Induction of Regulatory T Cells: A Role for Probiotics and Prebiotics to Suppress Autoimmunity

Mitesh Dwivedi[a], Prasant Kumar[a], Naresh C Laddha[b], E. Helen Kemp[c*]

[a]C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Tarsadi, Surat, Gujarat-394350, India

[b]Department of Molecular Biology, Unipath Specialty Laboratory Ltd., Ahmedabad, Gujarat, India

[c]Department of Oncology and Metabolism, University of Sheffield, Sheffield, United Kingdom

***Corresponding author:** Dr. E. Helen Kemp, Department of Oncology and Metabolism, The Medical School, University of Sheffield, Beech Hill Road, Sheffield, S10 2RX, United Kingdom.

Tel: +44-1142712984

Fax: +44-1142712475

E-mail: e.h.kemp@sheffield.ac.uk

Running title: Role of Probiotics and Prebiotics to Induce Regulatory T cells

Keywords: Probiotics; Prebiotics; Regulatory T cells; $CD8^+$ T cells; Autoimmunity; Immune regulation.

Abbreviations: Tregs, regulatory T cells; iTregs, inducible regulatory T cells; nTregs, natural regulatory T cells; pTregs, peripheral Tregs; DC, dendritic cells; APC, antigen-presenting cells; Teffs, T effector cells; FoxP3, Foxhead box P3; CTLA-4, cytotoxic T lymphocyte antigen-4; GITR, glucocorticoid-induced tumour necrosis factor receptor-related protein; LAG-3, lymphocyte activation gene-3; MHC, major histocompatibility complex; IL, interleukin; TGF-β, transforming growth factor-β; IFN-γ, interferon- γ; TNF-α, tumor necrosis factor-α; GALT, gut-associated lymphoid tissue; TLRs, toll like receptors; RA, retinoic acid; GPRs, G protein-coupled receptors; HDACs, histone deacetylases; HATs, histone acetyl transferases; BLG, β-lactoglobulin; PSA, polysaccharide A ; DC-SIGN, dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin; SCFAs, Short chain fatty acids; FOS, fructooligosaccharides; β-GOS, β-galactooligosaccharide; ILT, immunoglobulin-like transcript; PGE2, prostaglandin E2; COX2, cyclooxygenase-2.

Words count (text): 7,795

Word count (abstract): 157

Tables: 2

Figures: 3

References: 198

ABSTRACT

Regulatory T cells (Tregs) are comprised of a heterogeneous population of cells that play a vital role in suppressing inflammation and maintaining immune tolerance. Given the crucial role of Tregs in maintaining immune homeostasis, it is probably not surprising that many microbial species and their metabolites have the potential to induce Tregs. There is now great interest in the therapeutic potential of probiotics and prebiotics based strategies for a range of autoimmune disorders. This review will summarise recent findings concerning the role of probiotics and prebiotics in induction of Tregs to ameliorate the autoimmune conditions. In addition, the article is focused to explain the different mechanisms of Treg induction and function by these probiotics and prebiotics, based on the available studies till date. The article further proposes that induction of Tregs by probiotics and prebiotics could lead to the development of new therapeutic approach towards curbing the autoimmune response and as an alternative to detrimental immunosuppressive drugs.

Contents

1. Introduction

Human body harbours 100 trillion commensal microbes, exceeding the number of host cells by more than 10-fold, with a huge amount of genetic information i.e. 100-fold more genes than the human genome. With this enormous pool of genetic information, the microbiota may influence human life at many levels, far beyond host immunity and metabolic functions [1]. The microbiota is an important factor in human development and maintenance of the immune response. Recent studies explaining the interactions between microbiota and the host immune system have revealed the fundamental importance of the microbiome in shaping host immune responses [2].

Immune aberrations leading to undesirable response to body are major considerations for the therapeutic targets of many autoimmune diseases. However, approaches leading to less side effects and the effective targetness, avoiding the alteration of normal functions of body are a way forward. In today's scenario the approach for controlling the immune hyper-response mainly involves the natural ways which do not cause any side effects and help the body to maintain the immune homeostasis. One of such approaches includes use of probiotics with conjunction of prebiotics. The term probiotic refers to living microorganisms which, when consumed in adequate amounts, confer health benefits to the host [3]. A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well being and health [4]. The administration of microbes or microbial metabolites for the prevention and treatment of aberrant immune response is gaining importance [5]. The protective effects associated with these microbes are mediated by multiple mechanisms involving T cells, dendritic cells (DCs) and epithelial cells. Recent studies have suggested that commensal microbiota is actively involved in the development

of adaptive immune responses by programming many aspects of $CD4^+$ T cell differentiation [6].

An increasingly recognised feature of immunoregulatory microbes is their ability to induce regulatory T cells (Tregs) [7]. However, the molecular basis of such microbe-mediated Treg induction remains partly understood. The induction of Tregs by probiotics is highlighted by the amelioration of inflammation and diseases, although it has an effect on several cell types including epithelial cells, DCs and T cells. Tregs are a subset of $CD4^+$ lymphocytes that play a key role in maintaining peripheral tolerance *in vivo* through the active suppression of self-reactive T cell activation and expansion [8]. Thus, these cells are critical towards maintaining immune balance and restricting aberrant inflammation [9, 10]. The objective of this review article is to highlight the importance of Tregs in autoimmunity and exploring its use in curbing the autoimmune diseases through probiotic and prebiotic approaches i.e. explaining the impact of probiotic and prebiotic and the associated metabolites on the differentiation and function of Tregs. The understanding of these phenomena could provide us the insight into the therapeutic and/or preventive strategy for the several autoimmune disorders.

2. Regulatory T cells (Tregs) and autoimmunity

A number of T-cell subsets with immunoregulatory activities have been described, and their roles in certain animal autoimmune disease models have been shown and are likely to be of relevance in human immune-mediated diseases [11]. Natural Treg cells play a key role in maintaining peripheral tolerance *in vivo* through the active suppression of self-reactive T-cell activation and expansion (Fig. 1) [12, 13]. These naturally occurring T-cells can control actively and dominantly the activation and function of autoreactive T-cells that have

escaped from the thymus and can prevent development of the autoimmune diseases (Fig. 1). Dysfunction or deficiency of Tregs has been reported in several autoimmune diseases, both systemic [14, 15] and organ-specific [16]. Recently, the use of Tregs for therapeutic purpose in the treatment of autoimmune diseases is gaining importance. In a review article, Dwivedi et al., [17] have discussed the therapeutic potential of Tregs in treatment of vitiligo (an autoimmune skin depigmenting disorder) including the use of probiotics for the induction of Tregs, in addition to adoptive transfer therapy using Tregs, especially heat-shock protein-specific Tregs.

There are five major classes of Tregs: thymic derived $CD4^+CD25^+FoxP3^+$ natural Tregs (nTregs), $CD4^+CD25^+FoxP3^+$ induced Tregs (iTregs), Tr1 type Tregs (IL-10 dependent), Th3 type Tregs (TGF-β dependent, LAP^+) and $CD8^+$ Tregs (Fig. 2) [18]. The most intriguing Tregs are those showing $CD4^+CD25^{hi}FoxP3^+$ phenotype. FoxP3 has been found to be a specific marker for Tregs. $CD4^+CD25^{hi}$ cells constitute 4-10% whereas $CD4^+CD25^{low}$ cells comprise 22-23% of peripheral blood lymphocytes [19, 20]. Inhibition of effector cells proceeds via cell-cell contact by the former subset and via the secretion of inhibitory cytokines, mainly TGF-β and IL-10 by the latter. After T-cell receptor (TCR)-mediated stimulation, $CD4^+CD25^{hi}FoxP3^+$ Tregs suppress the activation and proliferation of other $CD4^+$ and $CD8^+$ T-cells in an antigen non-specific manner [21, 22]. Recently, Cortes et al. [23] identified a novel $FoxP3^+CD69^+$ Treg subset capable to maintain immune tolerance and protect to developing inflammation. The Treg identification, frequently based on the removal of contaminating activated effector T cells, includes the expression of the IL1 receptor type I/II (CD121a/CD121b) which has been used to distinguish $FoxP3^+$ Tregs from activated $FoxP3^+$ or $FOXP3^-$ effector T cells [24]. Similarly, CD49d has been used to identify contaminating activated effector T cells [25]. Moreover, the Treg population has also been subdivided according to the expression of

inducible co-stimulator (ICOS) [26, 27] and the expression of FoxP3 and CD45RA, a marker of naive T cells. $FoxP3^{high}CD45RA^{-}$ cells are activated/memory Tregs, positive for Ki-67—a nuclear protein expressed by proliferating cells [28].

Other markers expressed by Treg include glucocorticoid-induced tumour necrosis factor receptor-related protein (GITR; also known as TNFRSF18, and CD357) and lymphocyte activation gene-3 (LAG-3) [29] and CD127 [30]. GITR is expressed at high levels in activated T cells and Tregs. However, a recent review article discussing data from mouse and human studies has suggested that GITR is a crucial player in the differentiation of thymic Tregs (tTregs), and expansion of both tTregs and peripheral Tregs (pTregs) [31]. Moreover, the authors have suggested two potential new approaches for treating autoimmune diseases consisting of the *in vivo* expansion of $GITR^{+}$ Tregs by GITR-triggering drugs and *in vitro* expansion of autologous or heterologous $GITR^{+}$ Tregs to be infused in the patients [32]. Tregs may also express LAG-3 (CD223), a homolog of the major histocompatibility complex (MHC)-II coreceptor, CD4, but with higher binding affinity. The direct interaction of LAG-3 with MHC-II maintains the immaturity of DCs by reducing MHC-II–peptide presentation to naive T cells [33]. LAG-3 is also required for suppression activity of Tregs as suggested by study of Huang et al. [29], in which antibodies to LAG-3 inhibited the suppression by induced Tregs both *in vitro* and *in vivo*. In addition, natural $CD4^{+}CD25^{+}$ Tregs expressed LAG-3 upon activation, which was significantly enhanced in the presence of effector cells, whereas $CD4^{+}CD25^{+}$ Tregs from $LAG\text{-}3^{(-/-)}$ mice exhibited a reduced regulatory activity [29].

Furthermore, toll like receptors (TLRs), mainly expressed on antigen-presenting cells (APC) as a bridge linking innate and adaptive immunity, also exist on Tregs [34]. These Tregs play a major role in tuning inflammatory response to infections, in guarding against autoimmunity and play a role in tumor progression as well [35]. Moreover, increased

$CD4^{+}CD25^{high}FoxP3^{+}$ Tregs and their involvement in preventing effective antitumor immune responses in non-small-cell lung cancer (NSCLC) patients' [36] suggests that Tregs play a critical role in suppressing the activated T cell responses. It is possible that high affinity T-cells previously escaped clonal deletion in lymph nodes were inadvertently allowed to enter the circulation, emigrating to the target cell to inflict damage. An ongoing autoimmune response may be allowed to further develop and mature in the absence of functional Tregs [37]. In the absence of Tregs, cytotoxic T-cells with increasing affinity for their targets, continuously proliferate and migrate towards novel target cells, causing autoimmune destruction.

Though there are several proposed mechanisms with experimental support, yet no single mechanism is responsible for the full range of biological phenomena involving Tregs [38]. It is likely that in different milieu distinct mechanisms and different alternative subsets of regulatory cells are involved in tuning the immune response (Fig. 2) [39]. The putative mechanisms of Treg functions are: (i) modulation of antigen-presenting cell (APC) activity through Treg engagement of co-stimulatory receptors on the surface of APC, leading to weak or abrogated signals from APC to naive/effector cells; (ii) Treg secretion of cytokines, such as IL-10 and TGF-β, suppressing the activity of effector cells and APC; (iii) under certain circumstances, Tregs could have a direct cytotoxic effect, through the production of perforin/granzyme and induction of apoptosis in effector cells [by the interaction between Galectin-9 (Gal-9) and the T cell immunoglobulin and mucin domain-3 (TIM-3)]; (iv) Tregs may also cause metabolic disruption, for example stimulating APCs to produce enzymes that consume essential amino-acids, preventing naive/effector cell proliferation, and in the presence of TGF-β may induce the expression of FoxP3 in naive cells (i.e., they become

Tregs); (v) Tregs could also compete with effectors cells for APC signals or cytokines, such as IL-2 [40].

In recent years, numerous autoimmune and immune-mediated diseases have been shown to present significant number depletion and/or function impairment of Tregs. The decreased Treg cell numbers and impairment of Tregs function have been reported in patients with rheumatoid arthritis (RA) [41], systemic lupus erythematosus (SLE) [42,43,15], autoimmune thyroid disease (AITD) [44], multiple sclerosis (MS) [45,46], immune thrombocytopenic purpura (ITP) [47] and vitiligo [48-50]. Recently, Quaglino et al. [51] have reported a significant down-regulation of circulating Treg cells in a variety of immune-mediated skin diseases [such as psoriasis, scleroderma, bullous pemphigoid, graft-versus-host disease (GvHD) and inflammatory bowel disease (IBD)-related dermatoses]. These studies suggest for a deregulation of Tregs in maintaining immune homeostasis and contributing to immune tolerance breakdown in autoimmunity.

However, recently, Grant et al. [52] have raised important issues regarding the technical challenges associated with studying human Tregs, which have resulted to reports that are at times inconsistent or even contradictory and need to be taken care of in future studies associated with Tregs. For example, no combination of the different surface markers of Treg enables researchers to identify a homogenous Treg population, since the majority of the defining markers such as CD25, FOXP3 and CTLA-4 are also increased during effector T cell activation. In addition, assays related to evaluate the suppressive capacity of Tregs have also been challenged due to small cell numbers as well as different isolation methods implicates for Tregs have considerable effects on the study such as their yield, purity, concomitant use, and analysis of Treg markers. Moreover, the authors have emphasized that the most

profound challenges associated with Treg research is to assess frequency, phenotype or function at the site of inflammation [53].

3. Probiotics and Immune Regulation

Probiotic is derived from the Greek word meaning 'supporting life' and is first described as selective non-pathogenic living microorganisms or components of bacteria in food supplements, including some commensal bacterial flora, which have beneficial effects on host health and disease prevention and/or treatment [54,55]. Metchnikoff [56] first suggested that the consumption of lactic acid bacteria may benefit the human host's immune system. In addition to the major probiotic group of lactic acid bacteria, probiotic activity has been found to be associated with *Lactobacilli* (*LGG*, *gasseri*, *salivarius*), *Lactococci*, *Bifidobacteria* (*bifidum*, *longum*, *infantis*), *Streptococcus* (*thermophilus*, *cremoris*, *faecium*, *infantis*), *Enterococcus* (*faecium*), non-pathogenic *E. coli* Nissle 1917, *Bacillus coagulans* and *Saccharomyces* strains (*boulardii* and *cerevisiae*) [56,57].

One of the facts is that approximately 70% of our body's immune system is located in the gut [58]. Most of the immune system development begins as the baby born and continues to mature within a few years of life, when it comes in contact with microbial antigens and diets [59]. Important effects of these microorganisms and their products have been demonstrated not only in the gut, but also in adipose tissue, immune and nervous systems [60-62]. Probiotics in gut do several physiological functions inside the body system, mainly metabolic, trophic, and immunologic functions [63]. Gut are loaded with millions and trillions of microbes where they degrade undigested carbohydrates to produce important immunoregulatory molecules such as short-chain fatty acids (SCFAs) and synthesize essential vitamins [64,65]. Moreover, studies with germ-free (GF) animals suggested that

the microbiota is necessary for the development and regulation of immunity in the gut where it prevents the development of inappropriate inflammation [66-68].

Several studies have reported that modifications in the proportions of microorganisms in the gut (qualitatively or quantitatively) and, consequently, in the concentrations of the compounds produced and released by them in the lumen, play a role in the development of pathological conditions including inflammatory bowel disease (IBD), colon cancer, and type 1 and 2 diabetes mellitus [61,62,69]. Some of the compounds that have been implicated in the effects of microbiota on host cells are microbial-derived ligands of toll like receptors (TLRs) such as lipopolysaccharide (LPS) and flagellin, which activate, respectively, TLR-4 and -5 and modulate distinct aspects of host metabolism and immune responses [68]. Moreover, long term association of commensal microbes within the gut make them recognized by the innate immune system as harmless. Commensal microbes induce an unusual pattern of maturation of DCs such that these retain the ability to drive Treg. Moreover, it is likely that immunoregulatory disorders commonly occur first in those individuals whose innate immune systems are least efficient at driving Treg. The increased regulatory dendritic cells (DCreg) and Treg induced by 'Microbes reside in gut' lead to two immunoregulatory mechanisms mediated in part by release of IL-10 and TGF-β. Recently, Min and Rhee [70] have reviewed the influences of microbiota on the development of the gut mucosal immune system which include gut-associated lymphoid tissues (GALT), mucosal Barrier, Th17 cells, Tregs, DCs, innate lymphoid cells, IgA-producing B Cells, and plasma Cells. In addition, the role of gut microbiota in immune homeostasis and autoimmunity has extensively been reviewed by Wu and Wu [71].

4. Prebiotics and Immune Regulation

One approach to exploit the bacterial communities that influence beneficial immune response is to feed specific 'substrates' to the host that would preferentially expand beneficial bacteria—so-called 'prebiotics'. A prebiotic fibre is neither hydrolysed nor absorbed in the upper part of the gut, and becomes a selective substrate for one or a limited number of beneficial colonic bacteria [72]. Efficient prebiotics need to have a specific fermentation therein and thereby have the ability to alter the faecal microflora composition towards a more 'beneficial' community structure and impart a role in the immune system development and function [73]. Some of the extensively studied prebiotics are fructo-oligosaccharide (FOS) and inulin. The other prebiotic and fibre associated with immune response activation are galacto-oligosaccharides (GOS), lactulose, ressistant starch and arabinoxylan-oligosaccharides.

Short chain fatty acids (SCFAs), which are the major metabolic products of anaerobic bacteria fermentation of prebiotic modulate the production of inflammatory mediators by macrophages (Fig. 3). In general, SCFAs, such as propionate and butyrate, inhibit stimuli-induced expression of adhesion molecules, chemokine production and consequently suppress monocyte/macrophage and neutrophil recruitment, suggesting an anti-inflammatory action [74]. SCFAs, mainly butyrate, suppress the LPS- and cytokine-stimulated production of pro-inflammatory mediators including TNF-α, IL-6 and nitric oxide (NO). Butyrate also enhances the release of the anti-inflammatory cytokine IL-10 [75,76]. SCFAs reduce the *in vitro* adherence of monocytes and lymphocytes to human umbilical vein endothelial cells (HUVEC) [77]. In monocytes, butyrate also reduces the constitutive and IFN-γ-induced expression of lymphocyte function-associated antigen 3 (LFA-3) and intercellular adhesion molecule-1 (ICAM-1) [78]. Moreover, SCFAs regulate several

leukocyte functions including production of cytokines (TNF-α, IL-2, IL-6 and IL-10), eicosanoids and chemokines [e.g., MCP-1 (macrophage chemoattractant protein-1) and CINC-2(cytokine induce neutrophil chemoattractant-2)]. Studies have reported that the concentration of these fatty acids in the gut and blood may predispose to or prevents pathological conditions such as IBD, cancer and diabetes [79-81]. SCFAs also modulate the production of prostaglandin E2 (PGE2) and have been shown to stimulate the *in vitro* production of this eicosanoid by human monocytes [82]. PGE2 suppresses T cell receptor signalling and may play a role in resolution of inflammation [83]. PGE2 has been considered an anti-inflammatory prostanoid due to its ability to attenuate the production of IL-1β and TNF-α by macrophages and Th1 differentiation. The other SCFAs such as acetate and propionate at 30 mM reduce TNF-α production by LPS-stimulated human neutrophils [84]. Further, propionate and butyrate have been shown to inhibit the expression of pro-inflammatory mediators (TNF-α, CINC-2αβ and NO) in rat neutrophils, an effect that seems to involve attenuation of NF-kB activation [85]. Moreover, the prebiotics such as FOS, GOS, inulin and resistant starch have been shown to affect specific probiotic strains that drive the induction of Tregs by different mechanisms (Table 2) [86-89]. These reports suggest that prebiotics and their metabolites strongly drive the immune cells and their mediators towards homeostasis.

5. Mechanisms involved in Treg induction by Probiotics

Though the knowledge regarding probiotics and their use in mediating the immune disorders has expanded in the recent years, its application based approach in treating autoimmune disorders has not been extensively appreciated due to the lack of insight into the molecular mechanism of their effects on variety of immune cells and its wide

consequences. However, their critical role in regulating the regulators of our immune system such as 'regulatory T cells' have become recently focused by many research groups. Table 1 summarizes the studies which have emphasized the role of several probiotics in induction of Tregs and their function by different mechanisms. Few important mechanisms with concerning reports are detailed here.

5.1. Activation of Tolerogenic Dendritic Cells (DCs)

Given the important role of DCs in the orchestration of the immune response, it has been hypothesized that probiotic organisms modulate the immune response by influencing DC maturation. It has been suggested that some probiotics (*L. reuteri* and *L. casei*) influence monocyte-derived DCs to drive the development of Treg cells which produced increased levels of IL-10 (Fig. 3) [90]. The study suggested that the ability of these probiotics to induce Tregs by the DCs is due to their ability to bind to the lectin dendritic cell (DC)-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) (Fig. 3) [90]. In addition, extracellular protein such as S-layer protein A (SlpA) secreted by *L. acidophilus* NCFM has been shown to bind DC-SIGN and induce IL-10 production in DCs [91]. Another study also showed up-regulation of surface MHC class II and B7-2 (CD86), by *Lactobacilli sp.* which is indicative of DC maturation [92]. Moreover, in an animal model of inflammatory bowel disease (IBD), the probiotic VSL #3 (four *Lactobacilli*, three *Bifidobacteria*, and one *Streptococcal* strains) reduced the colitis severity due to the production of IL-10, and the generation of greater numbers of Treg cells expressing TGF-β [93].

Furtheremore, the *Bifidobacterium bifidum* (*B. Bifidum*) was found to be most potent polarizer *in vitro*-cultured neonatal DCs to drive Th1-cell responses involving increased IFN-*γ* producing T cells concomitant with reduction of IL-4-producing T-cells, suggesting its use in

prevention of allergic conditions [94,95]. Moreover, T-cells stimulated by *B. bifidum* resulted into matured DCs producing more IL-10 [96]. In addition, *Lactobacillus rhamnosus* (*L. rhamnosus*) was shown to modulate DC function to induce a novel form of T-cell hyporesponsiveness [97]. Another study in mice demonstrated that the probiotic preparation VSL #3 increased the expression of B7.1 (CD80), B7.2, CD40 and MHC class II molecules. A substantial increase in IL-10 levels was observed in the supernatant when DCs were incubated with the probiotics for three days [98]. These results demonstrate that probiotics possess the ability to modulate DC surface phenotype and cytokine release by blood DCs. In addition, one study showed increased production of IL-10 by murine colonic DCs following stimulation with *B. longum* implicating its important role in Treg induction and treatment of intestinal inflammatory diseases [99]. Furthermore, administration of a small consortium of probiotic bacteria, containing species of the *Lactobacillus*, *Streptococcus* and *Bifidobacterium* genera, upregulated IL-10, TGF-β and cyclooxygenase 2 (COX2) expression in DCs. These regulatory DCs can drive the differentiation of $FoxP3^+$ Treg cells, which in turn suppress a range of experimental inflammatory states [100].

These findings suggest that the probiotics act as anti-inflammatory agents by influencing DCs to induce a non-response state by promoting the development of Tregs and hence may be useful in the treatment of a number of inflammatory diseases, including atopic and autoimmune diseases.

5.2. Activation of Treg Associated Molecules

Specific Treg associated molecules such as Forkhead box P3 (FoxP3), Transforming growth factor (TGF)-β, Cytotoxic T lymphocyte antigen (CTLA)-4, and Interleukin (IL)-10 are involved in Treg cell proliferation, development and suppressive functions (Fig. 3). Adequate

expression of these molecules leads to induction of Tregs which can curb the effector T cells (Teffs) efficiently to prevent the autoimmunity [17]. The transcription factor-FoxP3 is a specific marker for Tregs and serves as the dedicated mediator governing Treg cell development and function [101]. TGF-β is important for imposing a regulatory phenotype on the Treg cell subset [102]. Studies have reported that TGF-β has the ability to induce $CD4^+CD25^-$ cells to become $CD4^+CD25^+$ Tregs *in vitro* [103,104], and TGF-β can induce FoxP3 expression in iTregs [105]. Additionally, other studies have clearly demonstrated that the suppressive capacity of $FoxP3^+$ Tregs *in vivo* is via a TGF-β-dependent mechanism [106,107]. Moreover, Tregs induced by IL-10 contribute to Treg cell-mediated immunosuppression principally by producing IL-10 [108]. CTLA-4 (CD152) is a T cell surface molecule involved in regulation of T cell activation [109] and plays a critical role in the maintenance of tolerance to self-antigens as suggested by knock-out mouse models [110,111].

Smits et al. [90] reported that *L. reuteri* and *L. casei* were involved in DC induced Treg cells production with increased levels of IL-10 and these Tregs were capable of inhibiting the proliferation of bystander T cells in an IL-10-dependent fashion. The study suggested their beneficial effect in the treatment of a number of inflammatory diseases, including atopic dermatitis and Crohn's disease. It has been demonstrated that oral consumption of *B. infantis* 35624 is associated with enhanced IL-10 secretion and FoxP3 expression in human peripheral blood (Fig. 3) [7]. Murine studies also demonstrated that *B. infantis* administration results in an increase in $CD4^+CD25^+FoxP3^+$ lymphocytes [112]. Moreover, it has been observed that, *in vitro* human DCs stimulated with *B. infantis* selectively promoted the upregulation of FoxP3 expression in naïve lymphocytes [7] thereby imparting them the Treg phenotype. Furthermore, *B. infantis* has been demonstrated to reduce symptom severity in patients with inflammatory bowel syndrome (IBS) in two placebo-controlled

human clinical studies [113,114]. Thus, the induction of $FoxP3^+$ Treg cells by *B. infantis* seems to be a robust and reproducible phenomenon, which could be useful in the treatment of autoimmune diseases.

Several studies have provided evidence that probiotics are involved in induction and differentiation of IL-10-dependent, TGF-β-bearing Tregs (Fig. 3) [115,116]. It has been suggested that probiotic *(L. reuteri* DSM 17938)-facilitated development of Tregs might play an important role in the prevention of necrotising enterocolitis, and Feleszko et al. [117] showed that probiotic bacteria (*L. rhamnosus* GG or *B. lactis* Bb-12) inhibit subsequent allergic sensitisation and airway disease in a murine model of asthma by inducing Tregs associated with increased TGF-β production. In a food allergy mouse model, oral administration of *B. bifidum* and *L. acidophilus* suppressed OVA-specific IgE production, which was caused by inducing Treg-associated TGF-β production [118]. Kim et al. [119] also reported that *B. lactis* AD011 (BL) increased the production of IL-10 whereas *L. casei* IBS041 (LC), and *L. acidophilus* AD031 (LA) increased TGF-β secretion when mouse DCs were cultured and stimulated with heat-killed probiotic bacteria. A mixture of three *Lactobacilli* strains (*L. paracasei* DSM 13434, *L. plantarum* DSM 15312 and DSM 15313) suppressed the progression and reversed the clinical and histological signs of experimental autoimmune encephalomyelitis by inducing $CD4^+CD25^+FoxP3^+$ Tregs and enhancing production of serum IL-10 and TGF-β levels [120].

Furthermore, the probiotics mixtures (*L. acidophilus*, *L. casei*, *L. reuteri*, *Bifidobacterium*, and *Streptococcus thermophilus*) induced the generation of $CD4^+FoxP3^+$ Tregs from the $CD4^+CD25^-$ population and increased the suppressor activity of naturally occurring $CD4^+CD25^+$ Tregs in mice [100]. Administration of the probiotics mixture induced both T-cell and B-cell hyporesponsiveness and down-regulated Th1, Th2, and Th17 cytokines

[100]. Interestingly, the report suggests that conversion of T cells into FoxP3$^+$ Tregs is directly mediated by DCregs that express high levels of IL-10 and TGF-β (Fig. 3). In another study, probiotic VSL#3 administration during a remission period ameliorated the severity of recurrent colitis in SJL/J mice by inducing an immunoregulatory response involving an early increase in the production of IL-10 and TGF-β-bearing T-regs [93].

Recently, the effects of *L. rhamnosus* on the progression of the clinical signs of atopic dermatitis to allergic asthma has been shown by suppressing Th2 and Th17 responses via CD4$^+$CD25$^+$FoxP3$^+$ Tregs [121]. In addition, the 17 strains belonging to *Clostridia* clusters IV, XIVa and XVIII, have been shown to induce TGFβ1 expression by intestinal epithelial cells. This TGFβ1-rich environment in turn increases the peripheral induction of, and IL-10 expression by Treg cells, particularly in the colonic mucosa [66,122] suggesting that a range of phylogenetically diverse commensal bacteria can suppress intestinal inflammation. Two other studies reported that oral administration of a probiotic strain e.g. *L. casei* could increase FoxP3$^+$ Tregs and enhance IL-10 and TGFβ production [123,124]. The study reported that the strain T120 of lactic acid bacteria was able to inhibit atopic disease in NC/Nga mice through enhanced production of IL-10 by spleenocytes and activation of Treg cells [123]. Karimi et al. [125] demonstrated that oral *L. reuteri* treatment increased the percentage and total number of CD4$^+$CD25$^+$FoxP3$^+$ T cells in spleens in allergic airway animal model which also had greater capacity to suppress T-effector cell proliferation and resulted into attenuation of the allergic airway and inflammatory response. A probiotic mix containing *L. acidophilus* and *B. longum* was also showed to be most effective in prevention of trinitrobenzene sulfonic acid (TNBS) induced colitis in BALB/c mice due to expansion of Treg cells and increased IL-10 production [126]. Furthermore, one study with *VSL#3*-treated patients with ileal pouch-anal anastomosis showed a significant reduction in ulcerative

colitis which was suggested to be due to a significant increase in the percentage of mucosal Treg cells [$CD4^+CD25^{high}$ and $CD4^+$LAP-T cells] and a significant increase in *FoxP3* mRNA expression [127].

In another study, a model of allergic contact dermatitis mediated by $CD8^+$ cytotoxic T lymphocytes and controlled by $CD4^+$ Treg cells was reported [128]. Daily oral administration of fermented milk containing *L. casei* or *L. casei* alone decreased skin inflammation by inhibiting the priming/ expansion of hapten-specific IFN-*γ*-producing $CD8^+$ effector T cells. This study provides the first evidence that oral administration of *L. casei* can reduce antigen-specific skin inflammation by controlling the size of the $CD8^+$ effector pool (Fig. 3). Moreover, one study where BALB/c mice was sensitized and skin inflammation was induced by topical allergen application, suggested that *E. coli* Nissle 1917 altered the local allergen-induced immune response (dermatitis) by $FoxP3^+$ cell increase and by favouring immunoregulatory cytokine patterns [129].

Recently, IL-27 has been shown to exert dual effects on Tregs by activating multiple signaling cascades, including the JAK-STAT and p38 MAPK pathways and suggested as a novel, promising target/agent for the treatment of autoimmune diseases [130]. IL-27 endows Tregs with IL-10 production and more effective suppression besides facilitating their survival and proliferation [131]. These STAT-1 mediated effects are attributable to IL-27-induced T-bet and CXCR3 coupled with increased IL-10 production in Treg, imparting these cells with the ability to control pathogenic Th1 effector responses at local sites [131,132]. IL-27 can also enhance the suppressive effect of Treg by increasing the expression of CTLA-4 and PD-1 [133], or LAG3 (on human Treg) [134] on their cell surface. Jeon et al. [135], have reported that *B. breve* activates intestinal $CD103^+$ DCs to produce IL-10 and IL-27 via the TLR2/MyD88 pathway thereby inducing IL-10-producing Tr1 cells in the large intestine and

thus prevents intestinal inflammation. In addition, probiotic strain that favors IL-12 and IL-27 is likely to skew immune response away from Th17 to Th1 [136]. Several strains of Lactobacillus (e.g., *L. acidophilus* [137], *L. gasseri* [138], and *L. rhamnosus* [139]) and Bifidobacterium (e.g., *B. lactis*, *B. breve* and *B. bifidum* [140,135]) have been suggested to possess this capacity.

The developmental pathways of Treg and Th17 cells are reciprocally interconnected and there is an important plasticity between these cells. Th17 cells represent a pro-inflammatory subset whereas Treg cells have an antagonist effect. Recently, a review article has discussed about Th17/Treg balance to play a major role in the development and the disease outcomes of autoimmune diseases [141]. The authors have suggested that targeting the Th17/Treg imbalance can be performed at different levels such as inhibition of pro-inflammatory cytokines and their receptors, of pathogenic cells or their specific signaling pathways. One of these mechanisms includes the transcription factors STAT5 and STAT3 which are known to control the differentiation of Tregs and Th17 cells, respectively. STAT5 promotes Treg cell development by enhanced expression of FoxP3. Interestingly, the study by Miettinen et al. [142] suggested that the extracellular Gram-positive bacteria activate transcription factors involved in cytokine signaling by two mechanisms: directly, leading to NF-ƙB activation, and indirectly via cytokines, leading to STAT activation. They demonstrated that both non-pathogenic *L. rhamnosus* GG and pathogenic *Streptococcus pyogenes* (group A streptococci) induce NF-ƙB and STAT DNA-binding activity in human primary macrophages. Moreover, IL-2 was considered regarding the suppression of Th17 polarization, and thus regulating the balance between regulatory and inflammatory immune cells [143]. More specifically, IL-2 promotes STAT-5 mediated suppression of IL-17 production while concurrently enabling Treg expansion and survival. Thus, these signaling

pathways could provide novel target molecules for the control of Treg cells in the treatment of autoimmune diseases. Interestingly, intake of probiotics (*L. bulgaricus* and *S. thermophilus*) has been shown to increase IL-2 production by blood lymphocytes [144,145]. In addition, *L. acidophilus* was also shown to increase the number of IL-2 and IL-12 producing cells [146], suggesting the crucial role of probiotics in induction of Tregs by modulating these signaling pathways.

Overall, these studies have suggested that Treg associated molecules and signaling pathways are activated by the probiotics which in turn could able to drive the induction and proliferation of active Tregs along with their suppressive functions. Hence, these findings open an opportunity to implicate the therapeutic potential of oral administration of a combination of probiotics in the management of autoimmune diseases through induction of Tregs. However, further animal as well as human studies are required to provide the mechanistic and therapeutic details for the possible use of probiotics in autoimmune diseases.

5.3. Stimulation of Toll-like Receptors (TLRs)

Toll-like receptors (TLRs) are a family of pattern recognition receptors present on gut lymphoid and epithelial cells that mediates innate immune responses to bacterial molecular patterns and, thereby, orchestrates acquired immunity. Marschan et al. [147] suggested that the transient protection offered by probiotics against IgE-associated allergic diseases is based on stimulation of TLRs. In particular, the findings of the study emphasized the role of chronic microbial exposure as an immunomodulator protecting from allergy through activation of Treg cells by TLR stimulation [147]. In addition, studies on TLR-2/-3/-4/-7 and -9 suggested that TLR stimulation could prevent the onset of type 1 diabetes in non-obese

diabetes mice and it mediates anti-inflammatory effects of probiotics in murine experimental colitis. [148,149]. The lactic acid bacteria species such as *B. bifidum*/*infantis* and *L. salivarius* were shown to be capable of activating TLR-2 [150]. Forsythe et al. [151] demonstrated that oral treatment of *L. reuteri* could drive the activation of TLR-9 which resulted into attenuation of asthmatic response in a mouse model of allergic airway inflammation. Further the study suggested that the anti-inflammatory response was dependent on TLR-9–mediated activation of the tolerogenic enzyme indoleamine 2,3-dioxygenase (IDO). It is suggested that TLR-9 in turn can activate the DCs which induce the Tregs. Indeed, DCs expressing IDO contribute to the generation and maintenance of peripheral tolerance by depleting autoreactive T cells and by inducing Treg responses [152].

Another study performed by Aumeunier et al. [153] on the disease-modifying effects of a set of natural or synthetic TLR agonists using two experimental non-obese diabetes mouse models, OVA- induced asthma and spontaneous autoimmune type 1 diabetes, showed that probiotics stimulate TLR- mediated effects which involve immunoregulatory cytokines such as IL-10 and TGF-β and different subsets of Treg cells, notably $CD4^{+}CD25^{+}FoxP3^{+}$ T cells for TLR-4 agonists (Fig. 3).

Furthermore, Round et al. [154], identified polysaccharide A from the intestinal commensal bacterium *Bacteroides fragilis,* as a bacteria-derived molecule which associated with the intestinal epithelium through host mucins and influenced lymphoid organogenesis and T cell differentiation to enhance mucosal tolerance which promoted intestinal colonization by *B. fragilis* and suppressed the pathogen-mediated colitis [155]. Moreover, Mazmanian et al. [156] suggested that these immunomodulatory functions depend on the expression of polysaccharide A (PSA), which can stimulate DCs through TLR2 [154]. The study further reported that $FoxP3^{+}$ $CD4^{+}$ Treg cells produced IL-10 and anti-inflammatory

cytokines in response to PSA that was presented by DCs; this also occurs through TLR2 signalling (Fig. 3) [154]. The findings of these studies suggest that TLRs could be one of the potential targets for probiotic mediated induction of the Tregs.

6. Mechanisms involved in Treg induction by Prebiotics

Several studies have revealed an intricate relationship between Tregs and microbial metabolism. Different metabolites and nutrients are produced in the interaction such as vitamins, polysaccharides and short chain fatty acids (SCFA). These by products obtained during interaction of prebiotics and probiotics regulate Treg generation, trafficking, and function through modulating mainly FoxP3 induction and Treg suppressive activity [157,158].

6.1. Short chain fatty acids (SCFAs) in induction of Tregs

One of the mechanisms by which probiotics maintains immune homeostasis is through production of the short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate; generated as end products of the fermentation of dietary fibers (Fig. 3). Several studies have provided evidence that SCFAs have beneficial effects on immune regulation and prevent inflammatory and autoimmune responses, because the host SCFA receptors and target molecules are expressed in immune cells. Among SCFAs, butyrate has received most attention for its effects on colonic health and immune homeostasis [159]. Studies have indicated the significant alteration in the number of butyrate-producing bacteria in colon of patients with inflammatory disorders [160,161]. Moreover, colonic irrigation with butyrate suppressed inflammation during ulcerative colitis [159] suggesting the role of the prebiotics in immune regulation.

Different bacterial families in the large intestine can produce SCFAs as end products of the fermentation of complex carbohydrates such as dietary fiber. One recent study has shown that common bacterial metabolites-SCFAs selectively expand Treg cells in the large intestine [162]. The study observed that feeding germfree mice three SCFAs (propionate, acetate, butyrate), individually or in combination, increased the frequency and number of FoxP3 Treg cells in the large intestine to an amount similar to that observed in conventionally reared animals. *Clostridia* species (cluster IV and XIVa) are a prominent source of acetate, butyrate, and to a lesser extent propionate in the colon and increase the development of Treg cells (those that do not express Helios) by inducing epithelial cells to produce TGF-β [122]. It has been suggested that bacterial-driven Treg cell generation in the intestine occurs locally and result in Treg cells that do not express the transcription factor Helios. The majority of the expanded Treg cell population expressed the Helios, which suggests that they acquired FoxP3 expression in the thymus. Interestingly the study reported that propionate also increased the expression of FoxP3 and IL-10, but not TGF-β in colonic Treg cells, indicating that SCFAs can also selectively enhance the Treg cell function. In addition, it has been shown that SCFAs inhibits LPS-induced production of TNF-α and IFN-γ in human PBMCs in a concentration-dependent manner [82]. These reports suggest the crucial role of SCFAs in induction of Tregs. Some of the mechanisms of SCFA mediated induction of Tregs are discussed here.

6.1.1. SCFAs induction of Tregs by GPR43 and GPR109A expression

There are distinct pathways through which intestinal bacteria can influence intestinal Treg cells (Fig. 3). One of the pathways involves the cell surface receptors for SCFAs, such as G protein-coupled receptors (GPCRs)- GPR43 and GPR109A (also known as

hydroxycarboxylic acid receptor 2 or HCA2), which are G protein coupled and are expressed in colonic epithelium, adipose tissue, and immune cells [163,164]. Maslowski et al. [62] showed that GPR43-deficient mice undergo severe colonic inflammation and colitis in DSS-induced colitis model and the GPR43 agonist acetate protects germ-free mice from DSS-induced colitis. Although GPR43 is activated by all three SCFAs, GPR109A (encoded by Niacr1) is activated only by butyrate (Fig. 3) [163,165]. In addition to butyrate GPR109A is also activated by niacin (vitamin B3) [163,164]. However, in colonic lumen, butyrate is generated at high concentrations (10–20 mM) by gut microbiota and serves as an endogenous agonist for GPR109A [166,167]. Interestingly, Gpr109a in immune cells plays a nonredundant function in niacin-mediated suppression of inflammation and atherosclerosis [168]. Gut microbiota also produce niacin. Niacin deficiency in humans results in pellagra, characterized by intestinal inflammation, diarrhoea, dermatitis, and dementia [169]. It is of great clinical relevance that lower abundance of GPR109A ligands, niacin and butyrate in gut is associated with colonic inflammation. Recently, Singh et al. [170] have demonstrated an anti-inflammatory and anticancer function for Gpr109a in colon. In particular, the study observed that butyrate activated Gpr109a signaling imposed anti-inflammatory properties in colonic antigen-presenting cells, which in turn induced differentiation of Treg cells and IL-10-producing T cells suggesting that depletion of gut microbiota or dietary fiber increased the risk for inflammatory and autoimmune disorders.

It is suggested that a combination of *Clostridia* species (cluster IV and XIVa) stimulates local differentiation of Treg cells, whereas administration of SCFAs leads to GPR43-dependent accumulation of thymus-derived Treg cell populations. Smith et al. [162] reported that propionate showed a superior effect on Treg cell expansion compared to acetate and butyrate, which may be due to its higher affinity [171] for GPR43, a receptor for

short chain fatty acids [172]. The study showed that intestinal Treg cells also express GPR43 (primarily expressed by adipocytes, intestinal epithelial cells, and leukocytes), which is driven by the presence of bacteria [162]. Interestingly, the observed effect of SCFAs on Treg cell expansion was abrogated in mice lacking GPR43 suggestive of its involvement in Treg induction through short-chain fatty acids [162]. These reports indicate that these GPCRs may regulate the Treg induction by specific SCFA and its concentration modulated by prebiotics and probiotics (Fig. 3).

6.1.2. SCFAs induction of Tregs by inhibition of histone deacetylases (HDACs)

Gene expression is regulated by the modulation of histone acetylation by histone acetyl transferases (HATs) and histone deacetylases (HDACs). SCFAs produced by gut microbes inhibit HDACs activity and consequently modulate the gene expression (Fig. 3). SCFAs function as non-competitive inhibitors of HDACs. Butyrate is the most potent inhibitor of HDAC, with a maximum efficiency of approximately 80% inhibition of HDAC1/2, whereas acetate is the least potent inhibitor of HDAC [173]. Propionate has a maximum inhibitory efficiently of approximately 60%. It has been shown that butyrate and propionate selectively inhibit HDAC1 and HDAC3. The HDACs, together with the HATs, controls the degree of protein acetylation. By inhibiting the HDAC activity, SCFAs increase the acetylation of histone and non-histone proteins including NFκB, MyoD, p53 and NFAT and, consequently, modulate gene expression [174].

The HDAC inhibition increases the production and suppressive function of $FoxP3^{+}$ Tregs, at least in part, by promoting the acetylation of FoxP3 protein itself (Fig. 3). FoxP3 acts as a repressor of transcription and is both an essential and sufficient regulator of the development and function of Tregs [175]. Acetylation of FoxP3 is required for effective

binding of FoxP3 to the promoter of the interleukin-2 (IL-2) gene and the suppression of IL-2 expression [176]. Study by Li et al. [175] showed that transcriptional repression by FoxP3 involves a HAT-HDAC complex that includes histone acetyl transferase TIP60 (Tat-interactive protein, 60 kDa) and class II HDACs: HDAC7 and HDAC9. In particular, the N-terminal 106-190 aa of FoxP3 are required for TIP60-FoxP3, HDAC7-FoxP3 association, as well as for the transcriptional repression of FoxP3 via its forkhead domain. FoxP3 can be acetylated in primary human Tregs, and TIP60 promotes FoxP3 acetylation *in vivo*. The HAT-deficient mutant and knockdown endogenous TIP60 based experiments suggested that a minimum FoxP3 ensemble containing native TIP60 and HDAC7 is necessary for IL-2 production regulation in T cells [175].

Studies have reported that SCFAs' mediated HDAC inhibition leads to induction of Tregs and its suppressive function (Fig. 3). Recently, Smith et al. [162] have shown that SCFAs such as butyrate are natural inhibitors of HDAC 6 and 9, consistent with the promotion of Treg cell generation and function [177]. In addition, recent studies have also shown that propionate and butyrate produced by gut microbes promote the generation of peripheral Tregs with regard to the inhibition of HDAC [178,179]. Moreover, Furusawa et al. [179] have suggested that gut microbe-derived butyrate induces the differentiation of colonic Tregs by enhancing histone H3 acetylation in the promoter and conserved non-coding sequence regions of the *FOXP3* locus, and reduces the development of colitis. In addition, butyrate, through its HDAC inhibition activity, potentiates the capability of DCs to induce Treg differentiation by repressing the expression of LPS response genes [178], and also inhibits macrophage activation by rendering them hyporesponsive to TLR stimulation [180]. As the inflammatory response is related to the development of several autoimmune

diseases, the SCFAs and their target molecules constitute potential therapeutic targets for the treatment of these diseases.

6.1.3. SCFAs induction of Tregs by prostaglandin E2 (PGE2)

SCFAs present multiple effects in different cells involved in the inflammatory and immune responses. One of such effects is to modulate the production of PGE2-a key mediator in the initiation and resolution of inflammation and in the transition from innate to acquired immune responses (Fig. 3) [82,181]. Cox et al. [82] showed that SCFAs can induce human monocyte release of PGE2. PGE2 is suggested to play a critical role in inflammatory bowel disease (IBD) via the EP4 receptor (i.e. one of the four PGE2 receptor subtypes) [182].

Prostaglandins are small-molecule derivatives of arachidonic acid (AA), produced by cyclooxygenases (COX; constitutively active cyclooxygenase COX1 and inducible COX2) and PG synthases [183]. COX enzymes play a critical role in converting arachidonic acid released from the cell membrane into prostaglandins. Perez et al. [184] reported that butyrate upregulates Kupffer cell PGE2 production by upregulating inducible COX-2 mRNA levels and modulates immune function (Fig. 3). Earlier, elevated levels of PGE2 and COX-2 activity leading to induction of Tregs and FoxP3, expansion of Tregs, and enhanced suppressive functions have been described (Fig. 3) [185]. Thus, COX2-PGE2-Treg represents an immunosuppressive network.

PGE2 has been shown to promote the development of Tregs in humans and in mice (Fig. 3) [185-187]. Interestingly, PGE2 is also involved in mediating the suppressive activity of Tregs [188]. It is involved in suppression of T cell receptor signaling [83] and promotes the induction of suppressive cytokine IL-10 and to directly suppress the production of multiple

proinflammatory cytokines [189]. IL-10 acts as a controller of PGE2 secretion, resulting in the paradoxical role of IL-10 in the reversal of the PGE2-mediated macrophage dysfunction. In addition to promoting *de novo* Treg differentiation from naive precursors, PGE2 also promotes the interaction of DCs with Tregs [190], suggesting that it may also promote the expansion of pre-existing Tregs. Thus, PGE2 can promote the activation, maturation, and migration of DCs, the central cells during the development of Ag-specific immunity, and suppresses both innate and Ag-specific immunity at multiple molecular and cellular levels [189]. Baratelli et al. [191] reported that PGE2 enhances the *in vitro* inhibitory function of human purified $CD4^+CD25^+$ Treg cells and induces a regulatory phenotype in $CD4^+CD25^-$ T cells thereby modulating FoxP3 expression and Treg function in human lymphocytes. PGE2 also induces expansion of Tr1 cells and modulates their activity, thus contributing to creating and sustaining a tolerogenic environment [186].

A subset of iTreg expressing ectonucleotidases CD39 and CD73 is able to hydrolyze ATP to 5' -AMP and adenosine (ADO) and thus mediate suppression of those immune cells which express ADO receptors. iTreg can also produce PGE2. The mechanisms responsible for iTreg-mediated suppression involve binding of ADO and PGE2 produced by iTreg to their respective receptors expressed on T effector cells (Teff), leading to the up-regulation of adenylate cyclase and cAMP activities in Teff and to their functional inhibition [192,193]. Thus, targeting the production, degradation, and responsiveness to PGE2 and its regulator COX2 may provide tools to modulate the patterns of immunity through Tregs in a wide range of inflammatory and autoimmune diseases.

6.2. Microbial polysaccharides in induction of Tregs

Bacteroides fragilis contains an immunomodulatory polysaccharide A (PSA) on its outer membrane which is known to increase immunological tolerance through inducing the differentiation of FoxP3$^+$ Tregs (Fig. 3). *B. fragilis*-derived PSA engages toll-like receptor 2 (TLR2) expressed by T cells, which promotes peripheral Treg (pTreg) generation from naive CD4$^+$ T cells in GALT and peripheral lymphoid organs [154]. In addition, it also enhances Treg production of regulatory cytokines including IL-10 and TGF-β2. Moreover, PSA also suppresses intestinal IL-17 production and protects against colitis [155]. Studies have reported that PSA administration or *B. fragilis* colonization protects mice from intestinal inflammatory diseases [155,194], and ameliorates experimental autoimmune encephalomyelitis (EAE), a murine model for human multiple sclerosis (MS) [195,196]. These findings suggest that PSA production by *B. fragilis* induces the generation of Tregs with enhanced immunoregulatory activity and protects the host from inflammatory diseases.

6.3. Microbial metabolites generated by the breakdown of food

Metabolites generated by the breakdown of food by probiotic organisms may have immunomodulatory effects. It was demonstrated that *L. paracasei* NCC2461 participates in the β-lactoglobulin (BLG) oral tolerance process in mice, attributable to the hydrolysis of BLG into peptides, which stimulate the production of IL-10 (Fig. 3) [197,198]. Thus, the increased IL-10 production in turn may drive the induction of Tregs in addition to its immunosuppressive effects.

7. Conclusions

Given the role of a single bacterial species which can have a drastic impact on host immunity, use of probiotics and prebiotics seems to have the positive immuno-ameliorative effects for curbing the autoimmunity. In recent years, the immunoregulatory properties of probiotics influencing health and disease have emerged as an area of scientific and clinical importance. Evidences reveal that the probiotics have a profound effect on the host immune system and can affect autoimmune-related diseases. In particular, the ability to induce Tregs by different mechanisms, probiotics in conjunction with prebiotics may prove to be an effective immunoregulatory approach to suppress autoimmunity. Though, animal model studies involving GF (germ free) animals, provides a power tool for such mechanistic studies, much more details with animals and human trials is necessary to demonstrate them as a successful therapeutic armentarium. The exciting advances and future development based on molecular mechanism of immunoregulation by these probiotics may provide new opportunities to develop novel Treg-based immunological interventions for the treatment of inflammatory and autoimmune diseases.

Conflict of interest

The authors declare no conflict of interest.

Take-home messages

- Understanding the interaction of probiotics with the host immune system is necessary, especially the regulatory immune system, as the rate of many immune disorders are raising at an alarmingly high speed.
- Several studies have suggested the potential role of probiotics and prebiotics in induction of Tregs, however, sufficient human clinical trials are required to confirm their role as an effective therapeutics.
- More intensive investigations are now required to understand the molecular mechanisms of Treg induction by probiotics and prebiotics to establish an effective immune mediated therapy for autoimmune disorders.

Acknowledgments

We are grateful to Uka Tarsadia University, Maliba Campus, Tarsadi, Gujarat, India for providing the facilities needed for the preparation of this article.

Funding sources

No funding sources were involved in preparation of this article.

References

[1] Tilg H, Moschen AR. Food, immunity, and the microbiome. Gastroenterology 2015;148:1107-9.

[2] Mazmanian SK, Lee YK. Interplay between Intestinal Microbiota and Host Immune System. J Bacteriol Virol 2014;44:1-9.

[3] Fuller R. Probiotics in man and animals. J Appl Bacteriol 1989; 66:365-78.

[4] de Vrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. Adv Biochem Eng Biotechnol 2008;111:1-66.

[5] Konieczna P, Akdis CA, Quigley EM, Shanahan F, O'Mahony L. Portrait of an immunoregulatory *Bifidobacterium*. Gut Microbes 2012a;3:261-6.

[6] Geuking MB, Köller Y, Rupp S, McCoy KD. The interplay between the gut microbiota and the immune system. Gut Microbes 2014;5:411-8.

[7] Konieczna P, Groeger D, Ziegler M, Frei R, Ferstl R, Shanahan F, et al. *Bifidobacterium infantis* 35624 administration induces FoxP3 T regulatory cells in human peripheral blood: potential role for myeloid and plasmacytoid dendritic cells. Gut 2012b;61:354-66.

[8] Levings M, Sangregorio R, Roncarolo M. Human CD25(+)CD4(+) T regulatory cells suppress naive and memory T cell proliferation and can be expanded in vitro without loss of function. J Exp Med 2001;193:1295-302.

[9] Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 2009;9:313-23.

[10] Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? Science 2010;330:1768-73.

[11] Maloy K, Powrie F. Regulatory T-cells in the control of immune pathology. Nat Immunol 2001;2:816-22.

[12] Klarquist, J, Denman CJ, Hernandez C, Wainwright DA, Strickland FM, Overbeck A, et al. Reduced skin homing by functional Treg in vitiligo. Pigment Cell Melanoma Res 2010;23:276-86.

[13] Sakaguchi S. Naturally arising CD4(+) regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 2004;22:531-62.

[14] Antiga E, Kretz C, Klembt R, Massi D, Ruland V, Stumpf C, et al. Characterization of regulatory T cells in patients with dermatomyositis. J Autoimmun 2010;35:342-50.

[15] Miyara M, Amoura Z, Parizot C, Badoual C, Dorgham K, Trad S, et al. Global natural regulatory T cell depletion in active systemic lupus erythematosus. J Immunol 2005;175: 8392-400.

[16] Chi LJ, Wang HB, Wang WZ. Impairment of circulating CD4(+)CD25(+) regulatory T cells in patients with chronic inflammatory demyelinating polyradiculoneuropathy. J Peripher Nerv Syst 2008;13:54-63.

[17] Dwivedi M, Kemp EH, Laddha NC, Mansuri MS, Weetman AP, Begum R. Regulatory T cells in vitiligo: Implications for pathogenesis and therapeutics. Autoimmun Rev. 2015;14:49-56.

[18] Weiner HL, da Cunha AP, Quintana F, Wu H. Oral tolerance. Immunol Rev 2011;241:241-59.

[19] Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S. Decidual and peripheral blood $CD4^{+}CD25^{+}$ regulatory T-cells in early pregnancy subjects and spontaneous abortion cases. Mol Hum Reprod 2004;10:347-53.

[20] Sugiyama H, Gyulai R, Toichi E, Garaczi E, Shimada S, Stevens SR, et al. Dysfunctional blood and target tissue $CD4^+$ $CD25^{high}$ regulatory T-cells in psoriasis: mechanism underlying unrestrained pathogenic effector T cell proliferation. J Immunol 2005;174:164-73.

[21] Thornton A, Shevach E. Suppressor effector function of $CD4^+$ $CD25^+$ immunoregulatory T-cells is antigen nonspecific. J Immunol 2000;164:183-90.

[22] Thornton C, Harrington L, Mangan P, Gavrieli M, Murphy K. Th17: an effector CD4 T cell lineage with regulatory T cell ties. Immunity 2006;24:677-88.

[23] Cortés JR, Sánchez-Díaz R, Bovolenta ER, Barreiro O, Lasarte S, Matesanz-Marín A, et al. Maintenance of immune tolerance by $Foxp3^+$ regulatory T cells requires CD69 expression. J Autoimmun 2014; 55:51-62.

[24] Tran DQ, Andersson J, Hardwick D, Bebris L, Illei GG, Shevach EM. Selective expression of latency-associated peptide (LAP) and IL-1 receptor type I/II (CD121a/ CD121b) on activated human $FOXP3^+$ regulatory T cells allows for their purification from expansion cultures. Blood 2009;113:5125-33.

[25] Kleinewietfeld M, Starke M, Di Mitri D, Borsellino G, Battistini L, Rotzschke O, et al. CD49d provides access to "untouched" human $Foxp3^+$ Treg free of contaminating effector cells. Blood 2009;113:827-36.

[26] Kornete M, Piccirillo CA. Critical co-stimulatory pathways in the stability of $Foxp3^+$ Treg cell homeostasis in Type I diabetes. Autoimmun Rev 2011;11: 104-11.

[27] Xu WD, Pan HF, Ye DQ, Xu Y. Targeting IRF4 in autoimmune diseases. Autoimmun Rev 2012;11:918-24.

[28] Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, et al. Functional delineation and differentiation dynamics of human $CD4^+$ T cells expressing the FoxP3 transcription factor. Immunity 2009;30:899-911.

[29] Huang CT, Workman CJ, Flies D, Pan X, Marson AL, Zhou G, et al. Role of LAG-3 in regulatory T cells. Immunity 2004;21:503-13.

[30] Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, Sasson S, Landay A, et al. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. J Exp Med 2006;203:1693-700.

[31] Ronchetti S, Ricci E, Petrillo MG, Cari L, Migliorati G, Nocentini G et al. Glucocorticoid-induced tumour necrosis factor receptor-related protein: a key marker of functional regulatory T cells. J Immunol Res 2015;2015:171520.

[32] Petrillo MG, Ronchetti S, Ricci E, Alunno A, Gerli R, Nocentini G et al. $GITR^+$ regulatory T cells in the treatment of autoimmune diseases. Autoimmun Rev 2015;14:117-26.

[33] Liang B, Workman C, Lee J, Chew C, Dale BM, Colonna L et al. Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. J Immunol 2008;180:5916-26.

[34] Zhang Q, Qian F, Liu H, Zhou L, Huang M, Zhang X, et al. Expression of surface markers on peripheral CD4+CD25high T-cells in patients with atopic asthma: role of inhaled corticosteroid. Chin Med J 2008;121:205-12.

[35] Afzali B, Lombardi G, Lechler R, Lord G. The role of T helper 17 (Th17) and regulatory Tcells (Treg) in human organ transplantation and autoimmune disease. Clin Exp Immunol 2007;148:32-46.

[36] Li L, Chao QG, Ping LZ, Xue C, Xia ZY, Qian D, et al. The prevalence of $FOXP3^+$ regulatory T-cells in peripheral blood of patients with NSCLC. Cancer Biother Radiopharm 2009;24:357-67.

[37] Dejaco C, Duftner C, Grubeck-Loebenstein B, Schermer M. Imbalance of regulatory T cells in human autoimmune diseases. Immunol 2006;117:289-300.

[38] Miyara M, Sakaguchi S. Natural regulatory T cells: mechanisms of suppression. Trends Mol Med 2007;13:108-16.

[39] Toda A, Piccirillo CA. Development and function of naturally occurring $CD4^+CD25^+$ regulatory T cells. J Leukoc Biol 2006;80:458-70.

[40] Caridade M, Graca L, Ribeiro RM. Mechanisms Underlying $CD4^+$ Treg Immune Regulation in the Adult: From Experiments to Models. Front Immunol 2013;4:378.

[41] Lawson CA, Brown AK, Bejarano V, Douglas SH, Burgoyne CH, Greenstein AS, et al. Early rheumatoid arthritis is associated with a deficit in the $CD4^+CD25^{high}$ regulatory T cell population in peripheral blood. Rheumatology (Oxford) 2006;45:1210-17.

[42] Liu MF, Wang CR, Fung LL, Wu CR. Decreased $CD4^+CD25^+$ T cells in peripheral blood of patients with systemic lupus erythematosus. Scand J Immunol 2004;59:198-202.

[43] Hu S, Xiao W, Kong F, Ke D, Qin R, Su M. Regulatory T cells and their molecular markers in peripheral blood of the patients with systemic lupus erythematosus. J Huazhong Univ Sci Technolog Med Sci 2008;28:549-52.

[44] Glick AB, Wodzinski A, Fu P, Levine AD, Wald DN. Impairment of regulatory T-cell function in autoimmune thyroid disease. Thyroid 2013;23:871-8.

[45] Feger U, Luther C, Poeschel S, Melms A, Tolosa E, Wiendl H. Increased frequency of $CD4^+$ $CD25^+$ regulatory T cells in the cerebrospinal fluid but not in the blood of multiple sclerosis patients. Clin Exp Immunol 2007;147:412-18.

[46] Frisullo G, Nociti V, Iorio R, Patanella AK, Caggiula M, Marti A, et al. Regulatory T cells fail to suppress CD4T+-bet+ T cells in relapsing multiple sclerosis patients. Immunology 2009;127:418-28.

[47] Arandi N, Mirshafiey A, Jeddi-Tehrani M, Shaghaghi M, Sadeghi B, Abolhassani H, et al. Alteration in Frequency and Function of $CD4^+CD25^+FOXP3^+$ Regulatory T cells in Patients with Immune Thrombocytopenic Purpura. Iran J Allergy Asthma Immunol 2014;13:85-92.

[48] Dwivedi M, Laddha NC, Arora P, Marfatia YS, Begum R. Decreased regulatory T-Cells and $CD4^+/CD8^+$ ratio correlate with disease onset and progression in patients with generalized vitiligo. Pigment Cell Melanoma Res 2013;26:586-91.

[49] Lili Y, Yi W, Ji Y, Yue S, Weimin S, Ming L. Global activation of $CD8^+$ cytotoxic T lymphocytes correlates with an impairment in regulatory T cells in patients with generalized vitiligo. PLoS One 2012;7:e37513.

[50] Ben Ahmed M, Zaraa I, Rekik R, Elbeldi-Ferchiou A, Kourda N, Belhadj Hmida N, et al. Functional defects of peripheral regulatory T lymphocytes in patients with progressive vitiligo. Pigment Cell Melanoma Res 2012;25:99-109.

[51] Quaglino P, Ponti R, Novelli M, Savoia P, Fava P, Papini M, et al. Flow cytometric analyses of circulating regulatory T cells in patients with dermatitis herpetiformis and other immune mediated dermatoses. G Ital Dermatol Venereol 2013;148:197-201.

[52] Grant CR, Liberal R, Mieli-Vergani G, Vergani D, Longhi MS. Regulatory T-cells in autoimmune diseases: challenges, controversies and--yet--unanswered questions. Autoimmun Rev 2015;14:105-16.

[53] Buckner JH. Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. Nat Rev Immunol 2010;10:849-59.

[54] Matsuzaki T, Takagi A, Ikemura H, Matsuguchi T, Yokokura T. Intestinal microflora: probiotics and autoimmunity. J Nutr 2007;137:798S-802S.

[55] Kubota A, He F, Kawase M, Harata G, Hiramatsu M, Lino H. Diversity of intestinal bifidobacteria in patients with Japanese cedar pollinosis and possible influence of probiotic intervention. Curr Microbiol 2011;62:71-7.

[56] Metchnikoff E. The prolongation of life: Optimistic studies. G. P. Putnam & Sons, London 1907.

[57] Food and Agriculture Organization, World Health Organization. Report of joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. FAO/WHO Report, World Health Organization, 2001:10-11.

[58] Vighi G, Marcucci F, Sensi L, Di Cara G, Frati F. Allergy and the gastrointestinal system. Clin Exp Immunol 2008;153:3-6.

[59] Rautava S, Isolauri E. The development of gut immune responses and gut microbiota: effects of probiotics in prevention and treatment of allergic disease. Curr Issues Intest Microbiol 2002;3:15-22.

[60] Heijtz RD, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci USA 2011;108:3047-52.

[61] Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, et al. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. Science 2010;328:228-31.

[62] Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature 2009;461:1282-6.

[63] Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol 2010; 10:159-69.

[64] Topping DL, Clifton PM. Short chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev 2001;81:1031-64.

[65] Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature 2011;474:327-36.

[66] Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science 2011;331:337-41.

[67] Hill DA, Artis D. Intestinal bacteria and the regulation of immune cell homeostasis. Annu Rev Immunol 2010;28:623-67.

[68] Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol 2004;4:478-85.

[69] Uronis JM, Muhlbauer M, Herfarth HH, Rubinas TC, Jones GS, Jobin C. Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. PLoS One 2009;4:e6026.

[70] Min YW, Rhee PL. The Role of Microbiota on the Gut Immunology. Clin Ther 2015;37:968-75.

[71] Wu HJ, Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes 2012;3:4-14.

[72] Schley PD, Field CJ. The immune-enhancing effects of dietary fibres and prebiotics Brit J Nutr 2002;87:S221-30.

[73] Kolida S, Tuohy K, Gibson GR. Prebiotic effects of inulin and oligofructose. Brit J Nutr 2002;87:S193-7.

[74] Vinolo MA, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. Nutrients 2011a;3:858-76.

[75] Park JS, Lee EJ, Lee JC, Kim WK, Kim HS. Anti-inflammatory effects of short chain fatty acids in IFN-γ-stimulated RAW 264.7 murine macrophage cells: Involvement of NF-ĸB and ERK signaling pathways. Int Immunopharmacol 2007;7:70-77.

[76] Chakravortty D, Koide N, Kato Y, Sugiyama T, Mu MM, Yoshida T, et al. The inhibitory action of butyrate on lipopolysaccharide-induced nitric oxide production in RAW 264.7 murine macrophage cells. J Endotoxin Res 2000;6:243-47.

[77] Menzel T, Luhrs H, Zirlik S, Schauber J, Kudlich T, Gerke T, et al. Butyrate inhibits leukocyte adhesion to endothelial cells via modulation of VCAM-1. Inflamm Bowel Dis 2004;10:122-8.

[78] Bohmig GA, Krieger PM, Saemann MD, Wenhardt C, Pohanka E, Zlabinger GJ. n-Butyrate downregulates the stimulatory function of peripheral blood-derived antigen-presenting cells: A potential mechanism for modulating T-cell responses by short-chain fatty acids. Immunology 1997;92:234-43.

[79] Huda-Faujan N, Abdulamir AS, Fatimah AB, Anas OM, Shuhaimi M, Yazid AM, et al. The impact of the level of the intestinal short chain Fatty acids in inflammatory bowel disease patients versus healthy subjects. Open Biochem J 2010;4:53-8.

[80] Vernia P, Caprilli R, Latella G, Barbetti F, Magliocca FM, Cittadini M. Fecal lactate and ulcerative colitis. Gastroenterology 1988;95:1564-8.

[81] McIntyre A, Gibson PR, Young GP. Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. Gut 1993;34:386-91.

[82] Cox MA, Jackson J, Stanton M, Rojas-Triana A, Bober L, Laverty M, et al. Short-chain fatty acids act as antiinflammatory mediators by regulating prostaglandin E(2) and cytokines. World J Gastroenterol 2009;15:5549-57.

[83] Wiemer AJ, Hegde S, Gumperz JE, Huttenlocher A. A live imaging cell motility screen identifies prostaglandin E2 as a T cell stop signal antagonist. J Immunol (Baltimore, Md.: 1950) 2011;187:3663-70.

[84] Tedelind S, Westberg F, Kjerrulf M, Vidal, A. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: A study with relevance to inflammatory bowel disease. World J Gastroenterol 2007;13:2826-32.

[85] Vinolo MA, Rodrigues HG, Hatanaka E, Sato FT, Sampaio SC, Curi R. Suppressive effect of short chain fatty acids on production of proinflammatory mediators by neutrophils. J Nutr Biochem 2011b;22:849-55.

[86] Bringiotti R, Ierardi E, Lovero R, Losurdo G, Di Leo A, Principi M. Intestinal microbiota: The explosive mixture at the origin of inflammatory bowel disease? World J Gastrointest Pathophysiol 2014; 5: 550-59.

[87] Moro G, Arslanoglu S, Stahl B, Jelinek J, Wahn U, Boehm G. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. Arch Dis Child 2006;91:814-9.

[88] Bassaganya-Riera J, DiGuardo M, Viladomiu M, deHorna A, Sanchez S, Einerhand AWC, et al. Soluble Fibers and Resistant Starch Ameliorate Disease Activity in

Interleukin-10–Deficient Mice with Inflammatory Bowel Disease. J Nutr 2011;141:1318-25.

[89] Vulevic J, Drakoularakou A, Yaqoob P, Tzortzis G, Gibson RG. Modulation of the fecal microflora profile and immune function by a novel trans-galactooligosaccharide mixture (B-GOS) in healthy elderly volunteers. Am J Clin Nutr 2008;88:1438-46.

[90] Smits HH, Engering A, van der Kleij D, de Jong EC, Schipper K, van Capel TM, et al. Selective probiotic bacteria induce IL-10-producing regulatory T cells *in vitro* by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. J Allergy Clin Immunol 2005;115:1260-7.

[91] Konstantinov SR, Smidt H, de Vos WM, Bruijns SC, 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 USA 2008;105:19474-9.

[92] Christensen HR, Frokiaer H, Pestka JJ. Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. J Immunol 2002;168:171-8.

[93] Di Giacinto C, Marinaro M, Sanchez M, Strober W, Boirivant M. Probiotics ameliorate recurrent Th-1 mediated murine colitis by inducing IL-10 and IL-10-dependent TGF-β- bearing regulatory cells. J Immunol 2005;174:3237-46.

[94] Niers LE, Hoekstra MO, Timmerman HM, van Uden NO, de Graaf PM, Smits HH, et al. Selection of probiotic bacteria for prevention of allergic diseases: Immunomodulation of neonatal dendritic cells. Clin Exp Immunol 2007;149:344-52.

[95] Niers LE, Timmerman HM, Rijkers GT, van Bleek GM, van Uden NO, Knol EF, et al. Identification of strong interleukin-10 inducing lactic acid bacteria which down-regulate T helper type 2 cytokines. Clin Exp Allergy 2005;35:1481-9.

[96] Hart AL, Lammers K, Brigidi P, Vitali B, Rizzello F, Gionchetti P, et al. Modulation of human dendritic cell phenotype and function by probiotic bacteria. Gut 2004;53:1602-9.

[97] Braat H, van den Brande J, van Tol E, Hommes D, Peppelenbosch M, van Deventer S. *Lactobacillus rhamnosus* induces peripheral hyporesponsiveness in stimulated $CD4^+$ T cells via modulation of dendritic cell function. Am J Clin Nutr 2004;80:1618-25.

[98] Drakes M, Blanchard T, Czinn S. Bacterial probiotic modulation of dendritic cells. Infect Immun 2004;72:3299-3309.

[99] Rigby RJ, Knight SC, Kamm MA, Stagg AJ. Production of interleukin (IL)-10 and IL-12 by murine colonic dendritic cells in response to microbial stimuli. Clin Exp Immunol 2005;139:245-56.

[100] Kwon HK, Lee CG, So JS, Chae CS, Hwang JS, Sahoo A, et al. Generation of regulatory dendritic cells and $CD4^+FoxP3^+$ T cells by probiotics administration suppresses immune disorders. Proc Natl Acad Sci USA 2010;107:2159-64.

[101] Yagi H, Nomura T, Nakamura K, Yamazaki S, Kitawaki T, Hori S, et al. Crucial role of FOXP3 in the development and function of human $CD25^+CD4^+$ regulatory T-cells. Int Immunol 2004;16:1643-56.

[102] Joetham A, Takeda K, Taube C, Miyahara N, Matsubara S, Koya T, et al. Naturally occurring lung CD4(+)CD25(+) T cell regulation of airway allergic responses depends on IL-10 induction of TGF-β. J Immunol 2007;178:1433-42.

[103] Zheng SG, Gray JD, Ohtsuka K, Yamagiwa S, Horwitz DA. Generation ex vivo of TGF-β-producing regulatory T cells from $CD4^+CD25^-$ precursors. J Immunol 2002;169:4183-9.

[104] Fu S, Zhang N, Yopp AC, Chen D, Mao M, Chen D, et al. TGF-β induces FoxP3^{+} T-regulatory cells from CD4^{+}CD25^{-} precursors. Am J Transplant 2004;4:1614-27.

[105] Fantini MC, Becker C, Monteleone G, Pallone F, Galle PR, Neurath MF. Cutting edge: TGF-β induces a regulatory phenotype in CD4^{+}CD25^{-} T cells through FoxP3 induction and down-regulation of Smad7. J Immunol 2004;172:5149-53.

[106] Liang S, Alard P, Zhao Y, Parnell S, Clark SL, Kosiewicz MM. Conversion of CD4^{+} CD25^{-} cells into CD4^{+}CD25^{+} regulatory T cells in vivo requires B7 costimulation, but not the thymus. J Exp Med 2005;201:127-37.

[107] Chen W, Konkel JE. TGF-beta and 'adaptive' FoxP3^{+} regulatory T cells. J Mol Cell Biol 2010;2:30-6.

[108] O'Garra A, Vieira PL, Vieira P, Goldfeld AE. IL-10-producing and naturally occurring CD4^{+} Tregs: limiting collateral damage. J Clin Invest 2004;114:1372-8.

[109] Gribben JG, Freeman GJ, Boussiotis VA, Rennert P, Jellis CL, Greenfield E, et al. CTLA4 mediates antigen-specific apoptosis of human T cells. Proc Natl Acad Sci USA 1995;92:811-5.

[110] Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, et al. Lymphoproliferative disorders with early lethality in mice deficient in CTLA-4. Science 1995;270:985-8.

[111] Chen Z, Stockton J, Mathis D, Benoist C. Modeling CTLA4-linked autoimmunity with RNA interference in mice. Proc Natl Acad Sci USA 2006;103:16400-5.

[112] O'Mahony C, Scully P, O'Mahony D, Murphy S, O'Brien F, Lyons A, et al. Commensal-induced regulatory T cells mediate protection against pathogen-stimulated NF-ƙB activation. PLoS Pathog 2008;4:e1000112.

[113] O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, et al. *Lactobacillus* and *bifidobacterium* in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology 2005;128:541-51.

[114] Whorwell PJ, Altringer L, Morel J, Bond Y, Charbonneau D, O'Mahony L, et al. Efficacy of an encapsulated probiotic *Bifidobacterium infantis* 35624 in women with irritable bowel syndrome. Am J Gastroenterol 2006;101:1581-90.

[115] Schwartz RH. Natural regulatory T cells and self tolerance. Nat Immunol 2005;6:327-30.

[116] Issazadeh-Navikas S, Teimer R, Bokermann R. Influence of dietary components on regulatory T cells. Mol Med 2012;18:95-110.

[117] Feleszko W, Jaworska J, Rha RD, Steinhausen S, Avagyan A, Jaudszus A, et al. Probiotic-induced suppression of allergic sensitization and airway inflammation is associated with an increase of T regulatory-dependent mechanisms in a murine model of asthma. Clin Exp Allergy 2007;37:498-505.

[118] 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;18:1393-1400.

[119] Kim JY, Park MS, Ji GE. Probiotic modulation of dendritic cells co-cultured with intestinal epithelial cells. World J Gastroenterol 2012a;18:1308-18.

[120] Lavasani S, Dzhambazov B, Nouri M, Fåk F, Buske S, Molin G, et al. A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing regulatory T cells. PLoS One 2010;5:e9009.

[121] Kim HJ, Kim YJ, Lee SH, Yu J, Jeong SK, Hong SJ. Effects of *Lactobacillus rhamnosus* on allergic march model by suppressing Th2, Th17, and TSLP responses via CD4(+)CD25(+)FoxP3(+) Tregs. Clin Immunol 2014;153:178-86.

[122] Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 2013; 500:232-6.

[123] Hacini-Rachinel F, Gheit H, Le Luduec JB, Dif F, Nancey S, Kaiserlian D. Oral probiotic control skin inflammation by acting on both effector and regulatory T cells. PLoS One 2009;4:e4903.

[124] So JS, Lee CG, Kwon HK, Yi HJ, Chae CS, Park JA, et al. *Lactobacillus casei* potentiates induction of oral tolerance in experimental arthritis. Mol Immunol 2008;46:172-80.

[125] Karimi K, Inman MD, Bienenstock J, Forsythe P. *Lactobacillus reuteri*-induced regulatory T cells protect against an allergic airway response in mice. Am J Respir Crit Care Med 2009;179:186-93.

[126] 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 gamma-deltaT and regulatory T cells of intestinal intraepithelial lymphocytes. Inflamm Bowel Dis 2009;15:1526-36.

[127] Pronio A, Montesani C, Butteroni C, Vecchione S, Mumolo G, Vestri A, et al. Probiotic administration in patients with ileal pouch-anal anastomosis for ulcerative colitis is associated with expansion of mucosal regulatory cells. Inflamm Bowel Dis 2008;14:662-8.

[128] Chapat L, Chemin K, Dubois B, Bourdet-Sicard R, Kaiserlian D. *Lactobacillus casei* reduces $CD8^+$ T cell-mediated skin inflammation. Eur J Immunol 2004;34:2520-8.

[129] Weise C, Zhu Y, Ernst D, Kühl AA, Worm M. Oral administration of *Escherichia coli* Nissle 1917 prevents allergen-induced dermatitis in mice. Exp Dermatol 2011;20:805-9.

[130] Meka RR, Venkatesha SH, Dudics S, Acharya B, Moudgil KD. IL-27-induced modulation of autoimmunity and its therapeutic potential. Autoimmun Rev 2015;14:1131-41.

[131] Hall AO, Beiting DP, Tato C, John B, Oldenhove G, Lombana CG, et al. The cytokines interleukin 27 and interferon-gamma promote distinct Treg cell populations required to limit infection-induced pathology. Immunity 2012;37:511-23.

[132] Yoshida H, Hunter CA. The immunobiology of interleukin-27. Annu Rev Immunol 2015;33:417-43.

[133] Moon SJ, Park JS, Heo YJ, Kang CM, Kim EK, Lim MA, et al. *In vivo* action of IL-27: reciprocal regulation of Th17 and Treg cells in collagen-induced arthritis. Exp Mol Med 2013;45:e46.

[134] Do JS, Visperas A, Sanogo YO, Bechtel JJ, Dvorina N, Kim S, et al. An IL-27/Lag3 axis enhances Foxp3 regulatory T cell-suppressive function and therapeutic efficacy. Mucosal Immunol 2015; doi: 10.1038/mi.2015.45.

[135] Jeon SG, Kayama H, Ueda Y, Takahashi T, Asahara T, Tsuji H, et al. Probiotic *Bifidobacterium breve* induces IL-10-producing Tr1 cells in the colon. PLoS Pathog 2012;8:e1002714.

[136] Sung CY, Lee NP, El-Nezami H. Regulation of T helper 17 by bacteria: an approach for the treatment of hepatocellular carcinoma. Int J Hepatol 2012;2012:439024.

[137] Chen CC, Chiu CH, Lin TY, Shi HN, Walker WA. Effect of probiotics *Lactobacillus acidophilus* on *Citrobacter rodentium* colitis: the role of dendritic cells. Pediatr Res 2009;65:169-75.

[138] Jan RL, Yeh KC, Hsieh MH, Lin YL, Kao HF, Li PH, et al. *Lactobacillus gasseri* suppresses Th17 pro-inflammatory response and attenuates allergen-induced airway inflammation in a mouse model of allergic asthma. Br J Nutr 2012;108:130-9.

[139] Veckman V, Miettinen M, Pirhonen J, Sirén J, Matikainen S, Julkunen I. *Streptococcus pyogenes* and *Lactobacillus rhamnosus* differentially induce maturation and production of Th1-type cytokines and chemokines in human monocyte-derived dendritic cells. J Leukoc Biol 2004;75:764-71.

[140] López P, González-Rodríguez I, Gueimonde M, Margolles A, Suárez A. Immune response to *Bifidobacterium bifidum* strains support Treg/Th17 plasticity. PLoS One 2011;6:e24776.

[141] Noack M, Miosse P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. Autoimmun Rev 2014;13:668-77.

[142] Miettinen M, Lehtonen A, Julkunen I, Matikainen S. Lactobacilli and Streptococci activate NF-ƙB and STAT signaling pathways in human macrophages. J Immunol 2000;164:3733-40.

[143] Laurence A, Tato CM, Davidson TS, Kanno Y, Chen Z, Yao Z, et al. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. Immunity 2007;26:371-81.

[144] Wheeler JG, Bogle ML, Shema SJ, Shirrell MA, Stine KC, Pittler AJ, et al. Impact of dietary yogurt on immune function. Am J Med Sci 1997;313:120-3.

[145] Mokrozub VV , Babenko LP, Sokolvyak OY, Lazarenko LM, Spivak MY. Effect of lactobacilli probiotic strains on IFN-γ, IL-2 and TNF-α- production in the peripheral blood cells culture of clinically healthy donors. EPMA Journal 2014;5:A135.

[146] Perdigon G, Maldonado Galdeano C, Valdez JC, Medici M. Interaction of lactic acid bacteria with the gut immune system. Eur J Clin Nutr 2002;56:S21-26.

[147] Marschan E, Kuitunen M, Kukkonen K, Poussa T, Sarnesto A, Haahtela T, et al. Probiotics in infancy induce protective immune profiles that are characteristic for chronic low-grade inflammation. Clin Exp Allergy 2008;38: 611-8.

[148] Gareau MG, Sherman PM, Walker WA. Probiotics and the gut microbiota in intestinal health and disease. Nat Rev Gastroenterol Hepatol 2010;7:503-14.

[149] Rachmilewitz D, Katakura K, Karmeli F, Hayashi T, Reinus C, Rudensky B, et al. Toll-like receptor 9 signaling mediates anti-inflammatory effects of probiotics in murine experimental colitis. Gastroenterology 2004;126:520-8.

[150] Hoarau C, Lagaraine C, Martin L, Velge-Roussel F, Lebranchu Y. Supernatant of *Bifidobacterium breve* induces dendritic cell maturation, activation, and survival through a Toll-like receptor 2 pathway. J Allergy Clin Immunol 2006;117: 696-702.

[151] Forsythe P, Inman MD, Bienenstock J. Oral treatment with live *Lactobacillus reuteri* inhibits the allergic airway response in mice. Am J Respir Crit Care Med 2007;175:561-9.

[152] Baban B, Hansen AM, Chandler PR, Manlapat A, Bingaman A, Kahler DJ, et al. Minor population of splenic dendritic cells expressing CD19 mediates IDO-dependent T cell suppression via type-I IFN signaling following B7 ligation. Int Immunol 2005;17:909-19.

[153] Aumeunier A, Grela F, Ramadan A, Pham Van L, Bardel E, Gomez Alcala A, et al. Systemic Toll-like receptor stimulation suppresses experimental allergic asthma and autoimmune diabetes in NOD mice. PLoS ONE 2010;5:e11484.

[154] Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, et al. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science 2011;332:974-77.

[155] Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature 2008;453:620-5.

[156] Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell 2005;122:107-18.

[157] Zeng H, Chi H. Metabolic control of regulatory T cell development and function. Trends Immunol 2015;36:3-12.

[158] Goldsmith JR, Sartor RB. The role of diet on intestinal microbiota metabolism: downstream impacts on host immune function and health, and therapeutic implications. J Gastroenterol 2014;49:785-98.

[159] Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer RJ. The role of butyrate on colonic function. Aliment Pharmacol Ther 2008;27:104-19.

[160] Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA 2007;104:13780-5.

[161] Wang T, Cai G, Qiu Y, Fei N, Zhang M, Pang X, et al. Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. ISME J. 2012;6:320-9.

[162] Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 2013;341:569-73.

[163] Blad CC, Tang C, Offermanns S. G protein-coupled receptors for energy metabolites as new therapeutic targets. Nat Rev Drug Discov 2012;11:603-19.

[164] Ganapathy V, Thangaraju M, Prasad PD, Martin PM, Singh N. Transporters and receptors for short-chain fatty acids as the molecular link between colonic bacteria and the host. Curr Opin Pharmacol 2013;13:869-74.

[165] Taggart AK, Kero J, Gan X, Cai TQ, Cheng K, Ippolito M, et al. (D)-beta-Hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. J Biol Chem 2005;280:26649-52.

[166] Thangaraju M, Cresci GA, Liu K, Ananth S, Gnanaprakasam JP, Browning DD, et al. GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. Cancer Res 2009;69:2826-32.

[167] Cresci GA, Thangaraju M, Mellinger JD, Liu K, Ganapathy V. Colonic gene expression in conventional and germ-free mice with a focus on the butyrate receptor GPR109A and the butyrate transporter SLC5A8. J Gastrointest Surg 2010;14:449-61.

[168] Lukasova M, Malaval C, Gille A, Kero J, Offermanns S. Nicotinic acid inhibits progression of atherosclerosis in mice through its receptor GPR109A expressed by immune cells. J Clin Invest 2011;121:1163-73.

[169] Hegyi J, Schwartz RA, Hegyi V. Pellagra: dermatitis, dementia, and diarrhea. Int J Dermatol 2004;43:1-5.

[170] Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, Shi H, et al. Activation of GPR109A, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity 2014;40:128-39.

[171] Le Poul E, Loison C, Struyf S, Springael JY, Lannoy V, Decobecq ME, et al. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. J Biol Chem 2003;278:25481-9.

[172] Kim S, Kim YM, Kwak YS. A novel therapeutic target, GPR43; where it stands in drug discovery. Arch Pharm Res 2012b;35:1505-9.

[173] Cousens LS, Gallwitz D, Alberts BM. Different accessibilities in chromatin to histone acetylase. J Biol Chem 1979;254:1716-23.

[174] Glozak MA, Sengupta N, Zhang X, Seto E. Acetylation and deacetylation of non-histone proteins. Gene 2005;363:15-23.

[175] Li B, Samanta A, Song X, Iacono KT, Bembas K, Tao R, et al. FOXP3 interactions with histone acetyltransferase and class II histone deacetylases are required for repression. Proc Natl Acad Sci U S A 2007;104:4571-6.

[176] Wang L, Tao R, Hancock WW. Using histone deacetylase inhibitors to enhance Foxp3(+) regulatory T-cell function and induce allograft tolerance. Immunol Cell Biol 2009; 87:195-202.

[177] Tao R, de Zoeten EF, Ozkaynak E, Chen C, Wang L, Porrett PM, et al. Deacetylase inhibition promotes the generation and function of regulatory T cells. Nat Med 2007;13:1299-307.

[178] Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature 2013;504:451-5.

[179] Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 2013;504:446-50.

[180] Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. Proc Natl Acad Sci USA 2014;111:2247-52.

[181] Willemsen LE, Koetsier MA, van Deventer SJ, van Tol EA. Short chain fatty acids stimulate epithelial mucin 2 expression through differential effects on prostaglandin E(1) and E(2) production by intestinal myofibroblasts. Gut 2003;52:1442-7.

[182] Nitta M, Hirata I, Toshina K, Murano M, Maemura K, Hamamoto N, et al. Expression of the EP4 prostaglandin E2 receptor subtype with rat dextran sodium sulphate colitis: colitis suppression by a selective agonist, ONO-AE1-329. Scand J Immunol 2002;56: 66-75.

[183] Kalinski P. Regulation of immune responses by prostaglandin E2. J Immunol 2012;188:21-28.

[184] Perez R, Stevenson F, Johnson J, Morgan M, Erickson K, Hubbard NE et al. Sodium butyrate upregulates Kupffer cell PGE2 production and modulates immune function. J Surg Res 1998;78:1-6.

[185] Sharma S, Yang SC, Zhu L, Reckamp K, Gardner B, Baratelli F, et al. Tumor cyclooxygenase-2/prostaglandin E2-dependent promotion of FOXP3 expression and $CD4^+$ $CD25^+$ T regulatory cell activities in lung cancer. Cancer Res 2005;65:5211-20.

[186] Bergmann C, Strauss L, Zeidler R, Lang S, Whiteside TL. Expansion of human T regulatory type 1 cells in the microenvironment of cyclooxygenase 2 overexpressing head and neck squamous cell carcinoma. Cancer Res 2007;67:8865-73.

[187] Soontrapa K, Honda T, Sakata D, Yao C, Hirata T, Hori S, et al. Prostaglandin E2-prostaglandin E receptor subtype 4 (EP4) signaling mediates UV irradiation induced systemic immunosuppression. Proc Natl Acad Sci USA 2011;108:6668-73.

[188] Mahic M, Yaqub S, Johansson CC, Taskén K, Aandahl EM. FOXP3$^+$ CD4$^+$ CD25$^+$ adaptive regulatory T cells express cyclooxygenase-2 and suppress effector T cells by a prostaglandin E2-dependent mechanism. J Immunol 2006;177:246-54.

[189] Phipps RP, Stein SH, Roper RL. A new view of prostaglandin E regulation of the immune response. Immunol Today 1991;12:349-52.

[190] Muthuswamy R, Urban J, Lee JJ, Reinhart TA, Bartlett D, Kalinski P. Ability of mature dendritic cells to interact with regulatory T cells is imprinted during maturation. Cancer Res 2008;68:5972-8.

[191] Baratelli F, Lin Y, Zhu L, Yang SC, Heuzé-Vourc'h N, Zeng G, et al. Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4$^+$ T cells. J Immunol 2005;175:1483-90.

[192] Whiteside TL, Jackson EK. Adenosine and prostaglandin E2 production by human inducible regulatoryT cells in health and disease. Front Immunol 2013;4:212.

[193] Su Y, Huang X, Raskovalova T, Zacharia L, Lokshin A, Jackson E, et al. Cooperation of adenosine and prostaglandin E2 (PGE2) in amplification of cAMP-PKA signaling and immunosuppression. Cancer Immunol Immunother 2008;57:1611-23.

[194] Round JL, Mazmanian SK. Inducible Foxp3$^+$ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci USA 2010;107:12204-9.

[195] Ochoa-Repáraz J, Mielcarz DW, Ditrio LE, Burroughs AR, Foureau DM, Haque-Begum S, et al. Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis. J Immunol 2009;183:6041-50.

[196] Ochoa-Repáraz J, Mielcarz DW, Wang Y, Begum-Haque S, Dasgupta S, Kasper DL, et al. A polysaccharide from the human commensal *Bacteroides fragilis* protects against CNS demyelinating disease. Mucosal Immunol 2010;3:487-95.

[197] Prioult G, Fliss I, Pecquet S. Effect of probiotic bacteria on induction and maintenance of oral tolerance to β-lactoglobulin in gnotobiotic mice. Clin Diagn Lab Immunol 2003;10: 787-92.

[198] Prioult G, Pecquet S, Fliss I. Stimulation of interleukin-10 production by acidic beta lactoglobulin-derived peptides hydrolyzed with *Lactobacillus paracasei* NCC2461 peptidases. Clin Diagn Lab Immunol 2004;11:266-71.

Table 1. Induction of regulatory T cells by different mechanisms mediated by probiotics.

Probiotics	**Different Mechanisms for Induction of Tregs**	**Outcome**	**Reference**
	I. Development of Tolerogenic Dendritic Cells		
Lactobacillus reuteri & *Lactobacillus casei*	Prime monocyte-derived dendritic cell; DC maturation through up-regulated surface MHC class II and B7-2 (CD86)	Increased Treg cells producing increased levels of IL-10	[90,92]
VSL #3	Matures dendritic cells (phenotype modulator); IL-10 production	Increased production of IL-10 by dendritic cells and suppression of Th1 cells; suggested for treatment of intestinal inflammatory diseases	[96,98]
Bifidobacterium bifidum	Prime neonatal dendritic cell (most potent polarizer)	Increased production of IL-10 by matured DC; suggested in prevention or treatment of atopic disease.	[94,95]
Lactobacillus rhamnosus	Modulates dendritic cell function (function modulator)	Decreased T cell proliferation and cytokine production (IL-2, IL-4) by matured DCs	[97]
Bifidobacterium longum	Increased IL-10 production by DC	Suggested in treatment of intestinal inflammatory diseases	[99]
	II. Induction of Treg Associated Molecules & Tregs		
E. coli Nissle1917	Increased FoxP3^{+} Tregs	Improved allergen-induced dermatitis	[129]
Probiotic mixture	Increased FoxP3^{+} Tregs & its suppressor activity	Therapeutical effects in experimental inflammatory bowel disease, atopic dermatitis, and rheumatoid arthritis	[100]

Lactobacillus casei DN-114 001	Increased FoxP3$^+$ Tregs, IL-10 & TGF-β production	Alleviates antigen-specific skin inflammation; reduced arthritis & delayed-type hypersensitivity	[123,124]
Lactobacillus reuteri & *Lactobacillus casei*	Increased DC-SIGN induced Tegs & IL-10	Suggested beneficial effects in the treatment of atopic dermatitis and Crohn's disease	[90]
VSL#3	Increased IL-10-dependent TGF-β-bearing Tregs and IL-10 production; increased IL-10 production; decreased expression of the costimulatory molecule CD80, and decreased IFN-γ production by T cells; increased FoxP3$^+$ Tregs	Ameliorate recurrent Th1-mediated murine colitis; increased anti-inflammatory effects; decreased Pouchitis	[93,96,127]
Mix 1 (*Lactobacillus acidophilus* & *Bifidobacterium longum*)	Increased FoxP3$^+$ Tregs and IL-10; decreased TNF-α	Prevention of trinitrobenzene sulfonic acid (TNBS)-induced colitis	[126]
Bifidobacterium infantis 35624	Increased FoxP3$^+$ Tregs; decreased TNF-α & IL-6	Reduced intestinal inflammation	[112]
Lactobacillus paracasei DSM 13434 & *Lactobacillus plantarum* DSM 15312	Increased FoxP3$^+$ Tregs, IL-10 & TGF-β production	Decreased experimental autoimmune encephalomyelitis (EAE)	[120]
Bifidobacterium lactis AD011 & *Lactobacillus acidophilus AD031*	Increased IL-10; decreased IL-4; Treg-associated TGF-β production	Prevention of allergy and atopic dermatits	[118,119]
Bacteroides fragilis	FoxP3$^+$ Tregs & IL-10 production	Increased	[194]

Clostridium sp. (clusters IV and XIVa)	FoxP3^{+} Tregs & TGF-β	Increased	[73]
Lactobacillus rhamnosus GG (ATCC 53103) & *Bifidobacterium lactis* (Bb-12)	Increased FoxP3^{+} Tregs and & TGF-β production	Suppression of allergic sensitization and airway inflammation	[117]
Lactobacillus reuteri (ATCC 23272)	Increased FoxP3^{+} Tregs	Attenuation of allergic airway response	[125]
III. Stimulation of Toll-like Receptors			
Bifidobacterium breve	Activation TLR-2 ; dendritic cell maturation & activation; increased IL-10 production	Control of excessive Th1 & Th2 polarization in atopic newborns	[150]
Lactobacillus reuteri	Activation of TLR-9; suggestive to induce Tregs via activation of the DCs	Inhibited the allergic airway response	[151]
VSL#3	Activation of TLR-3 and TLR-4; increased FoxP3 Tregs, IL-10 & TGF-β	Suppressed experimental allergic asthma and autoimmune diabetes	[153]

VSL#3: contains 8 different gram-positive organisms (four *Lactobacilli*, three *Bifidobacteria*, and one *Streptococcal* strains: *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus plantarum*, and *Streptococcus salivarius* subsp. *thermophilus*)

Table 2. Induction of regulatory T cells by different mechanisms mediated by prebiotics and probiotics.

Prebiotics	**Effect on Probiotic**	**Different Mechanisms for Induction of Tregs**	**Outcome**	**Reference**
Fructo-oligosaccharides (FOS)	Increased *Bifidobacteria*	Increased IL-10 positive DCs expressing TLR2 and TLR4	Reduced inflammation in IBD	[86]
Inulin	Increased *Biofidobacteria*, *Lactobacillus*, *Faecalibacterium prausnitzii* & *Eubacterium spp*	Increased IL-10 positive mucosal DCs	Reduced the incidence of atopic dermatitis	[87]
Resistant starch	Increased the growth of *Firmicutes*	Increased percentages of Tregs in spleen and Payer Patches	Reduced inflammation in IBS	[88]
B-galactooligosaccharides (β-GOS)	Increased *Bifidobacteria*	Increased the production of Tregs promoting cytokine IL-10 and significantly reduced production of IL-6, IL-1β, and TNF-α	Reduced inflammation in aging persons	[89]

Figure legends

Fig. 1. Effects of probiotics and prebiotics on immune regulation. Immune homeostasis is required to maintain the immune cells in adequate numbers by either their induction or suppression. The immune imbalance may lead to increase in particular immune cells population such as effector $CD8^+$ and $CD4^+$ T cells which may not be controlled by a deregulated Treg cells with defects in their numbers and/or function. This may lead to development of autoimmune conditions exhibiting the several deregulatory immune processes such as; increased pro-inflammatory factors, decreased anti-inflammatory factors, decreased Treg generation, expansion and activity, decreased tolerogenic DCs, increased cytotoxicity ($CD8^+$ T cells), increased Th1 activation and proliferation, increased Th17 activation, proliferation and increased co-stimulatory activity. However, given the role of probiotics and prebiotics in induction of Tregs help in establishing the immune homeostasis by increasing the Treg numbers and function which could lead to suppression of active $CD8^+$ and $CD4^+$ T cells. This results in decreased pro-inflammatory factors, increased anti-inflammatory factors, increased Treg generation, expansion and activity, increased tolerogenic DCs, decreased cytotoxicity ($CD8^+$ T cells), decreased Th1 activation and proliferation, decreased Th17 activation and proliferation and decreased co-stimulatory activity.

Fig. 2. Different types of regulatory T cells and their suppressive functions. Development of natural Treg cells (nTregs), naïve $CD4^+$ and $CD8^+$ T cells occurs in thymus. These T cells leave the thymus for the periphery where they colonize secondary lymphoid tissues and organs. nTregs specifically exhibits FoxP3 and CTLA-4 expression whereas naïve $CD4^+$ and $CD8^+$ T cells become inducible Tregs by induction with antigen presenting cells (APCs). These

inducible $CD4^+$ Tregs are further differentiated into iTreg (induced by IL-2, TGF-β and RA; exhibiting increased CD25 and FoxP3 expression), Tr1 (induced by IL-10; exhibiting low CD25 and no FoxP3 expression) and Th3 (induced by IL-10 and TGF-β; exhibiting no CD25 and FoxP3 expression) cells. Tr1 cells secrete high levels of IL-10 and probably TGF-β1 and Th3 cells secrete high levels of TGF-β1. Other naïve $CD4^+$ T cells differentiate into Th1, Th2 and Th17 cells which are induced by IL-12, IL-4 and IL-6, TGF-β respectively. The active suppression of these Th1, Th2 and Th17 cells as well as cytotoxic $CD8^+$ cells is carried out by all three types of inducible $CD4^+$ Treg cells by cell-cell contact, secretion of IL-10, TGF-β and probably IL-35. Inducible $CD8^+$ Treg cells are also developed from naïve $CD8^+$ cells by the same way as that of $CD4^+$ T cells. These are called as $CD4^+$ like $CD8^+$ iTregs, Tr1 like $CD8^+$ Treg and Th3 like $CD8^+$ Tregs and mediate the suppression of cytotoxic $CD8^+$ T cells and Th1, Th2 and Th17 cells in the similar manner. nTregs mediate the suppression of cytotoxic $CD8^+$ T cells and Th1, Th2 and Th17 cells via cell-cell contact, CTLA-4 and probably by secretion of IL-10, TGF-β and IL-35. Moreover, $CD8^+$ Treg cells induce the expression of the immunoglobulin-like transcripts: ILT3 and ILT4 in APCs, which negatively signal APC function. In addition, inducible $CD4^+$ Treg cells also inhibit the APC activity by decreasing the co-stimulatory receptors. nTreg cells via cell-cell contact directly inhibits the inflammatory DCs (APCs). nTregs also suppress cytotoxic $CD8^+$ cells by activating IL-10 secreting plasmacytoid DCs.

Figure 3. Different mechanisms of Treg induction by probiotics and prebiotics. (i) Probiotics induce the release of immunosuppressive cytokines such as TGF-β and IL-10 through TLR activation. Moreover, FoxP3 transcription factor along with TGF-β results into Treg induction and expansion leading to inhibition of activated $CD8^+$ and Th17 cells. (ii) Probiotics bind to

the lectin dendritic cell [DC-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)] and induce Tregs capable of secreting increased levels of IL-10. (iii) Probiotics metabolize the dietary fibers such as resistant starch, inulin, FOS, β-GOS etc. to release immunomodulatory short chain fatty acids (SCFAs): acetate, propionate and butyrate. These SCFAs activate the G protein-coupled receptors (GPCRs)- GPR43 and GPR109A on Treg cells. GPR43 is activated by all three SCFAs, however, GPR109A is activated only by butyrate. This interaction of SCFAs and GPCRs results into induction and expansion of Treg cells capable of suppressing the activated $CD8^+$ and Th17 cells population by secreting increased amounts of IL-10 and TGF-β. Moreover, SCFAs induction also involves inhibition of histone deacetylases (HDACs). Butyrate is the most potent inhibitor of HDAC among all three SCFAs. The HDAC inhibition increases the production and suppressive function of $FoxP3^+$ Tregs by promoting the acetylation of FoxP3 protein. In addition, SCFAs modulate the production of prostaglandin E2 (PGE2) which is involved in promoting *de novo* Treg differentiation from naive precursors, in suppression of T cell receptor signaling, promoting the induction of suppressive cytokine IL-10 and directly suppress the production of multiple proinflammatory cytokines. PGE2 also promotes the activation, maturation, and migration of dendritic cells (DCs) thereby promotes the interaction of DCs with Tregs and expansion of pre-existing Tregs. However, butyrate also induces the increased cyclooxygenase (COX-2) production which results into upregulation of PGE2 and affect the immunomodulatory effect of PGE2. (iv) Probiotic strain (*L. paracasei* NCC2461) is involved in hydrolysis of β-lactoglobulin (BLG) into peptides, which also stimulate the production of IL-10. (v) Microbial polysaccharide such as polysaccharide A (PSA) produced by *Bacteroides fragilis* induces the differentiation of suppressive $FoxP3^+$ Treg cells from naïve $CD4^+$ T cells through TLR-2 activation.

Figure 1

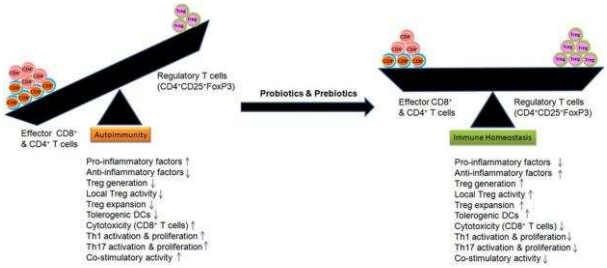

Regulatory T cells
(CD4+CD25+FoxP3)
Effector CD8+
& CD4+ T cells
Autoimmunity
Pro-inflammatory factors ↑
Anti-inflammatory factors ↓
Treg generation ↓
Local Treg activity ↓
Treg expansion ↓
Tolerogenic DCs ↓
Cytotoxicity (CD8+ T cells) ↑
Th1 activation & proliferation ↑
Th17 activation & proliferation ↑
Co-stimulatory activity ↑
Probiotics & Prebiotics
Effector CD8+
& CD4+ T cells
Regulatory T cells
(CD4+CD25+FoxP3)
Immune Homeostasis
Pro-inflammatory factors ↓
Anti-inflammatory factors ↑
Treg generation ↑
Local Treg activity ↑
Treg expansion ↑
Tolerogenic DCs ↑
Cytotoxicity (CD8+ T cells) ↓
Th1 activation & proliferation ↓
Th17 activation & proliferation ↓
Co-stimulatory activity ↓

Figure 2

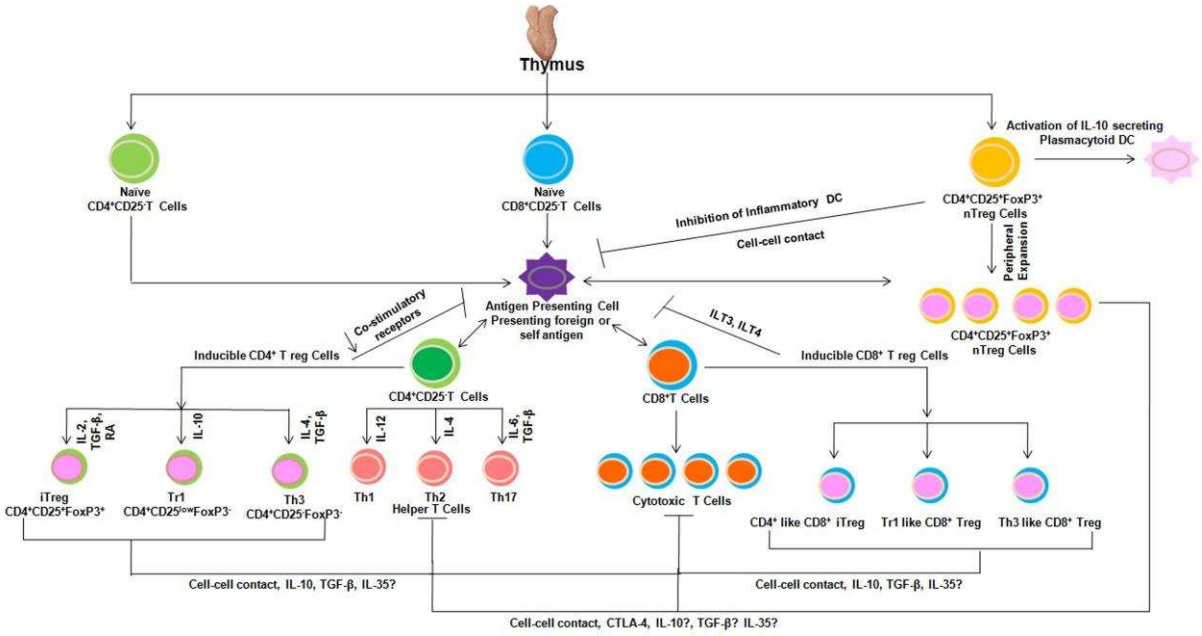
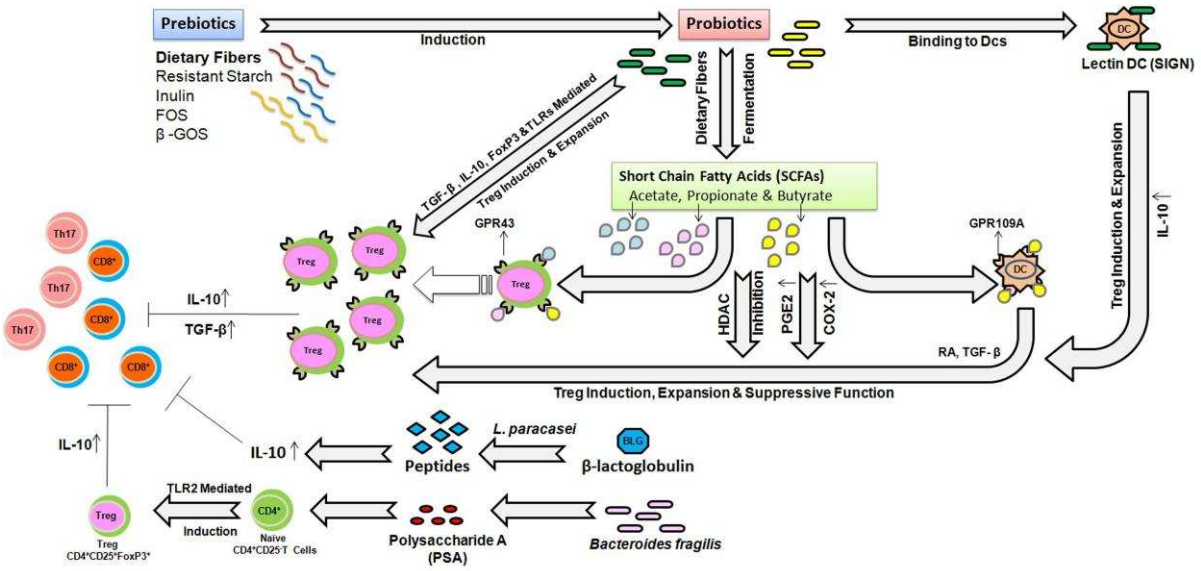

Thymus
Naïve
CD4+CD25- T Cells
Naïve
CD8+CD25- T Cells
Activation of IL-10 secreting
Plasmacytoid DC
CD4+CD25+FoxP3+
nTreg Cells
Inhibition of Inflammatory DC
Cell-cell contact
Peripheral
Expansion
Co-stimulatory
receptors
Antigen Presenting Cell
Presenting foreign or
self antigen
ILT3, ILT4
CD4+CD25+FoxP3+
nTreg Cells
Inducible CD4+ T reg Cells
Inducible CD8+ T reg Cells
CD4+CD25- T Cells
CD8+T Cells
IL-2,
TGF-β,
RA
IL-10
IL-4,
TGF-β
IL-12
IL-4
IL-6,
TGF-β
iTreg
CD4+CD25+FoxP3+
Tr1
CD4+CD25low FoxP3-
Th3
CD4+CD25-FoxP3-
Th1
Th2
Helper T Cells
Th17
Cytotoxic T Cells
CD4+ like CD8+ iTreg
Tr1 like CD8+ Treg
Th3 like CD8+ Treg
Cell-cell contact, IL-10, TGF-β, IL-35?
Cell-cell contact, IL-10, TGF-β, IL-35?
Cell-cell contact, CTLA-4, IL-10?, TGF-β? IL-35?

Figure 3


Prebiotics
Induction
Probiotics
Binding to Dcs
Lectin DC (SIGN)
Dietary Fibers
Resistant Starch
Inulin
FOS
β -GOS
Dietary Fibers
Fermentation
TGF-β , IL-10, FoxP3 & TLRs Mediated
Treg Induction & Expansion
Short Chain Fatty Acids (SCFAs)
Acetate, Propionate & Butyrate
GPR43
GPR109A
HDAC
Inhibition
PGE2
COX-2
Treg Induction & Expansion
IL-10
IL-10↑
TGF-β↑
RA, TGF-β
Treg Induction, Expansion & Suppressive Function
IL-10↑
IL-10↑
L. paracasei
Peptides
β-lactoglobulin
TLR2 Mediated
Induction
Naive
CD4+CD25-T Cells
Polysaccharide A
(PSA)
Bacteroides fragilis
Treg
CD4+CD25+FoxP3+