrointestinal disorders like irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), infectious diarrhea, *Clostridium difficile* colitis and antibiotic associated diarrhea and many other metabolic disorders such as obesity, diabetes and non-alcoholic fatty liver disease [5, 6]. Several mechanisms are involved in preventive activities of probiotics such as they modulate the immune system, regulate gut barrier and protect from pathogens [7]. For a probiotic to be successful it should have various qualities like it should be resistant to the low pH present in gastrointestinal tract, able to colonize in the gut, adhere to the epithelium and be able to activate the immune system. It should also have several other qualities such as it should be of human origin, non-pathogenic, noncariogenic and influence the local metabolic activity [4]. *Lactobacillus acidophilus* (LA) is one of the most common probiotics and is present in several commercially available food products and dietary supplements [8]. LA exhibits antimicrobial, anticarcinogenic and anti-inflammatory properties [8, 9]. LA has various properties that make it a good probiotic such as it is acid tolerant, bile tolerant, has lactase activity, can adhere to the human epithelial cells, lowers serum cholesterol level, prevents infection, modulates immune response, improves lactose metabolism of host, etc. [10]. Several commercially accessible strains of LA have probiotics ability like LA-1 to LA-5 (Chr. Hansen, Demark), NCFM (Dansico, Madison), SBT-2026 (Snow brand milk products, Japan), DDS-1 (Nebraska cultures, Nebraska), etc. LA NCFM is the most common LA strain and is regarded safe by the US Food and Drug Administration (FDA) [10]. LA has immunomodulatory properties and is considered for the treatment of various inflammatory diseases such as IBD, cancer, etc. [11, 12]. Use of probiotics for the prevention of bone loss has recently gain much attention. Probiotics prevent osteoporosis and other bone diseases like arthritis and periodontitis by influencing the immune system or via other mechanisms. Various studies have shown the potential of LA in preventing bone diseases [9]. Bone disorders like osteoporosis, rheumatoid arthritis (RA) and periodontitis are immune disorders and it is observed that LA has potential of preventing these disorders by modulating the immune system. Thus, LA can act as a therapeutic for the treatment of various bone fragilities.

In this chapter we summarized some of the mechanisms which are responsible for the health promoting effects of LA focussing primarily on immunomodulatory properties of LA. We also discussed the role of LA in preventing inflammatory bone loss and how modulation of gut microbiota and maintenance of gut integrity by LA can play a role in regulating bone health.

2. *Lactobacillus acidophilus*

LA is a type of lactic acid bacteria (LAB). LAB constitute a group of gram-positive, acid tolerant, catalase negative, non-sporulating and generally rod-shaped bacteria [13] that are frequently associated with dairy, meat and plants [14]. LAB produce lactic acid from carbohydrate fermentation which make them important in fermentation and agriculture-based industries. They are used for imparting unique textures and flavors and for preservation and acidification of different food

items [10]. LAB comprised a number of genera such as *Lactobacillus*, *Enterococcus*, *Streptococcus*, *Cornobacterium*, *Leuconostoc*, *Lactococcus*, *Bifidobacterium* and *Sporolactobacillus* which are further subdivided into species, subspecies, variants and strains [15, 16]. *Lactobacillus* is the largest genus of lactic acid bacteria having more than 145 species [17]. *Lactobacilli* is part of human microbial flora which colonizes in the human gastrointestinal and urinary tract [18]. *Lactobacillus* species are the first ones to colonize the gut after birth where they provide various health promoting effects. *Lactobacillus* species have various qualities that make them suitable as probiotics. They are resistant to stomach pH and bile juices, can adhere to the mucosa, inhibit growth of other harmful bacterial species and have immunomodulatory properties [19]. *Lactobacilli* encompass a wide range of species that have role in various biochemical and physiological functions [10]. LA is one of the most known species belonging to *Lactobacillus* genus. LA was earlier named as *Bacillus acidophilus* and first isolated in 1900 from the human infant feces by Moro [19]. Almost 80% of the yogurts in America have LA [19]. LA is rod shaped homofermentative anaerobic having size of approximatively 2–10 μM. LA is a thermophile and grows optimally at a temperature of 37 to 45^0 C and at pH range of 4–5 [16]. Highest growth is observed at pH between 5.5 and 6.0 whereas growth ceases at pH 4. Diet is one of the major source of LA in gut. Various commercially available food products such as yogurt and milk are supplemented with LA due to its probiotic value [19]. LA is part of human microbiota and is isolated from digestive, oral and vaginal areas but Claesson's characterization revealed that gastrointestinal tract is its main environment [19]. It is observed that LA supplementation to humans in heat killed form is completely safe. It is observed that heat killed LA provides protection to immunodeficient mice infected with *Candida albicans* [20]. Simakachorn et al. also reported that LA supplementation induces no adverse effect in children having diarrhea [21]. LA is found effective in the treatment of various inflammatory disorders like IBD, diabetes, cancer, etc. [19]. LA prevents these inflammatory disorders by regulating the immune homeostasis. Therefore, the immunomodulatory potential of LA can be used for the management of various disorders. From recent studies it is observed that LA also inhibits inflammatory bone loss and can prevent various bone fragilities such as osteoporosis, RA and periodontitis. Below we reviewed the various immune modifying properties of LA. Later in the chapter, we discussed the role of immune modification by LA in prevention of inflammation induced bone loss. Apart from immunomodulation LA also prevents dysbiosis and increase in gut permeability which are discussed later in the chapter.

2.1 LA role in modulating the immune system

LA has great immunomodulatory properties. Because of the immune modifying properties of LA it is considered for the treatment of various inflammatory diseases. LA can be an inexpensive therapeutic for treatment of numerous clinical manifestations involving malfunctioning of the immune system. Here we discuss various studies highlighting the importance of LA as a potential therapeutic for the prevention of several immune related disorders. It is observed that LA and *L. plantarum* supplementation for 60 days enhanced the expression of genes related to innate immune response in crayfish [22]. Feeding of probiotic "dahi" consisting of LA and *Bifidobacterium bifidum* reversed decrease in immune response in aging mice [23]. LA and *Bifidobacterium animalis* subspecies lactis decreased inflammation of intestinal epithelial cells by modifying the toll like receptor 2 (TLR2) mediated Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways [24]. Feeding of milk fermented with LA and *L. casei* increased both the phagocytic and lymphocytic

activity in swiss mice [25]. LA strain NCFM increased gram-positive immune response in *C. elegans* by modulating key immune signaling pathways such as p38 MAPK and β-catenin signaling pathways [26]. LA can be used for the prevention of obesity related effects. LA-KCTC3925 supplementation significantly attenuated the levels of splenic and hepatic cyclooxygenase-2 (COX-2) mRNA expression and intracellular adhesion molecule-1 (ICAM-1) expression in high fat diet induced obese mice [27]. LA supplementation generated non-specific immune response in germ free mice [28].

LA regulates the secretion of cytokines from various immune cells and maintains the balance between inflammatory and anti-inflammatory cytokines. It is observed that LA treatment significantly altered the production of interleukin (IL)-4 and interferon (IFN)-γ from splenocytes in the presence of purified tumor antigen. LA and *L. reuteri* modulated the cytokine response in neonatal gnotobiotic pigs infected with human rotavirus (HRV). LA and *L. reuteri* treatment in HRV infected pigs significantly enhanced the production of Th1 and Th2 cytokine responses as indicated by the higher concentration of IL-12, IL-10, IL-4 and INF-γ in these pigs. Treated pigs also had higher concentration of transforming growth factor (TGF)-β as compared to the controls. Thus, LA and *L. reuteri* supplementation can maintain immune homeostasis by regulating TGF-β level after HRV infection [29]. LA induced the production of cytokines such as IL-1β, TNF-α, IL-10 and IFN-γ from human peripheral blood mononuclear cells (PBMCs) [30]. LA significantly downregulated the production of anti-inflammatory cytokines and reactive oxygen species (ROS) whereas increased the production of anti-inflammatory cytokines from PBMCs isolated from Parkinson's disease patients [31]. Chen et al. showed that LA suppressed IL-17 production in experimental colitis model by suppressing expression of IL-23 and TGF-β1 and downstream phosphorylation of phospho-signal transducer and activator of transcription 3 (p-STAT3) [11]. It is observed that LA downregulated the expression of IL-1α, IL-1β, IL-8, monocyte chemoattract protein (MCP)-1 and C-X-C motif ligand 3 (CXCL3) in bovine mammary epithelial (BME) cells after Lipopolysaccharide (LPS) challenge. LA also increased the expression level of negative regulators of TLRs viz. toll interacting protein, ubiquitin-editing enzyme A20 and single immunoglobulin IL-1 single receptor in BME cells after LPS challenge [32]. Thus, LA can be a treatment option for bovine mastitis which is characterized by inflammation of the mammary glands. LA treatment significantly enhanced the expression of IL-1β, IFN-α, IFN-γ, interferon regulatory factor (IRF)-7, interferon-inducible transmembrane protein M3 and 2′,5′-oligoadenylate synthetase in chicken macrophages in response to avian influenza virus [33]. LA induced the production of TGF-β and inflammatory cytokines such as IL-6 and tumour necrosis factor (TNF-α) in dendritic cells (DCs) cocultured with intestinal epithelial cells [34]. It is observed that administration of LA strain L36 to germ-free mice induced higher expression of cytokines associated with Th2 cells such as IL-6, IL-5 and TGF-β and Th17 cells like IL-17A, IL-6 and TNF-α [35]. In dextran sodium sulphate (DSS) induced colitis LA administration suppressed the production of pro-inflammatory cytokines such as IL-6, TNF-α and IL-17 in colon tissues. *In vitro* LA treatment stimulated Tregs and the production of IL-10 [36]. LA treatment downregulated the expression of inflammatory cytokines, chemokines and myeloperoxidase in mice model of DSS induced colitis [37]. LA treatment also restored the number of colon goblet cells by inducing IL-10 expression and suppressing proinflammatory cytokines expression in DSS induced colitis [38]. LA treatment induced various antiviral cytokines and chemokines such as IL-1β, regulated upon activation, normal T cell expressed and presumably secreted (RANTES), macrophage colony stimulating factor (MCSF), eotaxin and IFN-α in lung and IL-17 in peyer's patches of influenza virus infected mice [39].

One of the mechanisms by which LA inhibits the progression of inflammatory diseases is by modulating T cells **(Figure 1)**. It is observed that LA-CGMCC 7282 along with *C. butyricum* CGMCC 7281 exerts strong anti-inflammatory effects and can prevent Th1 and Th2-type ulcerative colitis [40]. LA protected the β-lactoglobulin sensitized mice by reducing the allergic inflammation [41]. LA treatment was found to be positively associated with decreased mRNA expression of IL-17 and RORγt and reduced proliferation of Th17 cells under both *in vitro* and *in vivo* models of β-lactoglobulin allergy [41]. In case of HRV infection it is observed that varied doses of LA induced different effects. Wen et al. showed that low dose of LA enhanced IFN-γ producing T cell response but decreased Tregs response the production of TGF-β and IL-10 from Tregs. On the other hand, higher dose of LA upregulated Tregs response in gnotobiotic pigs infected with HRV [42].

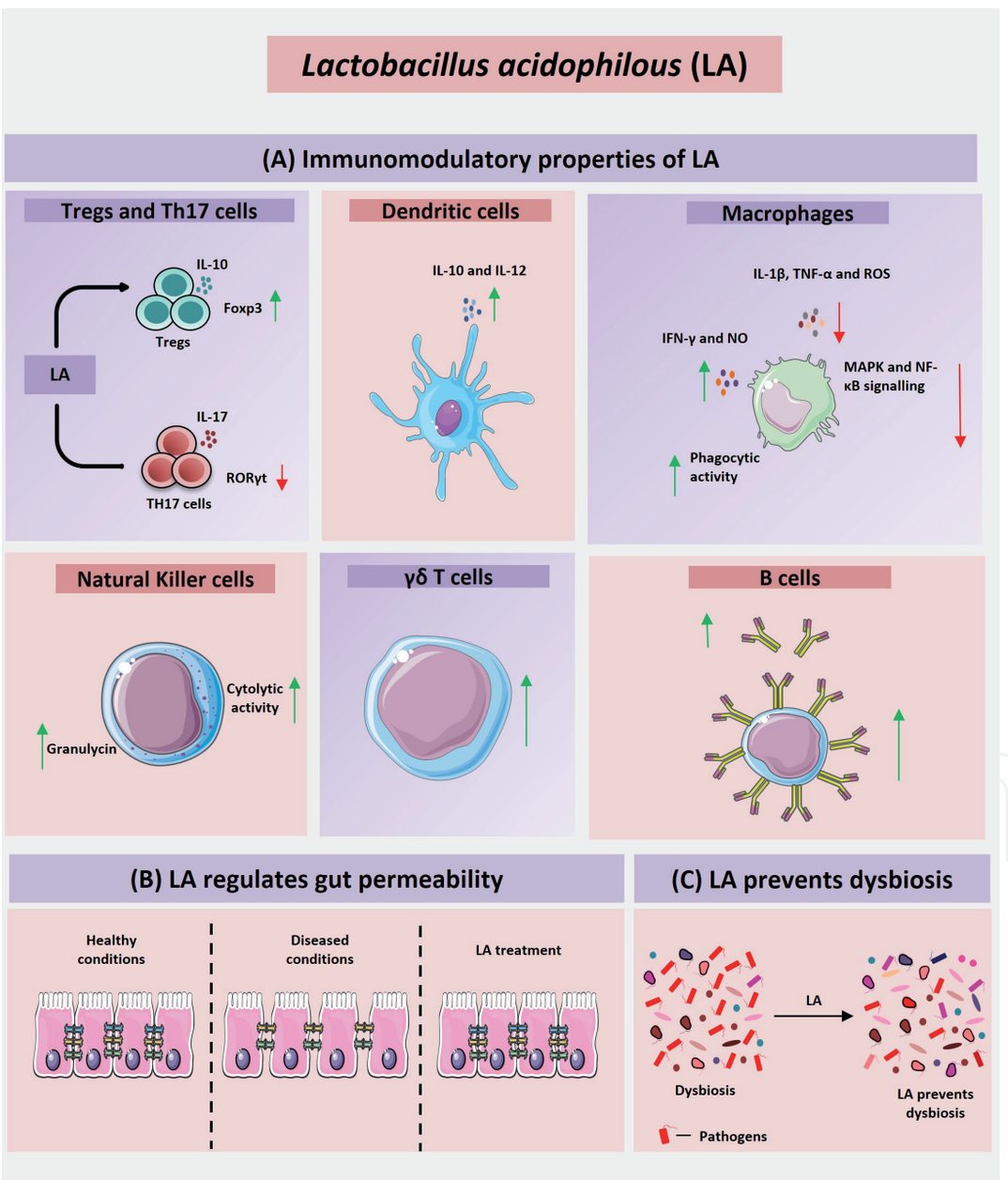

Figure 1.
Schematic diagram depicting immumodulatory properties and effect of LA on gut permeability and dysbiosis. **(A)** *LA influence the activity of various immune cells such as Tregs and Th17 cells, dendritic cells, macrophages, natural killer cells, γδ T cells and B cells.* **(B)** *LA prevents the increase in gut permeability which leads to various diseases such as IBD, IBS, etc.* **(C)** *LA restores gut microbiota composition in dysbiotic conditions.*

LA strain L-92 attenuated the progression of 2, 4-dinitroflurobenzene induced contact dermatitis by regulating Tregs in spleen and cervical lymph nodes. LA-L-92 administration also enhanced FoxP3, IL-10 and TGF-β levels as compared to the controls [43]. LA and *B. longum* administration to the colitis mice model upregulated the number of Tregs and γδ T cells in intraepithelial lymphocytes [44]. Li et al. showed that LA prevents β-immunoglobulin allergy by regulating the balance the Tregs/Th17 cells and activation of TLR2/NF-Kb signaling pathways [45]. It is observed that LA lysates administration in DSS induced colorectal cancer mice model suppressed macrophage (type 2 i.e. M2) polarization, increased the number of $CD8^+$ T cells and effector memory T cells and decreased the number of Tregs in tumor microenvironment [12]. It is reported that when LA is administered after saline challenge in pigs, it increased the number of leucocytes and $CD4^+$ T cells whereas when challenged with LPS decreased the number of both $CD4^+$ and $CD8^+$ T lymphocytes, leukocytes, expression of IL-6 and TNFα as compared to the control diet. LA modulates the activity of other immune cells also. LA enhanced the production of IL-10 and IFN-γ from splenocytes induced with concanavalin A (Con A) and significantly increased the phagocytic activity of peritoneal macrophages [46]. Surface layer protein (Slp) isolated from LA-NCFM reduced the production of IL-1β, TNF-α and ROS in LPS induced RAW 264.7 cells via suppression of MAPK and NF-κB signaling pathways. Slp also attenuated the production of nitic oxide (NO) and prostaglandin E2 (PGE_2) by inhibiting the expression of inducible nitric oxide synthase (iNOS) and COX-2 [40]. SLP derived from the LA-CICC6074 also like LA-NCFM decreased the secretion of TNF-α and enhanced the secretion of NO in RAW 264.7 cells [47]. LA stimulated M2 macrophages in peritoneal cavity and Tregs and Th2 cells in spleen of DSS treated mice [38]. Administration of LA, *L. rhamnosus* and *Bifidobacterium lactis* to the mice significantly enhanced the phagocytic activity of leukocytes and peritoneal macrophages as compared to the controls [48]. It is observed that non-LPS component of LA strain DSS-1 induced the IL-1α and TNF-α production by macrophages [49]. Moreover, LA treated macrophages showed higher expression of IFN-γ and costimulatory molecule CD40 [33]. LA induces activation and maturation of DCs [50]. LA stimulated the IFN-β response in DCs in a myeloid differentiation primary response 88 (Myd88) dependent manner [51]. Konstantinov et al., showed that major SlpA of LA-NCFM interacted with the DCs via their receptor dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) and modulated the function of DCs and T cells [52]. LA-NCFM upregulated the expression of defense genes in DCs such as IL-12 and IL-10 in a TLR-2 dependent manner [51]. LA administration also decreased the degranulation of mast cells and eosinophils [53]. It is observed that pre-treatment with LA-L-92 enhanced the natural killer (NK) cells activity in lung [39]. L-92 also reduced the number of neutrophils in lung of influenza treated mice [39]. It is reported that heat killed LA 205 increased the cytolytic activity of NK cells in a time and dose dependent manner. LA enhanced the cytotoxicity of NK cells by elevating the expression of granulysin which is cytolytic granule component in NK cells [54]. In elderly population administration of LA and *Bifidobacterium bifidum* enhanced the frequency of B cells in peripheral blood [55]. LA prevents various diseases by modulating the level of antibody production. LA can be beneficial in preventing food allergy. It is observed that LA-AD031 and *Bifidobacterium lactis* ADO11 administration significantly decreased the ovalbumin (OVA) specific IgE, IgA and IgG1 in OVA and cholera toxin sensitized mice [53]. Oral administration of heat killed LA attenuated hypersensitivity responses in bovine β-lactoglobulin sensitized mice model. LA administration decreased inflammatory cells and IgE production. Along with IgE production LA treatment enhanced mRNA expression levels of

CD25, FoxP3 and TGF-β whereas decreased the expression of IL-17A, RORγt and IL-10 in allergic group [56]. Intermediate dose of LA increased rotavirus specific IgA and IgG antibody secreting cells and memory B cells in response to rotavirus vaccine. Thus, LA administration can be used to improve the efficacy of rotavirus vaccine and thus can be effective against rotavirus diarrhea [57]. LA increase the IgA, IL-10 and IFNγ producing cells in small intestine [58]. LA improved the immunogenicity of Newcastle Disease vaccines (NDV). Chicks treated with both LA and vaccine have increased IgG and NDV antibody titres than the only vaccinated group [59]. Feeding of probiotic "dahi" (curd) containing LA and *L. casei* ameliorated the secretory IgA and lymphocyte proliferation in *Salmonella enteritidis* infected mice [60]. These probiotics also increased the proliferative response of splenocytes to LPS and con A [48]. Su et al. showed that LA SW1 could function as a promising immune adjuvant in DNA vaccine against foot and mouth disease (FMD). Oral LA-SW1 enhanced the levels of anti-FMDV antibody titres and FMDV neutralizing antibodies [61]. LA lysates also increased the antitumor activity of CTLA-4 monoclonal antibody [58]. LA not only promotes immune response but also inhibits unnecessary lethal immune responses. It is observed that LA along with *B. bifidus* or *B. infantis* suppressed the mitogen activated cell proliferation of splenocytes and PBMCs and arrested the cell cycle at G0/G1 phase. At higher concentrations these probiotics inhibited the mitogen activated overactive immune response and at lower concentration skewed the balance of Th1/Th2 balance towards Th1 [62].

On the basis of these above discussed studies, we can consider that LA has immune regulatory properties. LA has capabilities of regulating various innate and adaptive immune cells and therefore can maintain immune homeostasis. Altogether, these studies suggest that immunomodulatory properties of LA can be employed for regulating the disrupted immune homeostasis in various inflammatory diseases.

2.2 LA role in preventing dysbiosis

Trillions of microbes reside in human gastrointestinal tract. These microbes contribute in number of vital functions related to health. These are source of essential vitamins and nutrients. Microbes extract energy from food, modulate immune system and maintain gut permeability. Gut microbiota usually promotes human health but alteration in the gut lead to various clinical manifestations. Alteration in gut microbiota is termed as dysbiosis. Gut microbiota can be altered by various factors like diet, toxins, pathogens, drugs, antibiotics, etc. [63]. Dysbiosis is reported in various diseases like IBS, IBD, diabetes, obesity, cardiovascular diseases, asthma, allergy, etc. [63–67]. Dysbiosis is also observed in leukemia where selective modulation of *Lactobacillus* species is reported [68]. Dysbiosis is the reason for various vaginal diseases like aerobic vaginitis, bacterial vaginosis and vulvovaginal candidiasis. In vagina of reproductive aged women microbial homeostasis is maintained by the mutualistic relationship between microbes and the host which provide protection against vaginal infections by preventing the colonization of opportunistic pathogens [69]. LA, *L. iners* and *L. crispatus* are the most abundant bacterial species in vaginal tract [70]. Role of LA in preventing dysbiosis is reported by various studies. LA-DSS-1 administration improved the abundance of beneficial bacteria like *Lactobacillus* spp. and *Akkermansia* spp. in caecum [71]. In ulcerative colitis patients, supplementation of LA, *Lactobacillus salivarius* and *Bifidobacterium bifidus* along with anti-inflammatory drug mesalazine prevented intestinal dysbiosis [72]. LA also decreased dysbiosis and inflammation induced by *Salmonella typhimurium* infection in Th1 and Th2 biased mice [73]. LA reversed the alterations in the gut

microbiota composition caused due to administration of high fat diet in animals [74]. Oral administration of LA along with cobiotic ginger extract encapsulated in calcium-alginate beads modulated gut microbiota and prevented 1,2 dimethylhydrazine (DMH)/DSS induced colitis and precancerous lesions in rats [75]. Probiotic combination consisting of LA, *L. helveticus*, *L. gassari*, *L. crispatus* and *L. salivarius* prevented vaginal dysbiosis by restoring the altered vaginal microbiota to normal level. Probiotics combination enhanced the abundance of *Lactobacillus* while decreased the abundance of *Enterobacter* and *Enterococcus* [76]. Antibiotics use provide protection against wide number of pathogens but also disturb the intestinal microflora balance. On the contrary LA is found to be capable of restoring intestinal microbial homeostasis. It is observed that LA prevent the dysbiosis induced by antibiotic Azithromycin [77]. Synbiotic consisting of inulin, LA, *L. plantarum* W21, *L. lactis* and *Bifidobacterium lactis* W51 prevented stress induced dysbiosis and thus can be useful in preventing dysbiosis induced in stress related diseases like IBS and IBD [78]. LA administration is found effective in treatment of dyspepsia caused by dysbiosis [79]. It is observed that the oral intake of LA-GLA-14 and *L. rhamnosus* HN001 mixture along with bovine lactoferrin prevent vaginal dysbiosis and improve vaginal health. After oral ingestion both the LA-GLA-14 and *L. rhamnosus* HN001 colonize and restore the vaginal microbiota [69]. LA supplementation in mice increases short chain fatty acid (SCFA) producing bacteria and thus decreases the gram-negative bacteria [80].

2.3 LA role in maintaining gut permeability

Gut barrier is very important for the regulation of the immune homeostasis and for preventing the access of pathogens into the gut lumen. Through the leaky gut, pathogens invade into the lumen and lead to uncontrolled inflammation. Gut barrier is regulated by tight Junctions (TJs) which are present between the intestinal epithelial cells. TJs are transmembrane proteins and are divided into four groups: claudins, occludin, tricullin and junctional adhesion molecules (JAMs). Transmembrane TJs are linked with the actin cytoskeleton through the cytosolic scaffold proteins like zona occludens (ZOs) which are of three types ZO-1, ZO-2 and ZO-3 [81]. Alteration in expression of TJs leads to increase in gut permeability and intestinal inflammation which is responsible for various inflammatory diseases like IBD [82–86], colon cancer [87] and RA [88].

Several studies have shown that LA administration maintain gut permeability. It is observed that the mixture of LA-KLDS1.0901 and *L. plantarum* KLDS1.0344 prevents chronic alcohol liver injury in mice by improving the gut permeability. *Lactobacillus* mixture inhibits the increase in gut permeability and reduces the abundance of gram-negative bacteria resulting in decrease of LPS entering the portal vein thereby suppressing alcohol promoted liver inflammation [80]. LA along with *L. rhamnosus* and *B. bifidumi* prevented high fat diet induced increase in gut permeability and LPS translocation [74]. LA in combination with ginger extract restored colonic permeability in DMH-DSS induced colon cancer in Wistar rats [75]. Conditioned media of LA significantly prevented the increase in IL-1β induced increase in gut permeability. Conditioned media of LA inhibits IL-1β stimulated decrease in occludin and increase in claudin-1 expression and thus preserve intestinal permeability by normalizing the expression of occludin and claudin-1 [89]. Probiotic combination of LA, *L. reuteri*, *L. casei*, *Streptococcus thermophiles* and *Bifidobacterium bifidum* significantly reduced diabetes incidence and gut permeability [90]. Administration of probiotics LA and *Bifidobacterium infantis* to the pregnant women daily from embryonic day 15- to 2-week-old postnatally maintained the intestinal integrity of preweaned offspring. Thus, LA supplementation

to pregnant women can promote barrier function of developing offsprings [91]. LA and *Streptococcus thermophilus* enhanced the barrier function of epithelial cells and protected the epithelial cells from infection induced by enteroinvasive *E. coli* by limiting its adhesion and invasion [92].

3. Bone remodeling

Bone is a dynamic and metabolically active organ that is being remodeled throughout the life of the organisms. Bone remodeling is regulated by three different types of bone cells viz. osteoclasts (bone eating cells), osteoblasts (bone forming cells) and osteocytes. Osteoblasts are derived from multipotent stem cells that also give rise to fibroblasts, adipocytes, chondrocytes and myoblasts [93]. Osteoblasts are responsible for formation of osteoid matrix by depositing collagen which later on get calcified. The major constituent of osteoid matrix is type 1 collagen which provide resistance against fractures. Osteoid matrix also consists of various other non-collagenous proteins which are responsible for various critical functions of bone [94]. Osteoblast differentiation depends on a number of paracrine and transcription factors such as Runx2 and osterix and members of bone morphogenetic protein (BMP) family [94]. Osteoclasts are giant polynuclear cells that have special ability of resorbing bone [95]. Osteoclasts differentiate from monocytic progenitors that also give rise to cells of other monocytic lineages such as macrophages, dendritic cells, granulocytes and microglia. Osteoclast differentiation depends on two important cytokines: MCSF and receptor activator of nuclear factor κb ligand (RANKL). MCSF stimulate proliferation and differentiation of osteoclast progenitors. RANKL acts with the help of its receptor RANK and coupling molecule TNF receptor associated factor 6 (TRAF 6) to promote the differentiation and commitment of precursor cells [95]. Bone resorption starts when osteoclasts attach to the surface of bone and form a unique structure called sealing zone. Sealing zone permit the osteoclasts to form resorption space. Osteoclasts acidifies the resorption space to degrade the mineral and organic compartment of the bone. For this osteoclast secrete various lysosomal enzymes such as cathepsin K into the resorption space. To mediate bone resorption osteoclasts, form a specialized structure called as ruffled border that increase the surface area for active transport of H^+ through proton pump. Osteoclasts comparatively resorb a large area of bone and then die by apoptosis [94]. Osteocytes are osteoblasts that have been entrapped in the osteoid matrix during matrix calcification under the influence of bone specific alkaline phosphatase produced by osteoblasts [93, 94]. Osteocytes sense mechanical force and tissue strain and send signal to the other osteocytes and osteoblasts by forming cellular network termed as canaliculi permeating the entire bone matrix [93, 94]. Dynamic equilibrium between the osteoblasts and osteoclasts maintains bone integrity. Multiple interactions take place between the bone forming osteoblasts and bone resorbing osteoclasts to regulate the process of bone remodeling [96]. Osteoblasts positively regulate osteoclast differentiation by secreting RANKL and MCSF at pre-osteoblastic stage and negatively by secreting the RANKL decoy receptor osteoprotegerin (OPG). Bone remodeling restore microdamages and ensure the release of calcium and phosphorus in normal host physiology [97]. Bone remodeling consist of four phases viz. activation phase, resorption phase, reversal phase and formation phase [97]. In activation phase, MCSF and RANKL induce the differentiation of osteoclast progenitors into osteoclasts. During resorption phase pre-osteoclasts migrate at the surface of bone and get differentiated into mature osteoclasts and start resorbing bone. Resorption phase is followed by reversal phase where mononuclear cells remove the collagen remnants and prepare

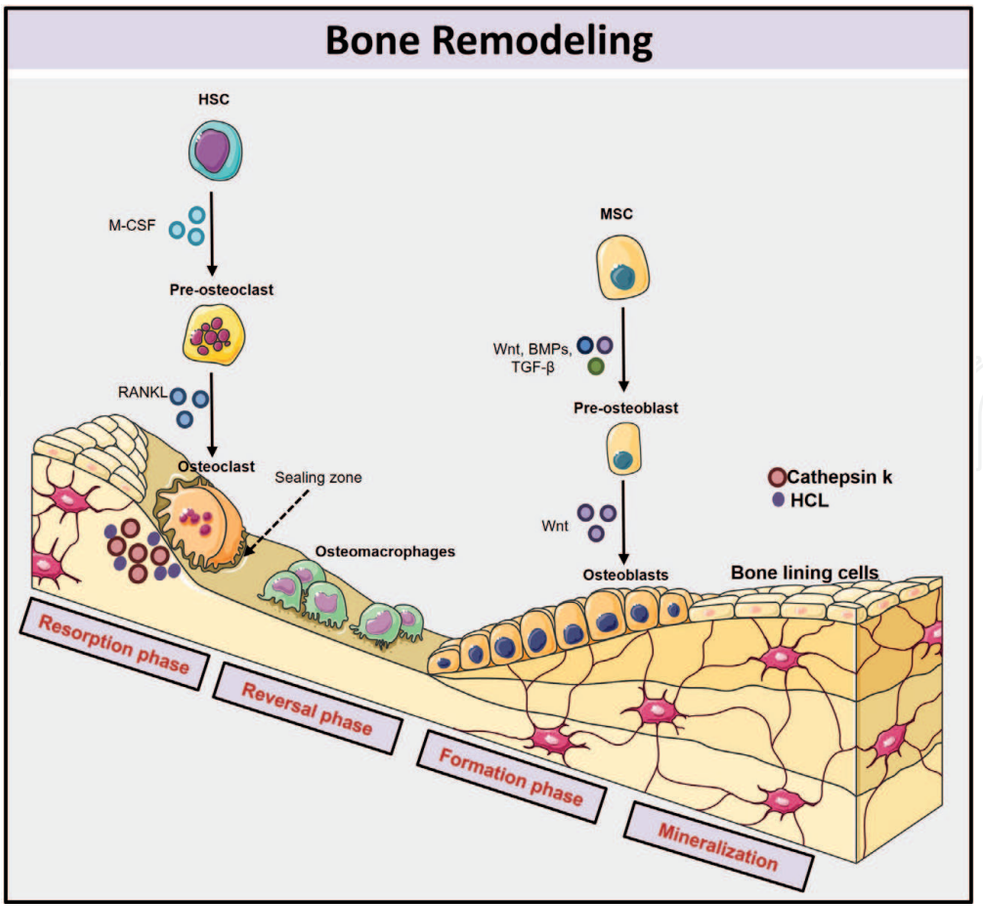

Figure 2.
Schematic representation of bone remodeling. Bone remodeling occurs in four phases viz. 1) Activation phase: MCSF and RANKL induce the differentiation of osteoclast progenitors into osteoclasts. 2) Resorption phase: Mature osteoclast with unique ruffled border starts resorption of bone by secreting cathepsin K, and H^+ in sealing zone. After resorption osteoclasts detach from the surface of bone and undergo apoptosis. 3) Reversal phase: During reversal phase osteoblasts precursor get differentiated into mature osteoblasts and are recruited to the resorption site. 4) Formation phase: Osteoblasts get occupied in the resorbed lacuna and start depositing the bone matrix. After formation phase osteoid gets mineralized and bone surface returns to resting phase with bone lining cells.

the surface for osteoblasts where they can next start the process of bone formation. Mononuclear cell also provides various signals for the differentiation and migration of osteoblasts [93, 97]. During formation phase osteoblasts replace the resorbed bone with new bone [97] (**Figure 2**). Bone remodeling is regulated by various factors such as hormones like estrogen and parathyroid hormone and immune cells like T cells and B cells. Below we next discusse the role of immune system in regulation of bone health and the potential of LA in preventing bone resorption via immunomodulation.

4. Bone and immune system

Bone is an immunomodulatory organ and various immune cells affect the development of bone. Bone cells and immune cells interact with each other in the bone marrow which is the common niche for the development of both bone and immune system. In bone marrow, bone cells and immune cells interact with each other and affects each other development. The interaction between the bone and immune system is now studied under a new field of immunology termed as Osteoimmunology, a term coined by Choi et al. in 2006. Impact of various immune cells and cytokines secreted by immune cells on bone development is now known [97]. It is observed

that cytokines such as IL-1, TNF-α, IL-6, IL-11, IL-15 and IL-17 induce bone resorption whereas cytokines such as IL-4, IL-10, IL13, IL-18, IFN-γ and granulocyte macrophage colony stimulating factor (GM-CSF) prevent bone loss. In various bone disorders the role of immune system has been discovered such as osteoporosis, RA and periodontitis. Osteoporosis is an inflammatory disease and several immune cells affect the development of osteoporosis. To study the immunology of osteoporosis we integrative biology started a novel field termed by us as "Immunoporosis" which deals specifically with the role of immune cells in osteoporosis [96]. Th17 cells and Tregs have most vital role in the development of bones and their balance is required for proper regulation of bone mass. $CD4^+FOXP3^+$ Tregs enhance bone mass by inhibiting osteoclastogenesis by directly suppressing the production of RANKL and MCSF [98]. Another mechanism by which $CD4^+FOXP3^+$ Tregs inhibit osteoclastogenesis or bone loss is by interacting with the CD80 and CD86 present on osteoclast precursors via CTLA-4, thereby inhibiting osteoclast differentiation [96]. Not only $CD4^+FOXP3^+$ Tregs, now the effect of $CD8^+FOXP3^+$ Tregs on bone is also discovered. It is observed that the $CD8^+$ Tregs prevent bone loss by inhibiting the formation of actin ring resulting in suppression of osteoclastogenesis [99]. Unlike Tregs, Th17 cells promote bone loss by inducing osteoclastogenesis via secretion of RANKL. Th17 also secrete IL-17 which induce bone loss by promoting RANKL expression on osteoclastogenesis supporting cells and by stimulating expression of inflammatory cytokines such as TNFα, IL-1 and IL-6 which further upregulate RANKL expression [9, 100]. Imbalance of Tregs and Th17 cells leads to bone loss which occurs during post-menopausal osteoporosis (PMO). Lack of estrogen promotes PMO. Estrogen prevents osteoporosis by inhibiting osteoclastogenesis but estrogen deficiency causes increased osteoclastogenesis by stimulating differentiation of Th17 cells. We also found in our studies that level of Th17 cells and inflammatory cytokines such as TNFα, IL-6, IL-17 and RANKL increased during post-menopausal osteoporosis [9, 101, 102]. Several studies have shown the role of Tregs and Th17 cells imbalanc in pathogenesis of RA [103]. The frequency of Th17 cells are enhanced in the joints and synovial fluid of RA patients [104] whereas the percentage of Tregs get significantly decreased in RA patients [105]. Similarly, the role of Tregs and Th17 cells imbalance is also found to be associated with periodontitis inflammation [106] and osteoarthritis [107]. Apart from these various other immune cells such as Th1, Th2, Th9 cells and γδ T cells are also involved in regulating bone health [97].

4.1 Role of LA in regulation of osteoimmune system

As immune system has such an important role in regulation of bone health, proper maintenance of immune homeostasis is very much required. Immune homeostasis for bone regulation is maintained by various factors such as estrogen hormone. Various strategies are used to prevent bone loss due to immune disruptions such as Denosumab, rituximab and TNF blockers [108, 109]. These strategies are proven effective but they also exert various adverse effects in the long run. Recently the use of probiotics is found to be effective in treatment of various inflammatory disorders such as IBD, obesity, diabetes, etc. [110–112]. Probiotics are also considered for the treatment of various bone disorders. LA is one of these probiotics. It is observed that LA has great potential of treating various bone pathologies. From comparison of different *Lactobacillus* species, it is observed that the effect of *Lactobacillus* on bone health is species dependent and LA has showed the most significant effect on bone parameters such as bone mineral density (BMD) and bone mineral content (BMC) among other *Lactobacillus* species [113]. In rat model of apical periodontitis, it is observed that level of alkaline phosphatase is significantly higher whereas the

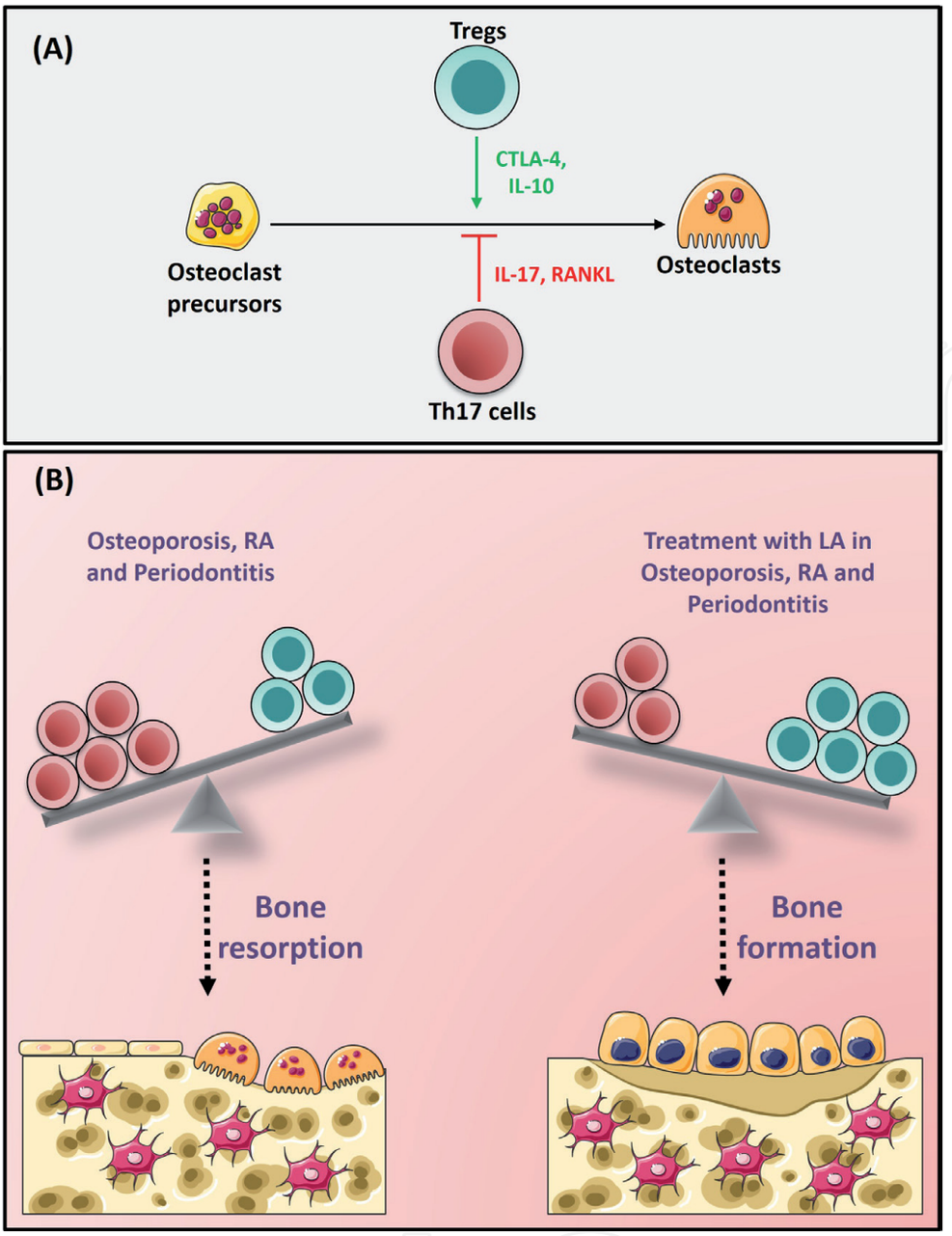

Figure 3.
Role of Tregs/Th17 cells axis in regulation of bone health: **(A)** *Tregs inhibit the differentiation of osteoclasts by secreting IL-10. Tregs also suppress osteoclastogenesis or bone loss by interacting with the CD80 and CD86 present on osteoclast precursors through cytotoxic T lymphocyte associated antigen 4 (CTLA-4). Th17 promote osteoclastogenesis via secretion of RANKL and IL-17. IL-17 induce expression of RANKL on osteoclastogenesis promoting cells.* **(B)** *In normal healthy conditions there is balance between Tregs and Th17 cells but during osteoporosis or other bone diseases like RA and periodontitis number of Th17 cells is increased which further leads to bone loss. LA treatment in these diseases restores the balance of Tregs and Th17 cells and thus prevent bone resorption.*

level of TRAP and RANKL is significantly lower in LA consumed groups [114]. It is reported that LA has antiarthritic properties and prevented Fruend's complete adjuvant mediated arthritis in female wistar rats [115]. It is observed that LA supernatant increased the proliferation of bone marrow stromal cells derived from rats [116]. It is observed in study from our group that LA can prevent bone loss by modulating the host immune system. We reported that LA improved both cortical and trabecular bone microarchitecture as well as enhanced the BMD and heterogeneity of bone in ovariectomized mice by skewing the Treg-Th17 cell balance **(Figure 3)**. LA administration promoted the development of anti-osteoclastogenic Tregs and inhibited the osteoclastogenic Th17 cells in ovariectomized mice. LA supplementation also

S.No.	Commercially available strains of LA	Source	Effect on bone	Reference
1.	ATCC 4356	ATCC	Modulated Treg-Th17 cell axis and inhibited the expression of inflammatory cytokines	[9]
2.	ATCC 314	ATCC	Prevented freund's complete adjuvant induced arthritis by decreasing the oxidative stress	[115, 118]
2.	ATCC 11975	ATCC	NR	—
3.	ATCC 4375D-5	ATCC	NR	—
4.	ATCC 53671	ATCC	NR	—
5.	ATCC 4355	ATCC	NR	—
6.	ATCC 4357	ATCC	NR	—
7.	ATCC 9224	ATCC	NR	—
8.	ATCC BAA-2832	ATCC	NR	—
9.	ATCC 13651	ATCC	NR	—
10.	ATCC 11975	ATCC	NR	—
11.	ATCC 832	ATCC	NR	—
12.	ATCC 43121	ATCC	NR	—
13.	ATCC 53544	ATCC	NR	—
14.	ATCC 53545	ATCC	NR	—
15.	ATCC 53546	ATCC	NR	—
16.	ATCC 4796	ATCC	NR	—
18.	ATCC 53671	ATCC	NR	—
19.	ATCC 700396	ATCC	NR	—
20.	LA-1	Chr. Hansen, Demark	Decreased the levels of inflammatory cytokines and enhanced the levels of anti-inflammatory cytokines in joints of osteoarthritic rats	[117]
21.	LA-2	Chr. Hansen, Demark	NR	—
22	LA-3	Chr. Hansen, Demark	NR	—
23	LA-4	Chr. Hansen, Demark	NR	—
24	LA-5	Chr. Hansen, Demark	NR	—
25	LA-14	Chr. Hansen, Demark	Decreased the inflammatory cytokines IL-1β and IL-6 in experimental apical periodontitis	[119]
26	DDS-1	Nebraska cultures, Nebraska	NR	—
27	NCFM	Dansico, Madison	NR	—
28	SBT-2026	Snow brand milk products, Japan	NR	—

ATCC: American Tissue Culture Collection.
NR: Not reported.

Table 1.
Different strains of LA and their effects on bone.

attenuated the expression of osteoclastogenic cytokines such as IL-6, IL-17, RANKL, TNF-α and increased the expression of anti-osteoclastogenic cytokines like IL-10 and IFN-γ. Thus, LA has therapeutic effects and it can be used as an osteoprotective agent [9]. LA prevented monosodium iodoacetate induced osteoarthritis and reduced cartilage destruction via inhibition of proinflammatory cytokines production [117]. LA supplementation along with *L. rhamnosus* significantly decreased the inflammatory cytokines IL-1β and IL-6 and enhanced the expression of IL-10 as compared to the controls in experimental apical periodontitis [114]. LA supplementation upregulated anti-inflammatory cytokines and downregulated inflammatory cytokines in serum in experimental arthritis model [118]. Thus, LA has great ability of preventing inflammatory bone loss and of regulating osteoimmune system **(Table 1)**.

5. Bone and dysbiosis

A number of microbes are localized in the gut. Some of them are beneficial for health whereas others are pathogenic and a balance of these microbes is required for normal physiological functioning of body. But due to several reasons like surgery, medications, irradiation and antibiotics this balance is dysregulated which leads to modifications in gut microbiota composition [3]. Dysbiosis is observed in various bone pathologies. Normally gut is dominated by four types of microbial phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria*. *Firmicutes* and *Bacteroidetes* constitutes over 90% of the total gut microbiota and dysregulation of *Firmicutes/Bacteroidetes* ratio affect various biological processes like bone remodeling. During osteoporosis *Firmicutes* counts significantly increases whereas counts of *Bacteroidetes* significantly decreases [120–122]. In osteoporosis increase in the number of *Faecalibacterium* and *Dialister* genera is also reported [123]. Dysbiosis is also observed in RA and periodontitis [124, 125]. Although, LA mainly prevents bone loss by regulating immune homeostasis it also restores the gut microbiota composition in various diseases as discussed above. Thus, it can be possible that LA can also inhibit bone resorption by preventing dysbiosis.

6. Bone health and gut permeability

Gut permeability has very important role in regulation of bone health. Various studies have shown that increase in gut permeability is associated with bone loss. Collins et al. group measured the intestinal permeability after 1, 4 and 8 weeks of ovx surgery and they found increased intestinal permeability one week after ovariectomy along with the increase in inflammatory cytokines like IL-1β and TNFα which are responsible for bone loss [126]. Estrogen deficiency during postmenopausal osteoporosis is responsible for increase in gut permeability. Estrogen has very significant role in regulating the gut barrier. It maintains gut barrier through its receptors which are present on the intestinal epithelial cells. There are two types of estrogen receptors: ERα and ERβ. ERβ has very important role in the regulation of TJs as $ER\beta^{-/-}$ mice has disrupted expression of tight junction proteins [39]. Various other studies proved the role of estrogen in regulation of gut barrier. Langen et al. reported decreased expression of ERβ and increased gut permeability in IBD patients [127]. Gut permeability decreases during oestrous phase whereas it is increased during dioestrus phase of rats and this increase in intestinal permeability during dioestrus phase can be prevented by treatment with oestradiol which upregulate the expression of occludin [128]. Estrogen and progesterone treatment

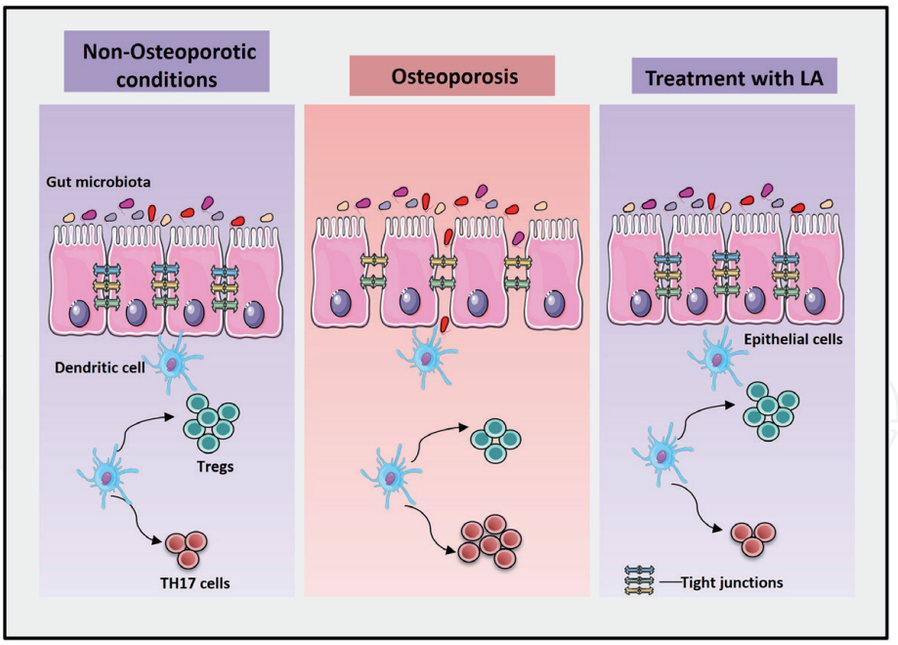

Figure 4.
Proposed mechanism of LA in bone health. In normal healthy condition there is no dysbiosis and no alteration in gut permeability which prevents gut inflammation and thus bone loss. During osteoporosis gut permeability increases which leads to dysbiosis and bone loss. LA regulate Tregs/Th17 cell axis, prevents dysbiosis and maintains gut permeability. Altogether, LA treatment prevents leaky gut and dysbiosis thereby restoring gut immune homeostasis in osteoporosis which inhibit bone loss.

decreases gut permeability and thus prevent secretion of inflammatory cytokines in IBD models [129]. LA prevents leaky gut in various diseases as discussed above and thus it can be possible that LA is effective in preventing leaky gut induced bone loss also. In summary we can say that by maintaining immune homeostasis and regulating both gut permeability and dysbiosis LA prevents bone resorption **(Figure 4)**.

7. Conclusion

In the last few years, several studies have delineated the role of LA in preventing a number of inflammatory and metabolic disorders. LA prevents these disorders through various mechanism such as by modulating the host immune system, by maintaining the gut permeability along with preventing dysbiosis. The role of LA in suppressing bone loss also highlights the importance of LA in regulating bone health. LA enhances bone mass and prevents several bone diseases like osteoporosis, arthritis and periodontitis via regulating the immune homeostasis. Thus, immunomodulatory property of LA is of utmost importance in management of various bone pathologies. LA can also prevent bone resorption by regulating the leaky gut and dysbiosis. Thus, LA has immense potential as a probiotic and can be used as a medical therapy for treatment of bone loss in humans but before that a lot of research is further needed to be done on the efficacy along with the associated pros and cons of LA on human health.

Acknowledgements

This work was financially supported by projects: DST-SERB (EMR/2016/007158), Govt. of India, Intramural project from All India Institute of Medical Sciences (A-798), New Delhi-India and AIIMS-IITD collaborative project

(AI-15) sanctioned to RKS. AB, LS, BV and RKS acknowledge the Department of Biotechnology, AIIMS, New Delhi-India for providing infrastructural facilities. AB thank DST SERB project for research fellowship, LS thank UGC for research fellowship.

Author contributions

RKS contributed in conceptualization and writing of the manuscript. AB, LS and BV participated in writing and editing of the review. RKS suggested and AB and LS created the illustrations. Figures are created with the help of https://smart.servier.com.

Conflicts of interest

The authors declare no conflicts of interest.

Author details

Asha Bhardwaj, Leena Sapra, Bhupendra Verma and Rupesh K. Srivastava*
Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), New Delhi, India

*Address all correspondence to: rupesh_srivastava13@yahoo.co.in; rupeshk@aiims.edu

References

[1] Ozen M, Dinleyici EC. The history of probiotics: the untold story. Benef Microbes. 2015 Jan 1;6(2):159-65.

[2] Gasbarrini G, Bonvicini F, Gramenzi A. Probiotics History. J Clin Gastroenterol. 2016 Nov;50(Supplement 2):S116-9.

[3] Williams NT. Probiotics. Am J Heal Pharm AJHP Off J Am Soc Heal Pharm. 2010 Mar;67(6):449-58.

[4] Gupta V, Garg R. Probiotics. Indian J Med Microbiol. 2009;27(3):202.

[5] Verna EC, Lucak S. Use of probiotics in gastrointestinal disorders: what to recommend? Therap Adv Gastroenterol. 2010 Sep 20;3(5):307-19.

[6] Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. Mechanisms of Action of Probiotics. Adv Nutr. 2019 Jan 1;10(suppl_1):S49-66.

[7] Suez J, Zmora N, Segal E, Elinav E. The pros, cons, and many unknowns of probiotics. Nat Med. 2019 May 6;25(5):716-29.

[8] Sanders ME, Klaenhammer TR. Invited Review: The Scientific Basis of Lactobacillus acidophilus NCFM Functionality as a Probiotic. J Dairy Sci. 2001;84(2):319-31.

[9] Dar HY, Shukla P, Mishra PK, Anupam R, Mondal RK, Tomar GB, et al. Lactobacillus acidophilus inhibits bone loss and increases bone heterogeneity in osteoporotic mice via modulating Treg-Th17 cell balance. Bone reports. 2018 Jun;8:46-56.

[10] Bull M, Plummer S, Marchesi J, Mahenthiralingam E. The life history of *Lactobacillus acidophilus* as a probiotic: a tale of revisionary taxonomy, misidentification and commercial success. FEMS Microbiol Lett. 2013 Dec 1;349(2):77-87.

[11] Chen L, Zou Y, Peng J, Lu F, Yin Y, Li F, et al. Lactobacillus acidophilus suppresses colitis-associated activation of the IL-23/Th17 axis. J Immunol Res. 2015;2015:909514.

[12] Zhuo Q, Yu B, Zhou J, Zhang J, Zhang R, Xie J, et al. Lysates of Lactobacillus acidophilus combined with CTLA-4-blocking antibodies enhance antitumor immunity in a mouse colon cancer model. Sci Rep. 2019;9(1):20128.

[13] Nuraida L. A review: Health promoting lactic acid bacteria in traditional Indonesian fermented foods. Vol. 4, Food Science and Human Wellness. Elsevier B.V.; 2015. p. 47-55.

[14] Carr FJ, Chill D, Maida N. The lactic acid bacteria: A literature survey. Vol. 28, Critical Reviews in Microbiology. CRC Press LLC; 2002. p. 281-370.

[15] Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature. 2011 May 12;473(7346):174-80.

[16] Anjum N, Maqsood S, Masud T, Ahmad A, Sohail A, Momin A. Lactobacillus acidophilus: Characterization of the Species and Application in Food Production. Crit Rev Food Sci Nutr. 2014 Jan 5;54(9):1241-51.

[17] Claesson MJ, van Sinderen D, O'Toole PW. Lactobacillus phylogenomics - Towards a reclassification of the genus. Int J Syst Evol Microbiol. 2008;58(12):2945-54.

[18] Slover CM. Lactobacillus: a Review. Clin Microbiol Newsl. 2008 Feb 15;30(4):23-7.

[19] María Remes Troche J, Coss Adame E, Ángel Valdovinos Díaz M,

Gómez Escudero O, Eugenia Icaza Chávez M, Antonio Chávez-Barrera J, et al. Lactobacillus acidophilus LB: a useful pharmabiotic for the treatment of digestive disorders. Therap Adv Gastroenterol. 2020;13:1756284820971201.

[20] Wagner RD, Pierson C, Warner T, Dohnalek M, Hilty M, Balish E. Probiotic Effects of Feeding Heat-Killed Lactobacillus acidophilus and Lactobacillus casei to *Candida albicans*-Colonized Immunodeficient Mice. J Food Prot. 2000 May 1;63(5):638-44.

[21] Simakachorn N, Pichaipat V, Rithipornpaisarn P, Kongkaew C, Tongpradit P, Varavithya W. Clinical evaluation of the addition of lyophilized, heat-killed Lactobacillus acidophilus LB to oral rehydration therapy in the treatment of acute diarrhea in children. J Pediatr Gastroenterol Nutr. 2000 Jan;30(1):68-72.

[22] Foysal MJ, Fotedar R, Siddik MAB, Tay A. Lactobacillus acidophilus and *L. plantarum* improve health status, modulate gut microbiota and innate immune response of marron (Cherax cainii). Sci Rep. 2020;10(1):5916.

[23] Kaushal D, Kansal VK. Age-related decline in macrophage and lymphocyte functions in mice and its alleviation by treatment with probiotic Dahi containing Lactobacillus acidophilus and Bifidobacterium bifidum. J Dairy Res. 2011 Nov;78(4):404-11.

[24] Li S-C, Hsu W-F, Chang J-S, Shih C-K. Combination of Lactobacillus acidophilus and Bifidobacterium animalis subsp. lactis Shows a Stronger Anti-Inflammatory Effect than Individual Strains in HT-29 Cells. Nutrients. 2019 Apr 27;11(5).

[25] Perdigón G, de Macias ME, Alvarez S, Oliver G, de Ruiz Holgado AP. Systemic augmentation of the immune response in mice by feeding fermented milks with Lactobacillus casei and Lactobacillus acidophilus. Immunology. 1988 Jan;63(1):17-23.

[26] Kim Y, Mylonakis E. Caenorhabditis elegans immune conditioning with the probiotic bacterium Lactobacillus acidophilus strain NCFM enhances gram-positive immune responses. Infect Immun. 2012 Jul;80(7):2500-8.

[27] Na HG, Park Y, Kim M-A, Lee JW, So G, Kim SH, et al. Secondary Fermented Extract of Chaga-Cheonggukjang Attenuates the Effects of Obesity and Suppresses Inflammatory Response in the Liver and Spleen of High-Fat Diet-Induced Obese Mice. J Microbiol Biotechnol. 2019 May 28;29(5):739-48.

[28] Neumann E, Oliveira MA, Cabral CM, Moura LN, Nicoli JR, Vieira EC, et al. Monoassociation with Lactobacillus acidophilus UFV-H2b20 stimulates the immune defense mechanisms of germfree mice. Brazilian J Med Biol Res = Rev Bras Pesqui medicas e Biol. 1998 Dec;31(12):1565-73.

[29] Azevedo MSP, Zhang W, Wen K, Gonzalez AM, Saif LJ, Yousef AE, et al. Lactobacillus acidophilus and *Lactobacillus reuteri* modulate cytokine responses in gnotobiotic pigs infected with human rotavirus. Benef Microbes. 2012 Mar 1;3(1):33-42.

[30] Vissers YM, Snel J, Zuurendonk PF, Smit BA, Wichers HJ, Savelkoul HFJ. Differential effects of Lactobacillus acidophilus and *Lactobacillus plantarum* strains on cytokine induction in human peripheral blood mononuclear cells. FEMS Immunol Med Microbiol. 2010 Jun 1;59(1):60-70.

[31] Magistrelli L, Amoruso A, Mogna L, Graziano T, Cantello R, Pane M, et al. Probiotics May Have Beneficial Effects in Parkinson's Disease: In vitro Evidence. Front Immunol. 2019;10:969.

[32] Fukuyama K, Islam MA, Takagi M, Ikeda-Ohtsubo W, Kurata S, Aso H, et al. Evaluation of the Immunomodulatory Ability of Lactic Acid Bacteria Isolated from Feedlot Cattle Against Mastitis Using a Bovine Mammary Epithelial Cells In Vitro Assay. Pathogens. 2020 May 25;9(5):410.

[33] Shojadoost B, Kulkarni RR, Brisbin JT, Quinteiro-Filho W, Alkie TN, Sharif S. Interactions between lactobacilli and chicken macrophages induce antiviral responses against avian influenza virus. Res Vet Sci. 2019 Aug;125:441-50.

[34] Kim JY. Probiotic modulation of dendritic cells co-cultured with intestinal epithelial cells. World J Gastroenterol. 2012;18(12):1308.

[35] Steinberg RS, Lima M, Gomes de Oliveira NL, Miyoshi A, Nicoli JR, Neumann E, et al. Effect of intestinal colonisation by two Lactobacillus strains on the immune response of gnotobiotic mice. Benef Microbes. 2014 Dec;5(4):409-19.

[36] Park J-S, Choi JW, Jhun J, Kwon JY, Lee B-I, Yang CW, et al. Lactobacillus acidophilus Improves Intestinal Inflammation in an Acute Colitis Mouse Model by Regulation of Th17 and Treg Cell Balance and Fibrosis Development. J Med Food. 2018 Mar;21(3):215-24.

[37] Kim W-K, Han DH, Jang YJ, Park S, Jang SJ, Lee G, et al. Alleviation of DSS-induced colitis via Lactobacillus acidophilus treatment in mice. Food Funct. 2021 Jan 7;12(1):340-50.

[38] Kim DH, Kim S, Lee JH, Kim JH, Che X, Ma HW, et al. Lactobacillus acidophilus suppresses intestinal inflammation by inhibiting endoplasmic reticulum stress. J Gastroenterol Hepatol. 2019 Jan;34(1):178-85.

[39] Goto H, Sagitani A, Ashida N, Kato S, Hirota T, Shinoda T, et al. Anti-influenza virus effects of both live and non-live Lactobacillus acidophilus L-92 accompanied by the activation of innate immunity. Br J Nutr. 2013 Nov;110(10):1810-8.

[40] Wang Y, Gu Y, Fang K, Mao K, Dou J, Fan H, et al. Lactobacillus acidophilus and Clostridium butyricum ameliorate colitis in murine by strengthening the gut barrier function and decreasing inflammatory factors. Benef Microbes. 2018 Sep 18;9(5):775-87.

[41] Wang J-J, Zhang Q-M, Ni W-W, Zhang X, Li Y, Li A-L, et al. Modulatory effect of Lactobacillus acidophilus KLDS 1.0738 on intestinal short-chain fatty acids metabolism and GPR41/43 expression in β-lactoglobulin-sensitized mice. Microbiol Immunol. 2019 Aug;63(8):303-15.

[42] Wen K, Li G, Bui T, Liu F, Li Y, Kocher J, et al. High dose and low dose Lactobacillus acidophilus exerted differential immune modulating effects on T cell immune responses induced by an oral human rotavirus vaccine in gnotobiotic pigs. Vaccine. 2012 Feb 1;30(6):1198-207.

[43] Shah MM, Saio M, Yamashita H, Tanaka H, Takami T, Ezaki T, et al. Lactobacillus acidophilus strain L-92 induces CD4(+)CD25(+)Foxp3(+) regulatory T cells and suppresses allergic contact dermatitis. Biol Pharm Bull. 2012;35(4):612-6.

[44] Roselli M, Finamore A, Nuccitelli S, Carnevali P, Brigidi P, Vitali B, et al. Prevention of TNBS-induced colitis by different Lactobacillus and Bifidobacterium strains is associated with an expansion of gammadeltaT and regulatory T cells of intestinal intraepithelial lymphocytes. Inflamm Bowel Dis. 2009 Oct;15(10):1526-36.

[45] Li A-L, Sun Y-Q, Du P, Meng X-C, Guo L, Li S, et al. The Effect of

Lactobacillus actobacillus Peptidoglycan on Bovine β-Lactoglobulin-Sensitized Mice via TLR2/NF-κB Pathway. Iran J Allergy Asthma Immunol. 2017 Apr;16(2):147-58.

[46] Paturi G, Phillips M, Kailasapathy K. Effect of probiotic strains Lactobacillus acidophilus LAFTI L10 and *Lactobacillus paracasei* LAFTI L26 on systemic immune functions and bacterial translocation in mice. J Food Prot. 2008 Apr;71(4):796-801.

[47] Zhang D, Wu M, Guo Y, Xun M, Wang W, Wu Z, et al. Purification of Lactobacillus acidophilus surface-layer protein and its immunomodulatory effects on RAW264.7 cells. J Sci Food Agric. 2017 Sep 1;97(12):4204-9.

[48] Gill HS, Rutherfurd KJ, Prasad J, Gopal PK. Enhancement of natural and acquired immunity by *Lactobacillus rhamnosus* (HN001), Lactobacillus acidophilus (HN017) and Bifidobacterium lactis (HN019). Br J Nutr. 2000 Feb;83(2):167-76.

[49] Rangavajhyala N, Shahani KM, Sridevi G, Srikumaran S. Nonlipopolysaccharide components) of Lactobacillus addophilus stimulate(s) the production of interleukin-1α and tumor necrosis factor-α by murine macrophages. Nutr Cancer. 1997 Jan;28(2):130-4.

[50] Elawadli I, Brisbin JT, Mallard BA, Griffiths MW, Corredig M, Sharif S. Differential effects of lactobacilli on activation and maturation of mouse dendritic cells. Benef Microbes. 2014 Sep;5(3):323-34.

[51] Weiss G, Maaetoft-Udsen K, Stifter SA, Hertzog P, Goriely S, Thomsen AR, et al. MyD88 drives the IFN-β response to Lactobacillus acidophilus in dendritic cells through a mechanism involving IRF1, IRF3, and IRF7. J Immunol. 2012 Sep 15;189(6):2860-8.

[52] Konstantinov SR, Smidt H, de Vos WM, Bruijns SCM, Singh SK, Valence F, et al. S layer protein A of Lactobacillus acidophilus NCFM regulates immature dendritic cell and T cell functions. Proc Natl Acad Sci U S A. 2008 Dec 9;105(49):19474-9.

[53] Kim JY, Choi YO, Ji GE. Effect of oral probiotics (Bifidobacterium lactis AD011 and Lactobacillus acidophilus AD031) administration on ovalbumin-induced food allergy mouse model. J Microbiol Biotechnol. 2008 Aug;18(8):1393-400.

[54] Cheon S, Lee KW, Kim KE, Park JK, Park S, Kim C, et al. Heat-killed Lactobacillus acidophilus La205 enhances NK cell cytotoxicity through increased granule exocytosis. Immunol Lett. 2011 May;136(2):171-6.

[55] De Simone C, Ciardi A, Grassi A, Lambert Gardini S, Tzantzoglou S, Trinchieri V, et al. Effect of Bifidobacterium bifidum and Lactobacillus acidophilus on gut mucosa and peripheral blood B lymphocytes. Immunopharmacol Immunotoxicol. 1992;14(1-2):331-40.

[56] Li A-L, Meng X-C, Duan C-C, Huo G-C, Zheng Q-L, Li D. Suppressive effects of oral administration of heat-killed Lactobacillus acidophilus on T helper-17 immune responses in a bovine β-lactoglobulin-sensitized mice model. Biol Pharm Bull. 2013;36(2):202-7.

[57] Liu F, Wen K, Li G, Yang X, Kocher J, Bui T, et al. Dual functions of Lactobacillus acidophilus NCFM as protection against rotavirus diarrhea. J Pediatr Gastroenterol Nutr. 2014 Feb;58(2):169-76.

[58] Paturi G, Phillips M, Jones M, Kailasapathy K. Immune enhancing effects of Lactobacillus acidophilus LAFTI L10 and *Lactobacillus paracasei* LAFTI L26 in mice. Int J Food Microbiol. 2007 Apr 1;115(1):115-8.

[59] Nouri Gharajalar S, Mirzai P, Nofouzi K, Madadi MS. Immune enhancing effects of Lactobacillus acidophilus on Newcastle disease vaccination in chickens. Comp Immunol Microbiol Infect Dis. 2020 Oct;72:101520.

[60] Jain S, Yadav H, Sinha PR, Naito Y, Marotta F. Dahi containing probiotic Lactobacillus acidophilus and Lactobacillus casei has a protective effect against Salmonella enteritidis infection in mice. Int J Immunopathol Pharmacol. 21(4):1021-9.

[61] Su J, Li J, Zheng H, You Y, Luo X, Li Y, et al. Adjuvant effects of L. acidophilus LW1 on immune responses to the foot-and-mouth disease virus DNA vaccine in mice. PLoS One. 2014;9(8):e104446.

[62] Li C-Y, Lin H-C, Lai C-H, Lu JJ-Y, Wu S-F, Fang S-H. Immunomodulatory effects of lactobacillus and Bifidobacterium on both murine and human mitogen-activated T cells. Int Arch Allergy Immunol. 2011;156(2):128-36.

[63] Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. Microb Ecol Heal Dis. 2015 Feb 2;26.

[64] Chong PP, Chin VK, Looi CY, Wong WF, Madhavan P, Yong VC. The Microbiome and Irritable Bowel Syndrome – A Review on the Pathophysiology, Current Research and Future Therapy. Front Microbiol. 2019 Jun 10;10.

[65] Tamboli CP. Dysbiosis in inflammatory bowel disease. Gut. 2004 Jan 1;53(1):1-4.

[66] Kesh K, Mendez R, Abdelrahman L, Banerjee S, Banerjee S. Type 2 diabetes induced microbiome dysbiosis is associated with therapy resistance in pancreatic adenocarcinoma. Microb Cell Fact. 2020 Dec 24;19(1):75.

[67] Nagpal R, Newman TM, Wang S, Jain S, Lovato JF, Yadav H. Obesity-Linked Gut Microbiome Dysbiosis Associated with Derangements in Gut Permeability and Intestinal Cellular Homeostasis Independent of Diet. J Diabetes Res. 2018 Sep 3;2018:1-9.

[68] Bindels LB, Beck R, Schakman O, Martin JC, De Backer F, Sohet FM, et al. Restoring specific lactobacilli levels decreases inflammation and muscle atrophy markers in an acute leukemia mouse model. PLoS One. 2012;7(6):e37971.

[69] Russo R, Edu A, De Seta F. Study on the effects of an oral lactobacilli and lactoferrin complex in women with intermediate vaginal microbiota. Arch Gynecol Obstet. 2018;298(1):139-45.

[70] Salinas AM, Osorio VG, Endara PF, Salazar ER, Vasco GP, Vivero SG, et al. Bacterial identification of the vaginal microbiota in Ecuadorian pregnant teenagers: an exploratory analysis. PeerJ. 2018;6:e4317.

[71] Vemuri R, Gundamaraju R, Shinde T, Perera AP, Basheer W, Southam B, et al. Lactobacillus acidophilus DDS-1 Modulates Intestinal-Specific Microbiota, Short-Chain Fatty Acid and Immunological Profiles in Aging Mice. Nutrients. 2019 Jun 7;11(6).

[72] Palumbo VD, Romeo M, Marino Gammazza A, Carini F, Damiani P, Damiano G, et al. The long-term effects of probiotics in the therapy of ulcerative colitis: A clinical study. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2016 Sep;160(3):372-7.

[73] Pradhan B, Guha D, Naik AK, Banerjee A, Tambat S, Chawla S, et al. Probiotics L. acidophilus and *B. clausii* Modulate Gut Microbiota in Th1- and Th2-Biased Mice to Ameliorate Salmonella Typhimurium-Induced Diarrhea. Probiotics Antimicrob Proteins. 2019;11(3):887-904.

[74] Bagarolli RA, Tobar N, Oliveira AG, Araújo TG, Carvalho BM, Rocha GZ, et al. Probiotics modulate gut microbiota and improve insulin sensitivity in DIO mice. J Nutr Biochem. 2017;50:16-25.

[75] Deol PK, Khare P, Singh DP, Soman G, Bishnoi M, Kondepudi KK, et al. Managing colonic inflammation associated gut derangements by systematically optimised and targeted ginger extract-Lactobacillus acidophilus loaded pharmacobiotic alginate beads. Int J Biol Macromol. 2017 Dec;105(Pt 1):81-91.

[76] Chen T, Xia C, Hu H, Wang H, Tan B, Tian P, et al. Dysbiosis of the rat vagina is efficiently rescued by vaginal microbiota transplantation or probiotic combination. Int J Antimicrob Agents. 2021 Jan 9;106277.

[77] Shoaib A, Dachang W, Xin Y. Determining the role of a probiotic in the restoration of intestinal microbial balance by molecular and cultural techniques. Genet Mol Res. 2015 Feb 20;14(1):1526-37.

[78] Konturek PC, Konturek K, Brzozowski T, Wojcik D, Magierowski M, Targosz A, et al. Participation of the intestinal microbiota in the mechanism of beneficial effect of treatment with synbiotic Syngut on experimental colitis under stress conditions. J Physiol Pharmacol. 2020 Jun;71(3).

[79] Kocián J. [Lactobacilli in the treatment of dyspepsia due to dysmicrobia of various causes]. Vnitr Lek. 1994 Feb;40(2):79-83.

[80] Li H, Shi J, Zhao L, Guan J, Liu F, Huo G, et al. *Lactobacillus plantarum* KLDS1.0344 and Lactobacillus acidophilus KLDS1.0901 Mixture Prevents Chronic Alcoholic Liver Injury in Mice by Protecting the Intestinal Barrier and Regulating Gut Microbiota and Liver-Related Pathways. J Agric Food Chem. 2021 Jan 13;69(1):183-97.

[81] Lee SH. Intestinal Permeability Regulation by Tight Junction: Implication on Inflammatory Bowel Diseases. Intest Res. 2015;13(1):11.

[82] Takeuchi K, Maiden L, Bjarnason I. Genetic aspects of intestinal permeability in inflammatory bowel disease. Novartis Found Symp. 2004;263:151-8; discussion 159-63, 211-8.

[83] Vu TH, Shipley JM, Bergers G, Berger JE, Helms JA, Hanahan D, et al. MMP-9/Gelatinase B Is a Key Regulator of Growth Plate Angiogenesis and Apoptosis of Hypertrophic Chondrocytes. Cell. 1998 May;93(3):411-22.

[84] Zeissig S, Burgel N, Gunzel D, Richter J, Mankertz J, Wahnschaffe U, et al. Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. Gut. 2007 Jan 1;56(1):61-72.

[85] Edelblum KL, Turner JR. The tight junction in inflammatory disease: communication breakdown. Curr Opin Pharmacol. 2009 Dec;9(6):715-20.

[86] Vetrano S, Rescigno M, Rosaria Cera M, Correale C, Rumio C, Doni A, et al. Unique Role of Junctional Adhesion Molecule-A in Maintaining Mucosal Homeostasis in Inflammatory Bowel Disease. Gastroenterology. 2008 Jul;135(1):173-84.

[87] Soler AP. Increased tight junctional permeability is associated with the development of colon cancer. Carcinogenesis. 1999 Aug 1;20(8):1425-32.

[88] Bjarnason I, So A, Levi AJ, Peters T, Williams P, Zanelli G, et al. Intestinal permeability and inflammation in rheumatoid arthritis: Effects of non-steroidal anti-inflammatory drugs. Lancet. 1984 Nov;324(8413):1171-4.

[89] Guo S, Gillingham T, Guo Y, Meng D, Zhu W, Walker WA, et al. Secretions of Bifidobacterium infantis and Lactobacillus acidophilus Protect Intestinal Epithelial Barrier Function. J Pediatr Gastroenterol Nutr. 2017;64(3):404-12.

[90] Kim TK, Lee J-C, Im S-H, Lee M-S. Amelioration of Autoimmune Diabetes of NOD Mice by Immunomodulating Probiotics. Front Immunol. 2020;11:1832.

[91] Yu Y, Lu J, Oliphant K, Gupta N, Claud K, Lu L. Maternal administration of probiotics promotes gut development in mouse offsprings. Aguila MB, editor. PLoS One. 2020 Aug 7;15(8):e0237182.

[92] Resta-Lenert S, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). Gut. 2003 Jul;52(7):988-97.

[93] Hadjidakis DJ, Androulakis II. Bone remodeling. In: Annals of the New York Academy of Sciences. Blackwell Publishing Inc.; 2006. p. 385-96.

[94] Walsh MC, Kim N, Kadono Y, Rho J, Lee SY, Lorenzo J, et al. Osteoimmunology: interplay between the immune system and bone metabolism. Annu Rev Immunol. 2006;24:33-63.

[95] Schett G, David J-P. The multiple faces of autoimmune-mediated bone loss. Nat Rev Endocrinol. 2010 Dec;6(12):698-706.

[96] Srivastava RK, Dar HY, Mishra PK. Immunoporosis: Immunology of Osteoporosis—Role of T Cells. Front Immunol. 2018;9:657.

[97] Dar HY, Azam Z, Anupam R, Mondal RK, Srivastava RK. Osteoimmunology: The Nexus between bone and immune system. Front Biosci (Landmark Ed. 2018 Jan 1;23:464-92.

[98] Zaiss MM, Axmann R, Zwerina J, Polzer K, Gückel E, Skapenko A, et al. Treg cells suppress osteoclast formation: A new link between the immune system and bone. Arthritis Rheum. 2007 Dec;56(12):4104-12.

[99] Shashkova E V., Trivedi J, Cline-Smith AB, Ferris C, Buchwald ZS, Gibbs J, et al. Osteoclast-Primed Foxp3 + CD8 T Cells Induce T-bet, Eomesodermin, and IFN-γ To Regulate Bone Resorption. J Immunol. 2016 Aug 1;197(3):726-35.

[100] Adamopoulos IE, Bowman EP. Immune regulation of bone loss by Th17 cells. Arthritis Res Ther. 2008;10(5):225.

[101] Dar HY, Pal S, Shukla P, Mishra PK, Tomar GB, Chattopadhyay N, et al. *Bacillus clausii* inhibits bone loss by skewing Treg-Th17 cell equilibrium in postmenopausal osteoporotic mice model. Nutrition. 2018;54:118-28.

[102] Sapra L, Dar HY, Bhardwaj A, Pandey A, Kumari S, Azam Z, et al. *Lactobacillus rhamnosus* attenuates bone loss and maintains bone health by skewing Treg-Th17 cell balance in Ovx mice. Sci Rep. 2021;11(1):1807.

[103] Kikodze N, Pantsulaia I, Chikovani T. The role of T regulatory and Th17 cells in the pathogenesis of rheumatoid arthritis (Review). Georgian Med News. 2016 Dec;(261):62-8.

[104] Leipe J, Grunke M, Dechant C, Reindl C, Kerzendorf U, Schulze-Koops H, et al. Role of Th17 cells in human autoimmune arthritis. Arthritis Rheum. 2010 Oct;62(10):2876-85.

[105] Zhang X, Zhang X, Zhuang L, Xu C, Li T, Zhang G, et al. Decreased regulatory T-cell frequency and interleukin-35 levels in patients with rheumatoid arthritis. Exp Ther Med. 2018 Oct 19;

[106] Gao L, Zhao Y, Wang P, Zhang L, Zhang C, Chen Q, et al. Detection of Th17/Treg cells and related factors in gingival tissues and peripheral blood of rats with experimental periodontitis. Iran J Basic Med Sci. 2017 Mar;20(3):294-300.

[107] Li Y, Luo W, Zhu S, Lei G. T Cells in Osteoarthritis: Alterations and Beyond. Front Immunol. 2017 Mar 30;8.

[108] Raterman HG, Lems WF. Pharmacological Management of Osteoporosis in Rheumatoid Arthritis Patients: A Review of the Literature and Practical Guide. Drugs Aging. 2019 Dec 21;36(12):1061-72.

[109] Elshahaly M, Wheater G, Naraghi K, Tuck SP, Datta HK, Ng W-F, et al. Changes in bone density and bone turnover in patients with rheumatoid arthritis treated with rituximab, a B cell depleting monoclonal antibody (HORUS TRIAL). BMC Musculoskelet Disord. 2013 Feb 14;14(S1):A10.

[110] Guandalini S, Sansotta N. Probiotics in the Treatment of Inflammatory Bowel Disease. Adv Exp Med Biol. 2019;1125:101-7.

[111] Wang Z-B, Xin S-S, Ding L-N, Ding W-Y, Hou Y-L, Liu C-Q, et al. The Potential Role of Probiotics in Controlling Overweight/Obesity and Associated Metabolic Parameters in Adults: A Systematic Review and Meta-Analysis. Evidence-Based Complement Altern Med. 2019 Apr 15;2019:1-14.

[112] Kocsis T, Molnár B, Németh D, Hegyi P, Szakács Z, Bálint A, et al. Probiotics have beneficial metabolic effects in patients with type 2 diabetes mellitus: a meta-analysis of randomized clinical trials. Sci Rep. 2020;10(1):11787.

[113] Montazeri-Najafabady N, Ghasemi Y, Dabbaghmanesh MH, Talezadeh P, Koohpeyma F, Gholami A. Supportive Role of Probiotic Strains in Protecting Rats from Ovariectomy-Induced Cortical Bone Loss. Probiotics Antimicrob Proteins. 2019;11(4):1145-54.

[114] Cosme-Silva L, Dal-Fabbro R, Cintra LTA, Dos Santos VR, Duque C, Ervolino E, et al. Systemic administration of probiotics reduces the severity of apical periodontitis. Int Endod J. 2019 Dec;52(12):1738-49.

[115] Amdekar S, Roy P, Singh V, Kumar A, Singh R, Sharma P. Anti-Inflammatory Activity of *Lactobacillus* on Carrageenan-Induced Paw Edema in Male Wistar Rats. Kanai T, editor. Int J Inflam. 2012;2012:752015.

[116] Samadikuchaksaraei A, Gholipourmalekabadi M, Saberian M, Abdollahpour Alitappeh M, Shahidi Delshad E. How does the supernatant of Lactobacillus acidophilus affect the proliferation and differentiation activities of rat bone marrow-derived stromal cells? Cell Mol Biol (Noisy-le-grand). 2016 Aug 31;62(10):1-6.

[117] Lee SH, Kwon JY, Jhun JY, Jung KA, Park SH, Yang CW, et al. Lactobacillus acidophilus ameliorates pain and cartilage degradation in experimental osteoarthritis. Immunol Lett. 2018 Nov 1;203:6-14.

[118] Amdekar S, Singh V, Kumar A, Sharma P, Singh R. Lactobacillus acidophilus Protected Organs in Experimental Arthritis by Regulating the Pro-inflammatory Cytokines. Indian J Clin Biochem. 2014 Oct;29(4):471-8.

[119] Cosme-Silva L, Dal-Fabbro R, Cintra LTA, Ervolino E, Plazza F, Mogami Bomfim S, et al. Reduced bone resorption and inflammation in apical periodontitis evoked by dietary supplementation with probiotics in rats. Int Endod J. 2020 Aug;53(8):1084-92.

[120] Wang J, Wang Y, Gao W, Wang B, Zhao H, Zeng Y, et al. Diversity analysis of gut microbiota in osteoporosis and osteopenia patients. PeerJ. 2017;5:e3450.

[121] Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010 Mar;464(7285):59-65.

[122] Yatsonsky Ii D, Pan K, Shendge VB, Liu J, Ebraheim NA. Linkage of microbiota and osteoporosis: A mini literature review. World J Orthop. 2019 Mar 18;10(3):123-7.

[123] Xu Z, Xie Z, Sun J, Huang S, Chen Y, Li C, et al. Gut Microbiome Reveals Specific Dysbiosis in Primary Osteoporosis. Front Cell Infect Microbiol. 2020;10:160.

[124] Picchianti-Diamanti A, Panebianco C, Salemi S, Sorgi M, Di Rosa R, Tropea A, et al. Analysis of Gut Microbiota in Rheumatoid Arthritis Patients: Disease-Related Dysbiosis and Modifications Induced by Etanercept. Int J Mol Sci. 2018 Sep 27;19(10):2938.

[125] Nath S, Raveendran R. Microbial dysbiosis in periodontitis. J Indian Soc Periodontol. 2013;17(4):543.

[126] Collins FL, Rios-Arce ND, Atkinson S, Bierhalter H, Schoenherr D, Bazil JN, et al. Temporal and regional intestinal changes in permeability, tight junction, and cytokine gene expression following ovariectomy-induced estrogen deficiency. Physiol Rep. 2017 May;5(9):e13263.

[127] Looijer-van Langen M, Hotte N, Dieleman LA, Albert E, Mulder C, Madsen KL. Estrogen receptor-β signaling modulates epithelial barrier function. Am J Physiol Liver Physiol. 2011 Apr;300(4):G621-6.

[128] Braniste V, Leveque M, Buisson-Brenac C, Bueno L, Fioramonti J, Houdeau E. Oestradiol decreases colonic permeability through oestrogen receptor β-mediated up-regulation of occludin and junctional adhesion molecule-A in epithelial cells. J Physiol. 2009 Jul 1;587(13):3317-28.

[129] van der Giessen J, van der Woude CJ, Peppelenbosch MP, Fuhler GM. A Direct Effect of Sex Hormones on Epithelial Barrier Function in Inflammatory Bowel Disease Models. Cells. 2019;8(3).