

# Chapter 17

## Probiotic Lactobacilli, Infection, and Immunomodulation



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**Abstract** Immunomodulatory agents are potentially believed to play important roles in infectious, allergic, and autoimmune diseases. The probiotic bacteria such as lactobacilli have immunomodulatory properties and are generally regarded as safe. Thus, they can be used as adjuvants for the treatment of infectious and allergic/autoimmune diseases. The immunomodulatory properties of five lactobacilli species such as *Lactobacillus casei*, *L. rhamnosus*, *L. paracasei*, *L. gasseri*, and *L. acidophilus* have been well studied. This book chapter summarizes the various studies which have reported immunomodulation by *Lactobacillus* species. Further, the immunomodulatory molecules produced by lactobacilli have been discussed. The immunomodulatory effects of *Lactobacillus* species are strictly strain-specific and in some cases yielded contrasting results in different hosts. Thus, use of lactobacilli as immunomodulatory agent for therapeutic use should be strictly backed by human clinical trials. Further, some of the immunomodulatory molecules are known to play role(s) in immunopathogenesis of allergic diseases. Thus probiotic lactobacilli species to be used as therapeutic agents should be screened for their ability to secrete harmful metabolites.

**Keywords** Allergic disease · Cytokine · GABA · Immunomodulatory · Lactobacilli · SCFA

### 17.1 Introduction

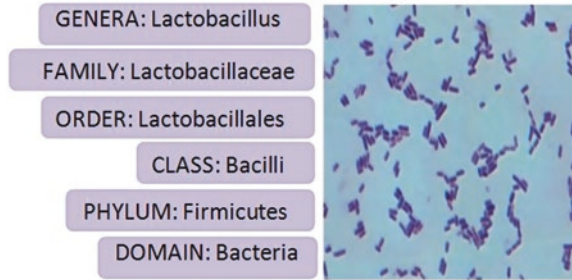
Lactic acid bacteria (LAB) are Gram-positive, catalase-negative, acid-tolerant bacteria belonging to phylum *Firmicutes* (Fig. 17.1). They produce lactic acid as major metabolic end product of carbohydrate fermentation. The genus *Lactobacillus* comprises the largest group of rod-shaped, non-sporulating and facultative anaerobes that contains 154 species. Lactobacilli are ubiquitous in nature and found in milk, fermented food products, and beverages. They comprise almost 0.01% of the gut

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**Fig. 17.1** The classification of *Lactobacillus* spp. and the Gram-stained microscopic view (1000×) of lactobacilli cells

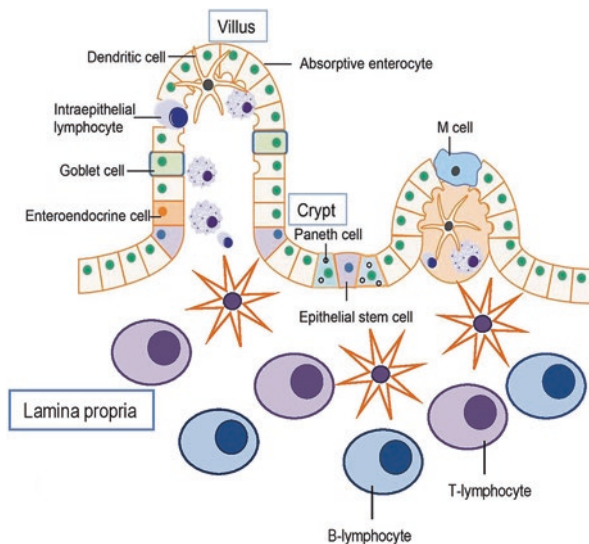


microbiome of human and animal tracts (Finegold et al. 1983). The proportion of lactobacilli in the vagina and oral cavity is quite high probably because of their ability to persist under harsh physiological conditions such as at low pH and their ability to bind to the vaginal epithelial cells.

Lactobacilli have a long history of safe consumption by humans through fermented foods, and therefore they have been accorded GRAS status (generally regarded as safe) by the World Health Organization (FAO/WHO 2001). They are the most common bacterial genera that are used as probiotics. Probiotics are defined as live microbial food supplements of human origin beneficially influencing human health by improving the intestinal microbial balance (FAO/WHO 2001). As the probiotic potential of bacteria is strain-specific, every lactobacilli strain should be screened for some characteristics such as the ability to survive and adhere to the intestinal tract, form strong biofilms, and auto-aggregate and co-aggregate with pathogens (Havenaar et al. 1992). *Lactobacillus* strains are known to benefit the host due to their various functional properties such as acting as microbial barriers against gastrointestinal pathogens, causing competitive exclusion of pathogen binding, and producing inhibitory compounds, such as organic acids, e.g., lactic acid and acetic acid, hydrogen peroxide, and cationic peptides, e.g., bacteriocins (Bermudez-Brito et al. 2012). They are also known to strengthen both the innate and adaptive immune system against pathogens as they have immunomodulatory properties. In this chapter five *Lactobacillus* spp. whose immunomodulatory properties have been widely reported worldwide will be reviewed, and further the various compounds of the lactobacilli that have immunomodulatory potential shall be discussed.

## 17.2 Gut-Associated Lymphoid Tissue (GALT)

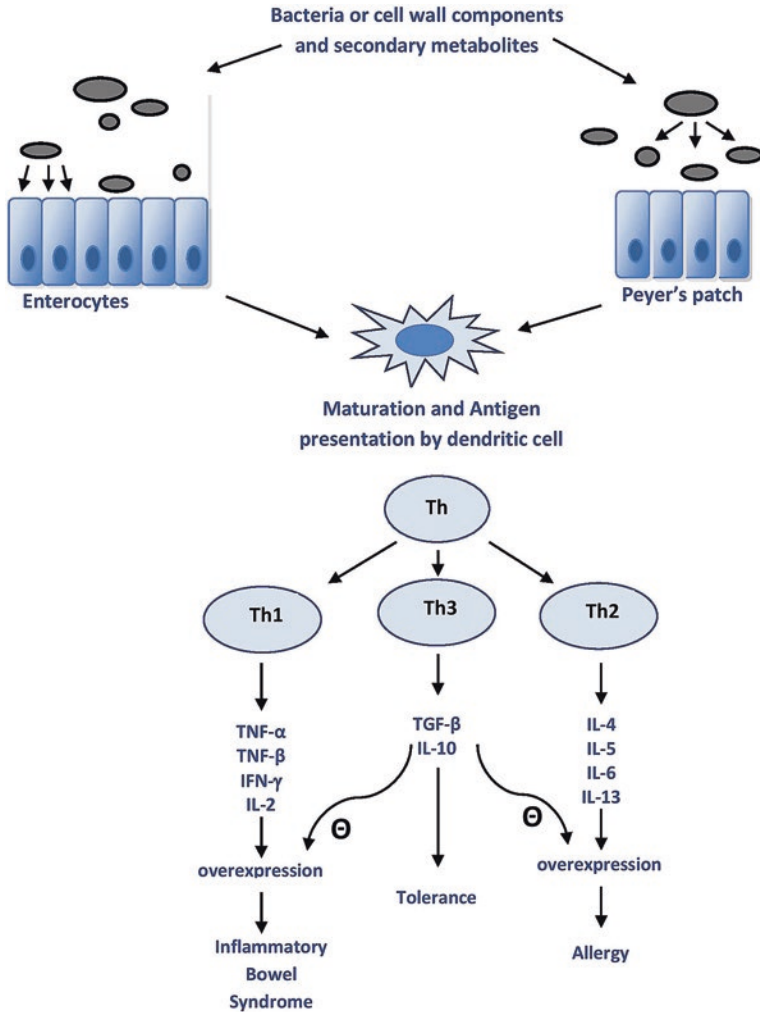
The lactobacilli form less than 1% of gut microflora. The various persistent species present in human feces are mostly *L. gasseri*, *L. crispatus*, *L. reuteri*, *L. salivarius*, and *L. ruminis*, and the sporadic species belong to *L. acidophilus*, *L. plantarum*, and *L. casei* group (*L. casei*, *L. paracasei*, and *L. rhamnosus*; Walter 2008). In the gut these lactobacilli species interact with the immune cells and are believed to cause immunomodulation. The gut mucosa consists of various immune cells that protect



**Fig. 17.2** The structure of small intestine mucosa along with associated immune cells

the host from the pathogens (Fig. 17.2). The epithelial cells of the gut contribute to innate immunity as they form tight junctions; secondly the mucus produced by goblet cells shields the epithelium from the action of gut enzymes, microorganisms, and other luminal contents. The intestinal epithelium contains a large population of lymphocytes known as intraepithelial lymphocytes (IELs), which recognize and eliminate infected epithelial cells and microorganisms. IELs are unique because they have high proportions of  $\gamma\delta$  T cells that do not require major histocompatibility complex (MHC) presentation of antigens for activation. Other important cells are Paneth cells located at the base of the crypts within the small intestine that secrete a vast array of antimicrobial proteins (Bevins and Salzman 2012) following microbial recognition via pattern recognition receptors, including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (Kobayashi et al. 2005). In the lamina propria (LP), dendritic cells (DCs) extend processes between the epithelial cells and sample the contents of the gut lumen, following which they activate lymphocytes (Niess and Reinecker 2006). The LP contains large numbers of dendritic cells and effector lymphocytes such as immunoglobulin A (IgA)-producing plasma cells and cluster of differentiation (CD) 4+ T cells.

The commensals present in the gut are important for the health of gut immune system because they are both immunostimulatory and immunomodulatory in nature. Immunostimulatory functions involve development of immune system, maintenance of healthy epithelial cell lining, recruitment of immune cells to the epithelium, and stimulation of the production of antimicrobial peptides and other innate immune effector molecules by immune cells. Immunomodulatory functions involve influencing the type of immune response through modulating the differentiation of immune effector cells (Fig. 17.3; Ivanov and Honda 2012).



**Fig. 17.3** Mechanism of bacterial immunomodulation of gut immune system

### 17.3 Immunomodulatory Role of Lactobacilli

Various reports have demonstrated that lactobacilli can modulate host’s immune system because they are potential adjuvants triggering mucosal and systemic immune responses. Lactobacilli have been shown to interact with various cells like natural killer (NK) cells, enterocytes, dendritic cells, macrophages, and T helper (Th) 1 and Th2 and T regulatory (Treg) cells and may modulate the immune response either toward pro- or anti-inflammatory type. The immunomodulatory effects of lactobacilli include their potential to activate, cause maturation, and induce cytokine production through interaction with immune cells. The immunomodulatory

properties of some lactobacilli such as *L. casei*, *L. rhamnosus*, *L. paracasei*, *L. gasei*, and *L. acidophilus* have been very well studied. These strains have the potential to be used as probiotics for the treatment of allergic and inflammatory diseases as shown by various animal studies.

### 17.3.1 Lactobacillus casei

*L. casei* is a facultative heterofermentative, acid-resistant, lactic acid-producing bacterial strain found in the mouth and intestinal tract of humans. *L. casei* can be isolated from cheddar cheese during maturation process. *L. casei* Shirota (LcS) is commercially available in probiotic-fermented milk drink, Yakult. Both probiotic and immunomodulatory roles of *L. casei* have been well documented.

Some reports suggested that LcS enhanced the NK cell activity in vitro (Dong et al. 2010) as well as stimulated the cytokines such as interleukin (IL)-10, IL-12, and tumor necrosis factor (TNF)- $\alpha$  and interferon (IFN- $\gamma$ ) production (Shida et al. 2006). Another report showed that *L. casei* DN-114001 elevated the number of CD4 Fox P3 Tregs in the mesenteric lymph nodes and reduced the production of the pro-inflammatory cytokines TNF- $\alpha$  and IFN- $\gamma$  (Zakostelska et al. 2011). Contrary findings were observed by Shida et al. (2002) in the food allergy mice model. Intraperitoneal injection of heat-killed LcS induced a rise in serum IL-12 levels, and the reduction in the levels of IgE and IgG by splenocytes was observed.

Kato et al. (1984) demonstrated that intraperitoneal application of *L. casei* in murine model activated macrophages by increasing their phagocytic and enzyme activities and also activated NK cells, which play an important role in tumor killing. In a human clinical trial, LcS-containing Yakult was orally administered to middle-aged and elderly individuals for 3 weeks, and the NK cell activity was determined. Although the numbers of NK cells and CD4+ and CD8+ T cells remained same, the NK cell activity was enhanced at 1 week, 3 weeks, and 6 weeks after the start of intake. However in elderly individuals, no effect on NK cell activity was observed (Takeda and Okumura 2007). In another similar report by Hashimoto et al. (1985), it was demonstrated in an in vitro assay that *L. casei* administration activated the Kupffer cells and immune cells associated with spleen, lung, and peritoneal macrophages. Perdigon et al. (1986) demonstrated that oral administration of *L. casei* led to lymphocytes and macrophage stimulation. Subsequent studies by Perdigon et al. (1990, 1991) showed that treatment with *L. casei*-activated cells in GALT even at low doses led to significantly higher production of secretory IgG in intestinal fluid, providing protection against infections such as *Salmonella*.

Herfías et al. (2005) showed that LcS had positive effect in case of the prevention of experimental ulcerative colitis in a mice model. Treatment with LcS showed an increase in colonic epithelial regeneration in murine model of ulcerative colitis in the chronic stage. In vivo effects of oral administration of LcS were tested in skin allergy mice model and experimental autoimmune encephalomyelitis (EAE) rat model. LcS abrogated Th1 response and thus proved effective in mice allergy model,

but it worsened the symptoms in EAE model (Baken et al. 2006). An ex vivo treatment of the gut mucosal cells isolated from the ileum of Crohn's disease patients with both *L. casei* and *L. bulgaricus* resulted in significant reduction in the numbers of CD4+ cells as well as TNF- $\alpha$  expression among IEL (Borrueal et al. 2002).

### 17.3.2 *Lactobacillus rhamnosus*

*L. rhamnosus* is a heterofermentative, facultative anaerobe found in abundance in the genitourinary tract of healthy females and fecal samples. *L. rhamnosus* GG (LGG) is the most extensively studied lactobacilli strain. LGG was originally isolated from human feces of healthy adults by Sherwood Gorbach and Barry Goldwin (Doron et al. 2005).

Pena and Versalovic (2003) demonstrated that LGG specifically inhibited TNF- $\alpha$  production in murine macrophages. Balejko et al. (2015) demonstrated that LGG cells immobilized in capsules as well as LGG metabolites activated the release of cytokine IL-10 and tumor growth factor (TGF)- $\beta$ 1 in human peripheral blood mononuclear cells (PBMCs) and downregulated the production of IFN- $\gamma$ . Kopp et al. (2008) demonstrated in an in vitro study that there was a significant increase in IL-10, IFN- $\gamma$  levels in the supernatant of LGG-treated PBMCs. However, another study showed that LGG was least effective among other probiotic bacteria such as *Leuconostoc mesenteroides* and *Streptococcus* spp. in the induction of various cytokines such as TNF- $\alpha$ , IFN- $\gamma$ , IL-12, and IL-10 in PBMC culture supernatants (Kekkonen et al. 2008).

Fong et al. (2015) studied the immunomodulatory effects of LGG on human dendritic cells (DCs), macrophages, and monocytes. Results showed that LGG downregulated the TLR2 mRNA levels of DCs and monocytes and both TLR2 and TLR8 mRNA levels of macrophages. The levels of IL-12, TNF- $\alpha$ , and IL-10 increased in both treated macrophages and monocytes, whereas the levels of Th2 cytokines, IL-4 and IL-25, decreased. Another report by Fong et al. (2016) showed immunomodulatory effect of LGG cells and LGG-derived soluble factors on human PBMCs + DC co-cultures. Both the treatments increased the TLRs on the surface of PBMCs and the pro-inflammatory Th1 and Th17 immune responses. In another study, immunomodulation of human DCs by *L. rhamnosus* Lcr35 was demonstrated (Evrard et al. 2011). Lcr35 treatment led to dose-dependent maturation of DCs that was associated with upregulation of membrane expression of CD86, CD83, human leukocyte antigen-antigen D related (HLA-DR), and TLR4. Further, Lcr35 induced strong dose-dependent increase of Th1/Th17 cytokine levels (TNF- $\alpha$ , IL-1 $\beta$ , IL-12, IL-23) but a low increase in IL-10 levels from DCs. In another study by Miettinen et al. (2000), LGG was shown to directly and very rapidly activate nuclear factor kappa B (NF- $\kappa$ B) in human macrophages that was not inhibited in the presence of protein synthesis inhibitor, cycloheximide.

To study the immunomodulatory effect of *Lactobacillus* spp. in mice, Kirjavainen et al. (1999) orally administered  $10^9$  colony-forming units (CFUs) of four different

*Lactobacillus* spp. (*L. acidophilus*, *L. casei*, *L. rhamnosus*, and *L. gasseri*) to mice for 7 days, *L. rhamnosus* along with *L. gasseri* and *L. casei* was shown to inhibit lipopolysaccharide (LPS)-mediated murine lymphocyte proliferation *ex vivo*. Wu et al. (2016) studied the effect of LGG in allergic murine model. The results showed that the levels of Th2 cytokines and IgE were significantly decreased in both serum and bronchoalveolar lavage fluid after treatment with LGG as compared to untreated OVA-sensitized mice.

The human clinical trials of LGG were also conducted. Kalliomaki et al. (2001, 2003, 2007) in a series of randomized placebo-controlled clinical trials demonstrated that the oral administration of LGG-containing capsules ( $10^{10}$  CFU daily) to mothers prenatally (2–4 weeks) and to postnatal pediatric subjects (6 months) in families with a history of atopic disease significantly lowered the risk of eczema at the age of 2, 4, and 7 years. However, allergic rhinitis and asthma tended to be more common in the LGG-treated group, and no significant differences were found in incidence of cow milk allergy. On the other hand, similar clinical trials with LGG repeated in Germany in families with atopic dermatitis did not yield any beneficial results (Fölster-Holst et al. 2006). The differences may be attributed to differences in the LGG strains and different population.

### 17.3.3 *Lactobacillus paracasei*

*L. paracasei* is a member of the normal human and animal gut microbiota. It is extensively used in the food industry as starter cultures for dairy products and also as probiotic (Marchand and Vandenplas 2000).

D'Arienzo et al. (2011) studied the immunomodulatory roles of five strains of *L. paracasei*. All the isolates had the ability to induce phenotypic maturation of DCs, increasing surface expression of CD11c and CD80 at levels comparable with LPS stimulation; however surface expression of CD86 was higher than that of LPS stimulation. The cytokine profile analysis showed variation among isolates. Isolate LMGP-17806 increased IL-12 production when co-administered with LPS, whereas in case of isolate ATCC334, IL-12 production was decreased. Similarly, IL-10 and IL-2 levels were increased when lactobacilli were co-administered with LPS. No significant differences were observed in case of TNF- $\alpha$  levels when lactobacilli were administered alone or along with LPS.

*L. paracasei* NCC2461 altered the cytokine profiles of murine CD4+ T lymphocytes. Dose-dependent inhibition of proliferative capacity of CD4+ T cells was seen after treatment with *L. paracasei*. Further, the *in vitro* stimulation of lymphocytes cultures with *L. paracasei* cells resulted in a dose-dependent increase in IL-10 and TGF- $\beta$  levels, whereas the Th1 and Th2 effector cytokine production such as IL-4, IL-5, and IFN- $\gamma$  decreased greatly (von de Weid et al. 2001).

In mice, oral administration of *L. paracasei* KW3110 increased IL-12 secretion and reduced IL-4 secretion from splenocytes, but no effect was seen in levels of IFN- $\gamma$  (Fujiwara et al. 2004). A study conducted by Zhu et al. (2016) demonstrated

the positive effect of oral administration of *L. paracasei* L9 on mouse systemic immunity by enhancing phagocytic activity of peritoneal macrophages and proliferation ratio of splenocytes, IgG levels in serum, and IgA levels in mucosa. L9 induced Th1-polarized immune response by elevating IFN- $\gamma$ /IL-10 ratio in mucosa as well as induced IL-12 in macrophages. Increased expression of TLR-2 mRNA in mucosa was also observed.

Immunomodulatory activity of *L. paracasei* subsp. *paracasei* NTU101 in enterohemorrhagic *E. coli* O157:H7-infected BALB/c mice was investigated by Tsai et al. (2010). Oral administration of *L. paracasei* to mice resulted in weight gain and lowered the cumulative morbidity rates. The upregulation of dendritic cells, Th cell activation, and antibody production in post- and pre-treated mice were observed as compared to untreated mice. *L. paracasei* downregulated the expression of TLRs on macrophages and pro-inflammatory cytokines and chemokines in post- and pre-feeding mice induced by *E. coli* infection, thus inhibiting the inflammation.

#### 17.3.4 *Lactobacillus gasseri*

*L. gasseri* is a homofermentative rod-shaped bacterium which can be isolated from human mouth, gut, and vagina. *L. gasseri* is known for weight maintenance and providing protection against pathogens. Research suggested that *L. gasseri* speeds up the metabolism, therefore resulting in weight loss (Kang et al. 2013). The other in vivo effects of *L. gasseri* include cholesterol-lowering effects (Ooi et al. 2010), alleviating symptoms of allergic responses and asthma (Chen et al. 2010), and reduction in menstrual pain in women suffering from endometriosis (Itoh et al. 2011).

Luongo et al. (2013) demonstrated the immunomodulatory abilities of *L. gasseri* OLL2809 and L13-Ia. Direct incubation of murine DCs with irradiated *L. gasseri* cells induced the secretion of cytokines IL-12, IL-10, and TNF- $\alpha$  in the supernatant. Further they explored the cross talk between gut enterocytes and DCs that is influenced by probiotic bacteria such as *L. gasseri*. The treatment of DCs with the supernatant of bacterial-conditioned murine enterocyte cell line completely suppressed the expression of all the three cytokines.

Human clinical trials with different strains of *L. gasseri* have been conducted. Chen et al. (2010) conducted a randomized, double-blind, placebo-controlled study on the oral administration of *L. gasseri* A5 to the children suffering from asthma and allergic rhinitis. The study concluded that probiotic intake significantly improved the pulmonary function tests and the disease symptoms and significantly decreased TNF- $\alpha$ , IFN- $\gamma$ , and IL-13 production by PBMCs was also observed. Another randomized, double-blind clinical trial conducted by Olivares et al. (2006) suggested that the consumption of the fermented product containing strains, *L. gasseri* CECT 5714 and *L. coryniformis* CECT 5711, boosted the immune system of healthy humans by increasing the numbers of phagocytic cells such as monocytes and neutrophils, as well as their phagocytic activity. The increase in proportion of NK cells,



IgA concentrations, and the levels of serum IL-10 and IL-4 was also observed after 2 weeks treatment. After 2 weeks of treatment, significant decrease was observed in serum IgE levels. IgE is involved in allergic responses; therefore decrease in IgE levels could be beneficial for allergic patients.

### 17.3.5 *Lactobacillus acidophilus*

*L. acidophilus* is a homofermentative bacterium that can be isolated from human vagina, gut, and mouth. As part of starter culture, it is used in the production of various dairy products such as acidophilus milk and cheese. *L. acidophilus* has been shown to inhibit the growth of *Candida albicans* by inhibiting the biofilm formation by *C. albicans* (Vilela et al. 2015).

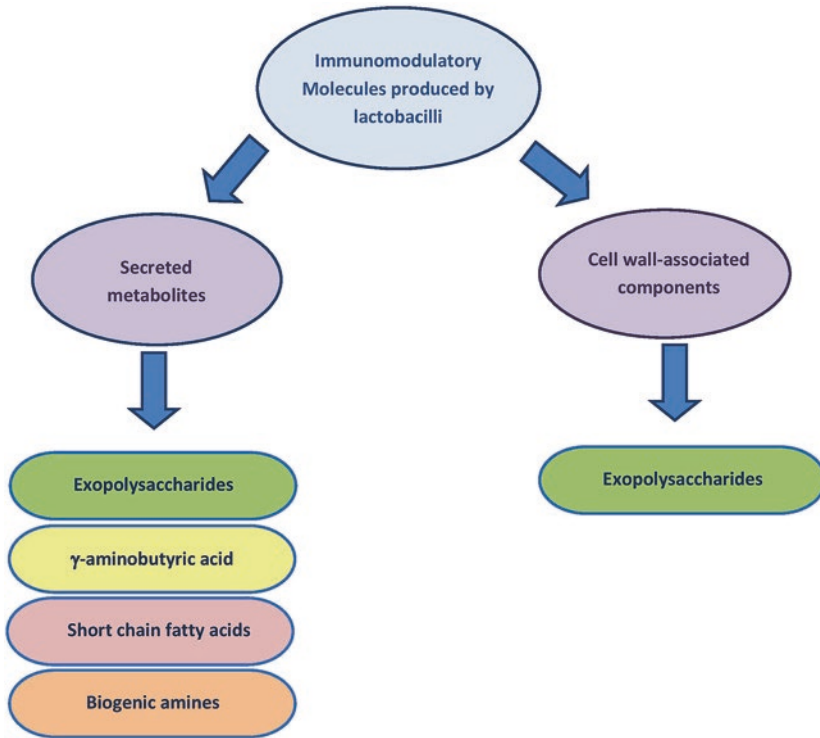
The immunomodulatory role of *L. acidophilus* was studied in in vitro cultures. *L. acidophilus* was shown to bind to DC via DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN) and activate concentration-dependent IL-10 production (Konstantinova et al. 2008). The binding of *L. acidophilus* cells with DCs with the mutant strain that lacked surface S-layer A protein and had dominant expression of S-layer protein B was significantly reduced. Also the DCs treated with mutant strain produced pro-inflammatory cytokines in higher amounts as compared to parental strain.

A study conducted by Gill et al. (2000) showed that mice fed on *L. acidophilus* ( $10^9$  CFUs) had enhanced phagocytic activity of blood leukocytes and macrophages as compared to controls. The levels of sera antibodies were also significantly enhanced. Further, the spleen cells isolated from the treated mice produced significantly higher amounts of IFN- $\gamma$  in response to concanavalin A, i.e., T-cell mitogen, as compared to control, whereas no significant increase was observed in IL-4 production. This study suggested that diet supplemented with these bacteria enhanced both cell-mediated and humoral immunity in healthy mice.

Maroof et al. (2012) showed that administration of *L. acidophilus* in breast cancer murine model can modulate immune responses and thereby cause reduction in tumor volume. The pro-inflammatory response due to enhanced production of IFN- $\gamma$  and the decrease in IL-4 levels from splenocytes was observed.

## 17.4 Immunomodulatory Molecules

The immunomodulatory functions of different cell wall-associated and secreted molecules of lactobacilli have been studied. The prominent ones among cell wall-associated immunomodulatory molecules are exopolysaccharides (EPS) and among those secreted are secretory EPS,  $\gamma$ -aminobutyric acid (GABA), short-chain fatty acids (SCFA), and biogenic amines (Fig. 17.4).



**Fig. 17.4** The various immunomodulatory molecules of lactobacilli

### 17.4.1 EPS

EPS are high molecular weight molecules composed of sugar residues produced by microorganisms. They are important for the structural integrity of biofilms and also determine the physiochemical properties of the biofilm. They are made up of either homo- or heteropolysaccharides. The food applications of EPS are well known; for example, EPS have been commercially used as additives to improve texture as well as viscosity of naturally fermented milk products and prevented syneresis in fermented milk products. The lactobacilli-derived EPS have many health-benefiting potentials such as having antitumor, anti-ulcer, antioxidant, cholesterol-lowering, and immunostimulating activities (Patten and Laws 2015; Kim et al. 2010).

Few research studies have shown the immunomodulatory activities of purified EPS and EPS-producing bacteria. In *in vitro* study in murine macrophage cell line RAW264.7 cells, the EPS of *L. paracasei* subsp. *paracasei* NTU 101 and *L. plantarum* NTU 102 stimulated the dose-dependent increase in pro-inflammatory cytokine production (TNF- $\alpha$ , IL-6, and IL-1 $\beta$ ). Further, EPS from both the cultures also increased the proliferative and phagocytic abilities of RAW264.7 cells (Liu et al. 2011). Similar results were obtained by Ciszek-Lenda et al. (2011), where they

reported that EPS derived from *L. rhamnosus* KL37 enhanced the production of both pro-inflammatory (TNF- $\alpha$ , IL-6, IL-12) and anti-inflammatory (IL-10) cytokines from murine macrophages. They also demonstrated that EPS made cells tolerant to subsequent stimulation by same stimulus.

Gorska et al. (2014) demonstrated the differential immunomodulatory properties of two EPS – L900/2 and L900/3 produced by *L. rhamnosus* LOCK 0900. Exposure of mouse bone marrow-derived dendritic cells (BM-DC) to both the EPS did not trigger the production of cytokines; however, they differentially modulated the immune responses of BM-DC to *L. plantarum*. L900/2 along with *L. plantarum* cells induced the production of IL-10 levels, whereas L900/3 along with *L. plantarum* led to enhanced levels of IL-12 secretion by BM-DC. Further they showed in an experimental-induced allergy mouse model that L900/3 abrogated the ovalbumin allergen-induced IL-4, IL-5, IL-10, and IL-13 production in the spleen and mesenteric lymph nodes and thus has the potential to be used as therapeutic agent for the treatment of allergy (Gorska et al. 2017).

Another report by Gorska et al. (2016a) showed immunomodulatory properties of two different EPS 919/A and 919/B isolated from *L. casei* in BM-DC. Both the EPS did not induce the production of cytokines IL-10 and IL-12 in BM-DC; however coincubation of BM-DC with *L. plantarum* and the EPS induced the production of IL-10, but no changes in IL-12 levels were observed.

In another study by Gorska et al. (2016b), the EPS were purified from different lactobacilli species isolated from the gut of mice model of inflammatory bowel disease and from the gut of healthy mice. The EPS from all the isolates induced human mononuclear DCs to secrete cytokine weakly as compared to whole bacteria. The treatment of DCs with purified EPS did not alter the cytokine profile except *L. johnsonii* E142 which induced murine BM-DC to produce of IL-10 and IL-12 and human mononuclear DCs to secrete IL-6, IL-10, and TNF- $\alpha$ .

The in vivo effects of EPS of *L. kefiranoferiens* known as kefir isolated from the fermented milk product kefir on the gut mucosal immunity were investigated (Vinderola et al. 2006). EPS in a dose-dependent manner induced the production of IgA in both the large and small intestine. It also induced the secretion of cytokines IL-4 and IL-12 in the intestinal fluid and slightly enhanced the number of IL4+, IL6+, and IL10+ T cells in the LP of the small and large intestine. In another study, oral administration of EPS of a particular strain of *L. delbrueckii* spp. *bulgaricus* OLL1073R-1 to mice induced the enhanced the NK cell activity, whereas, under in vitro conditions, it induced the mouse splenocytes to produce IFN- $\gamma$  (Makino et al. 2006, 2016).

### 17.4.2 GABA

GABA is a nonprotein amino acid produced by decarboxylation of glutamate by action of enzyme, glutamic acid decarboxylase (GAD). Immune cells like macrophages, lymphocytes, dendritic cells, and monocytes express GABA receptors,

through which GABA regulates their immune responses. There are two types of GABA receptors – GABA<sub>A</sub> and GABA<sub>B</sub> – expressed differentially on different immune cells. GABA<sub>A</sub> receptors are present on the cell surface of T cells and dendritic cells where they form functional channels through which they modulate proliferation, cytokine release, inflammatory response, and intracellular calcium concentrations.

GABA primarily functions as an inhibitor neurotransmitter in the brain of animals, but it is also known to have immunomodulatory functions in animals. Some of the reported immunomodulatory functions of GABA include downregulation of T-cell proliferation, reduction of the levels of IL-2, downregulation of Th1 proliferation, and therefore inhibition of delayed-type hypersensitivity response in vivo (Jin et al. 2013). GABA transporters (GAT) are also reported to be present on various immune cells. The deficiency of GAT in mice leads to increased T-cell proliferation and cytokine production. GABAergic agents, like topiramate and vigabatrin, lead to dose-dependent inhibition of cytokines IL-17 and IFN- $\gamma$  produced by T cells and TNF, IL-6, and IL-10 produced by either T cells or DCs and macrophages (Bhat et al. 2009). Some *Lactobacillus* spp. are known to have gene for GAD enzyme and thus have been shown to produce GABA in the culture supernatant (Barrett et al. 2012; Dhakal et al. 2012), and thus theoretically the GABA-producing strains can modulate the immune response.

### 17.4.3 SCFA

Lactobacilli are known to carry out saccharolytic conversion of nondigestible carbohydrate to produce SCFA that are two- to six-carbon volatile acids (Pessione 2012). The prominent SCFA are acetate, propionate, and butyrate. Amino acid fermentation can also lead to production of SCFA mainly acetate and butyrate production. Lactobacilli are known to synthesize SCFA by fermenting pyruvate and by the phosphoketolase route under heterofermenting conditions (Pessione 2012). SCFA can easily pass into the blood stream through the gut epithelium. Butyrate and propionate have been shown to induce a strong anti-inflammatory response in the human monocyte-derived dendritic cells in vitro (Nastasi et al. 2015). Transcriptomic analysis showed that treatment of mature dendritic cells with butyrate upregulated 458 genes and downregulated 322 genes, whereas propionate treatment upregulated 230 genes and downregulated 41 genes in total. The prominent genes downregulated belong to pro-inflammatory chemokines such as chemokine (C-C motif) ligand (CCL)-3, CCL4, CCL5, chemokine (C-X-C motif) ligand (CXCL)-9, CXCL10, and CXCL11. Also butyrate and propionate inhibited the expression of LPS-induced cytokines such as IL-6 and IL-12p40. In another report, it was shown that feeding SCFA to germ-free mice enhanced the levels of colonic Tregs (Smith et al. 2013) and feeding butyrate enhanced the levels of peripheral Tregs in antibiotic-treated mice (Arpaia et al. 2013). Treatment of BM-DC with butyrate led to the downregulation of LPS-induced pro-inflammatory mediators, such as nitric oxide,

IL-6, and IL-12, via acting on histone deacetylases thus probably playing an important role in gut tolerance (Chang et al. 2014).

### 17.5 Biogenic Amines

Biogenic amines are molecules with one or more amine groups. They are generated by decarboxylation of amino acids or by transamination or amination of ketones or aldehydes. Biogenic amines include monoamines such as histamine, serotonin, and three catecholamines (epinephrine, norepinephrine, and dopamine) and polyamines such as cadaverine, putrescine, spermine, and spermidine (Fig. 17.5).

Several studies have suggested that histamine receptors (HRs) are expressed not only on mast cell and basophils but also on other immune cells such as lymphocytes, neutrophils, macrophages, and DCs, therefore modulating the function of these cells in the immune system. Histamine has a role in the immunopathogenesis of allergies and anaphylaxis as it is known to cause vasodilation, smooth muscle contraction, and mucus production. However, as it downregulates the proliferation of Th1 cells and upregulates the proliferation of Th2 cells, it again has a role in allergic disease and asthma. Histamine induces the secretion of Th2 cytokines such as IL-4, IL-5, IL-10, and IL-13 and inhibits the production of Th1 cytokines such as IL-2, IFN- $\gamma$ , and IL-12 (Shahid et al. 2009). Lactobacilli are known to produce biogenic amines especially tyramine and putrescine (Lucas et al. 2007); however, the production of amines is strain-specific. There are reports that the absorption of biogenic amine in systemic circulation at high concentrations can result in toxicity due to enhanced release of adrenaline and noradrenaline that induces gastric acid secretion, increased blood glucose levels, and high blood pressure (Shalaby 1996) leading to hypertensive crisis ultimately causing end-organ damage in the heart or central nervous system (McCabe-Sellers et al. 2006; Blackwell 1963). Also increased levels of putrescine have been detected in gastric carcinomas caused by *Helicobacter pylori* (Shah and Swiatlo 2008).

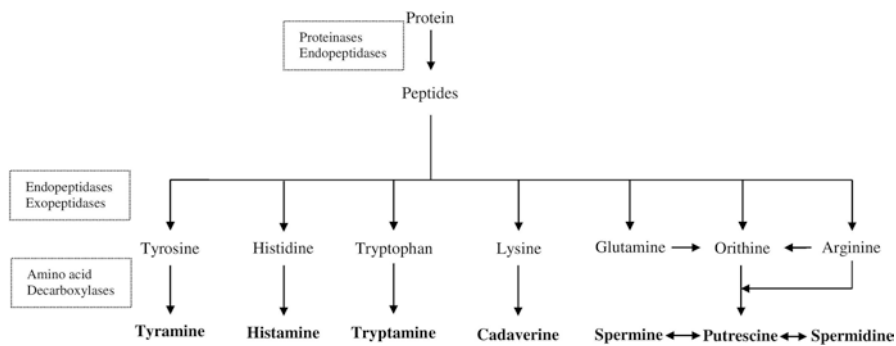


Fig. 17.5 Various biogenic amines and their precursors

## 17.6 Conclusions

Few *Lactobacillus* spp. have strong immunomodulatory abilities and thus can be potentially therapeutic in certain health conditions such as allergic, inflammatory autoimmune diseases, and infectious diseases. Further, due to their immunomodulatory nature, lactobacilli have been reported to enhance the effectiveness of several candidate mucosal vaccines against malaria, HIV, and infantile diarrhea as shown in animal models (Amdekar et al. 2010). However, the immunomodulation by lactobacilli depends on the bacterial strain and host, and it has been noted that at times in vitro studies do not correlate with in vivo results. Thus, human clinical trials of the therapeutic effectiveness of *Lactobacillus* spp. or its metabolites in patients are strongly recommended. Further, none of the clinical trials of *Lactobacillus* spp. in humans had shown any evidence of infection or side effects, thus, proving their GRAS status. Furthermore, the EPS of some *Lactobacillus* spp. have also shown immunomodulatory behavior; therefore they can be the safer alternatives as compared to live probiotic cells especially for the immunocompromised patients. As some lactobacilli strains are known to produce high amounts of biogenic amines, therefore probiotic lactobacilli should be screened for their ability to secrete biogenic amines.

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## References

- Amdekar S, Dwivedi D, Roy P, Kushwah S, Singh V (2010) Probiotics: multifarious oral vaccine against infectious traumas. *FEMS Immunol Med Microbiol* 58:299–306
- Arpaia N, Campbell C, Fan X, Dikiy S, van der Veecken J, deRoos P et al (2013) Metabolites produced by commensal bacteria promote peripheral regulatory T cell generation. *Nature* 504:451–455
- Baken KA, Ezendam J, Gremmer ER, Klerk R, Pennings JA, Matthee B et al (2006) Evaluation of immunomodulation by *Lactobacillus casei* Shirota: immune function, autoimmunity and gene expression. *Int J Food Microbiol* 112:8–18
- Balejko E, Bogacka A, Balejko J, Kucharska E (2015) Immunomodulation effect of metabolites from *Lactobacillus rhamnosus* GG on interleukins release in vitro. *J Food Nutr Res* 3:297–302
- Barrett E, Ross RP, O'Toole PW, Fitzgerald GF, Stanton C (2012)  $\gamma$ -aminobutyric acid production by culturable bacteria from the human intestine. *J Appl Microbiol* 113:411–417
- Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A (2012) Probiotics mechanisms of action. *Ann Nutr Metab* 61:160–174
- Bevins CL, Salzman NH (2012) Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. *Nat Rev Microbiol* 9:356–368
- Bhat R, Axtell R, Mitra A, Miranda M, Lock C, Tsien RW et al (2009) Inhibitory role for GABA in autoimmune inflammation. *Proc Natl Acad Sci* 107:2580–2585
- Blackwell B (1963) Hypertensive crisis due to monoamine-oxidase inhibitors. *Lancet* 2:849–850

- Borrueal N, Carol M, Casellas F, Antolin M, de Lara F, Espin E et al (2002) Increased mucosal tumour necrosis factor  $\alpha$  production in Crohn's disease can be downregulated ex vivo by probiotic bacteria. *Gut* 51:659–664
- Chang PV, Hao L, Offermanns S, Medzhitov R (2014) The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proc Natl Acad Sci* 111:2247–2252
- Chen YS, Jan RL, Lin YL, Chen HH, Wang JY (2010) Randomized placebo-controlled trial of *Lactobacillus* on asthmatic children with allergic rhinitis. *Pediatr Pulmonol* 45:1111–1120
- Ciszek-Lenda M, Nowak B, Srottek M, Gamian A, Marcinkiewicz J (2011) Immunoregulatory potential of exopolysaccharide from *Lactobacillus rhamnosus* KL37: effects on the production of inflammatory mediators by mouse macrophages. *Int J Exp Pathol* 92:382–391
- D'Arienzo R, Bozzella G, Rossi M, De Bellis P, Lavermicocca P, Sisto A (2011) Distinct immunomodulatory properties of *Lactobacillus paracasei* strains. *J Appl Microbiol* 111:1482–1491
- Dhakal R, Bajpai VK, Baek KH (2012) Production of GABA ( $\gamma$  – aminobutyric acid) by microorganisms: a review. *Braz J Microbiol* 43:1230–1241
- Dong H, Rowland I, Tuohy KM, Thomasand LV, Yaqoob P (2010) Selective effects of *Lactobacillus casei* Shirota on T cell activation, natural killer cell activity and cytokine production. *Clin Exp Immunol* 161:378–388
- Doron S, Snyderman DR, Gorbach SL (2005) *Lactobacillus GG*: bacteriology and clinical applications. *Gastroenterol Clin N Am* 34:483–498
- Evrard B, Coudeyras S, Dotsch Gilbert A, Charbonnel N, Alamé J, Tridon A et al (2011) Dose-dependent immunomodulation of human dendritic cells by the probiotic *Lactobacillus rhamnosus* Lcr35. *PLoS One* 6:1–12
- FAO/WHO (2001) Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report of a joint Food Agricultural Organisation/WHO expert consultation, Cordoba
- Finegold SM, Sutter VL, Mathisen GE (1983) Normal indigenous intestinal flora. In: Hentges DJ (ed) *Human intestinal microbiota in health and disease*. Academic, New York, pp 3–31
- Fölster-Holst R, Müller F, Schnopp N, Abeck D, Kreiselmaier I, Lenz T et al (2006) Prospective, randomized controlled trial on *Lactobacillus rhamnosus* in infants with moderate to severe atopic dermatitis. *Br J Dermatol* 155:1256–1261
- Fong FLY, Kirjavainen P, Wong VHY, El-Nezami H (2015) Immunomodulatory effects of *Lactobacillus rhamnosus* GG on dendritic cells, macrophages and monocytes from healthy donors. *J Funct Foods* 13:71–79
- Fong FL, Kirjavainen PV, El-Nezami H (2016) Immunomodulation of *Lactobacillus rhamnosus* GG (LGG)-derived soluble factors on antigen-presenting cells of healthy blood donors. *Sci Rep* 6:1–8
- Fujiwara D, Inoue S, Wakabayashi H, Fujii T (2004) The anti-allergic effects of lactic acid bacteria are strain dependent and mediated by effects on both Th1/Th2 cytokine expression and balance. *Int Arch Allergy Immunol* 135:205–215
- Gill HS, Rutherford KJ, Prasad J, Gopal PK (2000) Enhancement of natural and acquired immunity by *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (HN017) and *Bifidobacterium lactis* (HN019). *Br J Nutr* 83:167–176
- Gorska S, Schwarzer M, Jachymek W, Srutkova D, Brzozowska E, Kozakova H (2014) Distinct immunomodulation of bone marrow-derived dendritic cell responses to *Lactobacillus plantarum* WCFS1 by two different polysaccharides isolated from *Lactobacillus rhamnosus* LOCK 0900. *Appl Environ Microbiol* 80:6506–6516
- Gorska S, Hermanova P, Ciekot J, Schwarzer M, Srutkova D, Brzozowska E et al (2016a) Chemical characterization and immunomodulatory properties of polysaccharides isolated from probiotic *Lactobacillus casei* LOCK 0919. *Glycobiology* 26:1014–1024
- Gorska S, Sandström C, Wojas-Turek J, Rossowska J, Pajtasz-Piasecka E, Brzozowska E et al (2016b) Structural and immunomodulatory differences among lactobacilli exopolysaccharides

- isolated from intestines of mice with experimentally induced inflammatory bowel disease. *Sci Rep* 6:1–16
- Gorska S, Schwarzer M, Srutkova D, Hermanova P, Brzozowska E, Kozakova H et al (2017) Polysaccharides L900/2 and L900/3 isolated from *Lactobacillus rhamnosus* LOCK 0900 modulate allergic sensitization to ovalbumin in a mouse model. *Microb Biotechnol* 10:586–593
- Hashimoto S, Seyama Y, Yokokura T, Mutai M (1985) Cytotoxic factor production by Kupffer cells elicited with *Lactobacillus casei* and *Corynebacterium parvum*. *Cancer Immunol Immunother* 20:117–121
- Havenaar R, Brink BT, Huis in't Veld JHJ (1992) Selection of strains for probiotic use. In: *Probiotics*. Springer, Dordrecht, pp 209–224
- Herfías MV, Koninkx JF, Vos JG, Huis in't Veld JH, van Dijk JE (2005) Probiotic effects of *Lactobacillus casei* on DSS-induced ulcerative colitis in mice. *Int J Food Microbiol* 103:143–155
- Itoh H, Uchida M, Sashihara T (2011) *Lactobacillus gasseri* OL2809 is effective especially on the menstrual pain and dysmenorrhoeal in endometriosis patients: randomized, double-blind, placebo-controlled study. *Cytotechnology* 63:153–161
- Ivanov II, Honda K (2012) Intestinal commensal microbes as immune modulators. *Cell Host Microbe* 12:496–508
- Jin Z, Mendu SK, Birmir B (2013) GABA is an effective immunomodulatory molecule. *Amino Acids* 45:87–94
- Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E (2001) Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 357:1076–1079
- Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E (2003) Probiotics and prevention of atopic disease: 4-year follow-up of a randomized placebo-controlled trial. *Lancet* 361:1869–1871
- Kalliomaki M, Salminen S, Poussa T, Isolauri E (2007) Probiotics during the first 7 years of life: a cumulative risk reduction of eczema in a randomized, placebo- controlled trial. *J Allergy Clin Immunol* 119:1019–1021
- Kang JH, Yun SI, Park MH, Park JH, Jeong SY, Park HO (2013) Anti-obesity effect of *Lactobacillus gasseri* BNR17 in high-sucrose diet-induced obese mice. *PLoS One* 8(1):1–8
- Kato I, Yokokura T, Mutai M (1984) Augmentation of mouse natural killer cell activity by *Lactobacillus casei* and its surface antigens. *Microbiol Immunol* 28:209–217
- Kekkonen RA, Kajasto E, Miettinen M, Veckman V, Korpela R, Julkunen I (2008) Probiotic *Leuconostoc mesenteroides* sp. *cremoris* and *Streptococcus thermophilus* induce IL-12 and IFN-gamma production. *World J Gastroenterol* 14(8):1192–1203
- Kim Y, Oh S, Yun HS, Oh S, Kim SH (2010) Cell-bound exopolysaccharide from probiotic bacteria induces autophagic cell death of tumour cells. *Lett Appl Microbiol* 51:123–130
- Kirjavainen PV, El-Nezami HS, Salminen SJ, Ahokas JT, Wright PA (1999) The effect of orally administered viable probiotic and dairy lactobacillus on mouse lymphocyte proliferation. *FEMS Immunol Med Microbiol* 26:131–135
- Kobayashi KS, Chamailard M, Ogura Y, Henegariu O, Inohara N, Nunez G et al (2005) Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science* 307:731–734
- Konstantinova SR, Smidta H, de Vosa WM, Bruijnsb SCM, Singh SK, Valence F et al (2008) S layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T cell functions. *Proc Natl Acad Sci* 105:19474–19479
- Kopp MV, Goldstein M, Dietschek A, Sofke A, Heinzmann A, Urbanek R (2008) *Lactobacillus* GG has in vitro effects on enhanced interleukin-10 and interferon- $\gamma$  release of mononuclear cells but no in vivo effects in supplemented mothers and their neonates. *Clin Exp Allergy* 38:602–610
- Liu CF, Tseng KC, Chiang SS, Lee BH, Hsu WH, Pan TM (2011) Immunomodulatory and antioxidant potential of *Lactobacillus* exopolysaccharides. *J Sci Food Agric* 91:2284–2291



- Lucas PM, Blancato VS, Claisse O, Magni C, Lolkema JS, Lonvaud-Funel A (2007) Agmatine deiminase pathway genes in *Lactobacillus brevis* are linked to the tyrosine decarboxylation operon in a putative acid resistance locus. *Microbiology* 153:2221–2230
- Luongo D, Miyamoto J, Bergamo P, Nazzaro F, Baruzzi F, Sashihara T et al (2013) Differential modulation of innate immunity in vitro by probiotic strains of *Lactobacillus gasseri*. *BMC Microbiol* 13:298
- Makino S, Ikegami S, Kano H, Sashihara T, Sugano H, Horiuchi H et al (2006) Immunomodulatory effects of polysaccharides produced by *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1. *J Dairy Sci* 89:2873–2881
- Makino S, Goto A, Nakamura M, Ogawa M, Chiba Y, Hemmi J et al (2016) Enhanced natural killer cell activation by exopolysaccharides derived from yogurt fermented with *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1. *J Dairy Sci* 99:915–923
- Marchand J, Vandenplas Y (2000) Micro-organisms administered in the benefit of the host: myths and facts. *Eur J Gastroenterol Hepatol* 12:1077–1088
- Maroof H, Hassan ZM, Mobarez AM, Mohamadabadi MA (2012) *Lactobacillus acidophilus* could modulate the immune response against breast cancer in murine model. *J Clin Immunol* 32:1353–1359
- McCabe-Sellers BJ, Staggs CG, Bogle ML (2006) Tyramine in foods and monoamine oxidase inhibitor drugs: a crossroad where medicine, nutrition, pharmacy, and food industry converge. *J Food Compos Anal* 19:58–65
- Miettinen M, Lehtonen A, Julkunen I, Matikainen S (2000) Lactobacilli and Streptococci activate NF-kappa B and STAT signaling pathways in human macrophages. *J Immunol* 164:3733–3740
- Nastasi C, Candela M, Bonfeld CM, Geisler C, Hansen M, Krejsgaard T et al (2015) The effect of short-chain fatty acids on human monocyte-derived dendritic cells. *Sci Rep* 5:1–10
- Niess JH, Reinecker HC (2006) Dendritic cells in the recognition of intestinal microbiota. *Cell Microbiol* 8:558–564
- Olivares M, Díaz-Ropero MP, Gómez N, Lara-Villoslada F, Sierra S, Maldonado JA et al (2006) The consumption of two new probiotic strains, *Lactobacillus gasseri* CECT 5714 and *Lactobacillus coryniformis* CECT 5711, boosts the immune system of healthy humans. *Int Microbiol* 9:47–52
- Ooi LG, Ahmad R, Yuen KH, Liong MT (2010) *Lactobacillus gasseri* CHO220 and inulin reduced plasma total cholesterol and low-density lipoprotein cholesterol via alteration of lipid transporters. *J Dairy Sci* 93:5048–5058
- Patten DA, Laws AP (2015) *Lactobacillus*-produced exopolysaccharides and their potential health benefits: a review. *Benefic Microbes* 6:457–471
- Pena JA, Versalovic J (2003) *Lactobacillus rhamnosus* GG decreases TNF- $\alpha$  production in lipopolysaccharide-activated murine macrophages by a contact-independent mechanism. *Cell Microbiol* 5:277–285
- Perdigon G, Nader de Maclas ME, Alverarez S, Medici M, Oliver G (1986) Effect of a mixture of *Lactobacillus casei* and *Lactobacillus acidophilus* administered orally on the immune system in mice. *J Food Prot* 49:986–989
- Perdigon G, Macias ME, Alvarez S, Oliver G, Holgado AA (1990) Prevention of gastrointestinal infection using immunobiological methods with milk fermented with *Lactobacillus casei* and *Lactobacillus acidophilus*. *J Dairy Res* 57:255–264
- Perdigón G, Alvarez S, Holgado AA (1991) Immunoadjuvant activity of oral *Lactobacillus casei*: influence of dose on the secretory immune response and protective capacity in intestinal infections. *J Dairy Res* 58:485–496
- Pessione E (2012) Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows. *Front Cell Infect Microbiol* 2:1–15
- Shah P, Swiatlo E (2008) A multifaceted role for polyamines in bacterial pathogens. *Mol Microbiol* 68:4–16

- Shahid M, Tripathi T, Sobia F, Moin S, Siddiqui M, Khan RA (2009) Histamine, histamine receptors, and their role in immunomodulation: an updated systematic review. *Open Immunol J* 2:9–41
- Shalaby AR (1996) Significance of biogenic amines in food safety and human health. *Food Res Int* 29:675–690
- Shida K, Takahashi R, Iwadate E, Takamizawa K, Yasui H, Sato T et al (2002) *Lactobacillus casei* strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model. *Clin Exp Allergy* 32:563–570
- Shida K, Suzuki T, Kiyoshima-Shibata J, Shimada S, Nanno M (2006) Essential roles of monocytes in stimulating human peripheral blood mononuclear cells with *Lactobacillus casei* to produce cytokines and augment natural killer cell activity. *Clin Vaccine Immunol* 13:997–1003
- Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M et al (2013) The microbial metabolites, short chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 341:569–573
- Takeda K, Okumura K (2007) Effects of a fermented milk drink containing *Lactobacillus casei* strain shirota on the human NK-cell activity. *J Nutr* 137:791–793
- Tsai YT, Cheng PC, Pan TM (2010) Immunomodulating activity of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 in enterohemorrhagic *Escherichia Coli* O157:H7-infected mice. *J Agric Food Chem* 58:11265–11272
- Vilela SF, Barbosa JO, Rossoni RD, Santos JD, Prata MC, Anbinder AL et al (2015) *Lactobacillus acidophilus* ATCC 4356 inhibits biofilm formation by *C. albicans* and attenuates the experimental candidiasis in *Galleria mellonella*. *Virulence* 6:29–39
- Vinderola G, Perdigon G, Duarte J, Farnworth E, Matar C (2006) Effects of the oral administration of the exopolysaccharide produced by *Lactobacillus kefiranoferiens* on the gut mucosal immunity. *Cytokine* 36:254–260
- von de Weid T, Bulliard C, Schiffrin E (2001) Induction by a lactic acid bacterium of a population of CD4+ T cells with low proliferative capacity that produce transforming growth factor  $\beta$  and interleukin-10. *Clin Diagn Lab Immunol* 8:695–701
- Walter J (2008) Ecological role of lactobacilli in the gastrointestinal tract: implications for fundamental and biomedical research. *Appl Environ Microbiol* 74:4985–4996
- Wu CT, Chen PJ, Lee YT, Ko JL, Ko HL (2016) Effects of immunomodulatory supplementation with *Lactobacillus rhamnosus* on airway inflammation in a mouse asthma model. *J Microbiol Immunol Infect* 49:625–635
- Zakostelska Z, Kverka M, Klimesova K, Rossmann P, Mrazek J, Kopecny J et al (2011) Lysate of probiotic *Lactobacillus casei* DN-114 001 ameliorates colitis by strengthening the gut barrier function and changing the gut microenvironment. *PLoS One* 6:1–11
- Zhu Y, Zhu J, Zhao L, Zhang M, Guo H, Ren F (2016) Effect of oral administration of *Lactobacillus paracasei* L9 on mouse systemic immunity and the immune response in the intestine. *Arch Biol Sci* 68:311–318