

## Probiotics as regulators of inflammation: A review

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### ABSTRACT

A substantial and increasing body of clinical evidence supports the role of specific strains and mixtures of probiotics in the prevention and treatment of certain diseases. Several general mechanisms of action have been proposed, including supporting repair of hyperpermeable epithelial barriers, interfering with infection by pathogens, and restoring a healthful balance of commensal microbes to affect metabolism. Emerging evidence supports an additional role of probiotics as important modulators of immune system responses, including inflammation, at mucosal surfaces. In particular, by preventing or repairing ‘leaky’ epithelial barriers, probiotics can indirectly affect the inflammatory response by negating the source of pro-inflammatory stimuli associated with low-grade endotoxemia. They also enhance production of short chain fatty acids with anti-inflammatory properties (e.g. butyrate) as well as increase synthesis of antimicrobial peptides that influence inflammation resolution pathways in the mucosa. Furthermore, probiotics and some of their secreted metabolic products can act as ligands for innate immune system receptors, directly influencing key pro-inflammatory pathways. They also stimulate the differentiation and activity of important immune cells (e.g., dendritic cells, T cells), and subsequently increase production of important regulatory cytokines, including interleukin-10 (IL-10) and transforming growth factor-beta (TGF- $\beta$ ). Finally, there are limited but increasing animal studies and clinical trials demonstrating probiotics do affect common biomarkers of inflammation, including C-reactive protein, as well as signs and symptoms of the associated diseases suggesting they can have therapeutic benefit in the treatment of chronic inflammatory disease.

**Keywords:** probiotics, inflammation, endotoxemia, epithelial, cytokines, immune, butyrate, antimicrobial peptides

### INTRODUCTION

Our understanding of the beneficial roles of commensal microorganisms in sustaining human health has increased dramatically in recent times. It is evident a prolonged disruption in the

normal ecology of commensal microflora (often termed dysbiosis) can contribute to different diseases through a number of mechanisms. For example, a reduced diversity of 'healthy' microbiota and subsequent opportunistic overgrowth of potentially pathogenic commensal bacteria and fungi alters signaling to the immune system. This results in abnormal activation of certain immune cells (e.g., Th1, Th17, gamma delta T ( $\gamma\delta$ T) cells and innate lymphocytes) and repression of certain regulatory mechanisms (e.g., reduction of the activity and number of Treg and Tr1 cells, reduced immunoglobulin A (IgA) synthesis and lowered levels of major suppressive cytokines such as IL-10 and TGF- $\beta$ ) [1]. In general, the immunological outcome of dysbiosis is a shift away from the normal state of tolerance to an abnormal state of heightened reactivity towards commensal microbiota and dietary substances, resulting in chronic inflammation. There is a substantial and increasing body of scientific literature demonstrating that supplementation with probiotics can help treat dysbiosis, restore a healthy microbial ecology, and modulate the chronic inflammatory processes that can lead to disease.

## REVIEW

Probiotics can help regulate inflammation in a number of ways, but this paper will focus primarily on: 1) *indirect effects*, by maintaining or repairing epithelial barriers, enhancing production of short chain fatty acids with anti-inflammatory properties (e.g., butyrate) as well as increasing synthesis of antimicrobial peptides that influence inflammation resolution pathways in the mucosa and 2) *direct effects*, by binding innate immune system receptors and triggering pathways that affect the production of cytokines associated with inflammation.

### ***Indirect effects: 1) Repair of hyperpermeable epithelial barriers***

A hyperpermeable epithelial barrier in the gastrointestinal tract is proposed as a major cause of chronic inflammation in a recently described pathophysiological process termed low-grade or subclinical endotoxemia. This is also termed metabolic endotoxemia if the cause(s) of the hyperpermeability are metabolic in origin, including a high-fat diet. In general, low-grade endotoxemia means there is abnormal translocation of macromolecules, including Gram-negative bacteria across the barrier, resulting in the lipopolysaccharide (LPS) (also termed endotoxin) portion of their cell wall, entering circulation to act as a constant low-level pro-inflammatory stimulus. This is in contrast to the most commonly recognized abnormal translocation of LPS, where a relatively large amount (>10-200 ng/ml) enters systemic circulation to cause overwhelming systemic inflammation and the serious and potentially fatal condition termed sepsis [2]. The medical consequences of this persistent 'trickle' of LPS (e.g., 1pg-100pg/ml) are potentially enormous with metabolic endotoxemia recently referred to as "one of the key culprits in provoking a non-resolving low-grade inflammation" [2]. Indeed, many different chronic diseases, including obesity [3], diabetes [4], fatty liver disease and nonalcoholic steatohepatitis [5], atherosclerosis, Parkinson's disease [6], and cancer cachexia [7], are now correlated with metabolic endotoxemia.

Treatment of low-grade endotoxemia requires a comprehensive clinical approach that includes alteration of certain dietary choices and lifestyle behaviors, inclusion of medications with anti-inflammatory and/or drainage properties, but most importantly, repair of damaged epithelial barriers. A number of different natural health products will help repair a

hyperpermeable epithelial barrier, including glutamine [8], quercetin, zinc [9] (e.g., zinc carnosine [10]) and different herbal medicines, but undoubtedly the most studied product is probiotics.

There are a number of mechanisms whereby certain probiotic strains can improve the structure and function of intestinal epithelial barriers, including buttressing the physical barrier, increasing mucin production, promoting production of antimicrobial peptides and heat shock proteins, attenuating the negative effect of pathogenic microorganisms, and modulating signaling pathways that affect cell proliferation and survival [11, 12]. For a diagrammatic representation of the various mechanisms of action of probiotics refer to [11, 12]. Of particular interest is the ability of specific probiotic strains to significantly decrease tumor necrosis factor alpha (TNF- $\alpha$ ) [13, 14, 15], a pro-inflammatory cytokine that causes leaky barriers by directly impairing the integrity of the tight junctional complexes between epithelial cells. Other probiotic strains bolster mucosal membrane integrity by directly affecting expression of one or more of the important tight junctional proteins (e.g., zonulin-1, occludin and cingulin) [16, 17]. Various other cellular and molecular mechanisms (eg. release of metabolites and bioactive molecules, suppression of oxidative stress, interference with inflammatory pathways, increase expression of mucins, augment levels of IgA) are used by different probiotic strains to help protect and repair epithelial barriers [18, 19]. Several different probiotics including *Lactobacillus* species (eg. *L. rhamnosus* GG, *L. plantarum* DSM 9843, *L. reuteri* R2LC, *L. casei* DN-114001, *L. paracasei* NCC2461), *Bifidobacterium infantis*, *Saccharomyces boulardii* and *S. cerevisiae* UFMG 905 and *Escherichia coli* Nissle 1917 have been shown to protect and repair epithelial barriers *in vitro* [18, 19].

There also are a number of animal studies [20, 21] and clinical trials [22, 23, 24, 25, 26, 27] demonstrating the efficacy of different probiotics in repairing or preventing damage to epithelial barriers caused by different diseases or other stressors.

### ***Indirect effects: 2) Increase production of short chain fatty acids including butyrate***

Another indirect effect of probiotics on inflammation occurs via their ability to ferment certain types of fiber, thus increasing the production of short chain fatty acids, such as: propionate, acetic acid, and butyrate. Butyrate is particularly important as a modulator of inflammation, with anti-inflammatory effects demonstrated in intestinal epithelial cells, macrophages, and leukocytes [28]. For example, butyrate acts as an important signaling molecule, influencing pathways that block NF- $\kappa$ B-induced increases in pro-inflammatory cytokines by a number of different mechanisms [11]. Butyrate also regulates the production of certain cytokines (e.g., TNF- $\alpha$ , IL-2, IL-6, IL-10), eicosanoids and chemokines from leukocytes, and affects their ability to migrate to sites of inflammation [14]. Furthermore, butyrate is an important inhibitor of histone deacetylase and has the potential to regulate expression of numerous genes, including those that encode important inflammatory mediators, i.e., nitric oxide (NO), IL-6 and IL-12 [29]. It also was recently shown to induce the differentiation and expansion of regulatory T cells not only in the colon [30] but also in the periphery [31], suggesting butyrate plays an important role in maintaining the balance between tolerance and hyper-reactivity. Finally, butyrate is a primary fuel source for colonocytes, and therefore helps maintain epithelial barriers in a number of different ways [32]. This evidence that butyrate also has important anti-inflammatory properties

highlights the potential of using a carefully selected mixture of probiotic strains and compatible prebiotics (i.e. to formulate synbiotics) to maximize therapeutic efficacy [33].

***Indirect effects: 3) Promote synthesis of antimicrobial peptides***

Antimicrobial peptides (AMPs) are a group of small amphipathic peptides (e.g., defensins and cathelicidins) found in multicellular organisms such as plants and animals [34] and is produced by a wide variety of different cell types, including epithelial cells, leukocytes, intestinal Paneth cells [35] and keratinocytes [36, 37]. They were originally recognized and named for their role as potent broad-spectrum natural antimicrobials, providing a first line of defense at mucosal barriers, including those in the gastrointestinal and pulmonary tract [38]. Since their discovery, numerous additional functions have been determined, including important roles as immune signaling molecules that influence diverse functions, such as wound repair, expression of different chemokines and cytokines, recruitment of leukocytes, chemotaxis of mast cells, modulation of dendritic cell function and even modulation of adaptive immunity [39, 40]. In fact, AMPs are involved in such a widespread diversity of signaling that it is proposed they are “poorly named, antimicrobial peptides are multifunctional pillars around which the innate and adaptive immune response has evolved” [41]. Recently it was shown that these AMPs also act in prime inflammation resolution in the mucosa [42], suggesting they are part of an additional endogenous local pathway used to control an acute inflammatory response, bringing the associated inflamed tissue back to homeostasis. There are a number of different substances, including Vitamin D [43, 44], certain amino acids [45], butyrate [46], and probiotics [47], that are recognized as potent inducers of the synthesis and activity of AMPs.

Probiotics including *Escherichia coli* Nissle 1917, *Lactobacillus fermentum* PZ1162 and the mixture VSL# 3 were demonstrated to induce the secretion of AMP human beta-defensin -2 through pro-inflammatory pathways that involved the transcription factors NF- $\kappa$ B and AP-1 as well as MAPKs [47]. Several other types of AMPs including various bacteriocins are induced by various strains of probiotics (eg. *L. salivarius* DPC6005, *L. salivarius* UCC118, *L. plantarum* DDEN 11007, *L. plantarum* C11, *L. lactis* DPC3147) and demonstrated to have multiple roles in colonization, microbicidal activity as well as other signaling functions [48]. Although there is a need for further research, enhancing synthesis and secretion of AMPs might prove to be an additional indirect pathway whereby probiotics could help regulate an inflammatory response.

***Direct effects: 1) Act as ligands for innate immune system receptors such as Toll-like receptors and nucleotide-binding oligomerization domain receptors***

Probiotics also can have direct effects on the inflammatory response by acting as ligands for specific members of a large family of innate immune system receptors termed Toll-like receptors (TLRs), thereby influencing important signaling pathways, including NF- $\kappa$ B, mitogen-activated protein kinase (MAPK), phosphoinositide-3-kinase-protein kinase B/Akt (PI3K-PKB/Akt), and peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) [11]. TLR-2 and TLR-4 activation by probiotic microorganisms are recognized as particularly important receptors mediating the direct influence of probiotic and commensal bacteria on inflammatory signaling pathways [12, 49]; TLR-9 also is identified as an important receptor for mediating the anti-inflammatory effect of certain probiotic strains [12, 50].

Recent evidence suggests that some species of probiotics may release low molecular weight substances that can cross the epithelial cell membrane to interact with specific members of another large group of intracellular innate immune system receptors termed nucleotide-binding oligomerization domain receptors (NLRs) [12]. Triggering the downstream pathways associated with activation of NLRs represents another method whereby probiotics can affect inflammasome assembly and NF- $\kappa$ B associated signaling, thus the inflammatory response.

The ability of probiotics to influence NF- $\kappa$ B and PPAR $\gamma$  signaling pathways would especially have widespread effects on inflammation, as these two transcription factors are recognized as critically important intracellular hubs at the intersection of many different pathways leading to production of numerous proinflammatory signals, including cytokines and chemokines. Activation of TLRs and NLRs also leads to assembly of inflammasomes (i.e., intracellular molecular platforms found in many cells that have a number of different regulatory functions, but are particularly responsible for activating the enzyme caspase-1 involved in the final maturation of the key pro-inflammatory cytokine IL-1 $\beta$  and IL-18) [51].

The effects of probiotics are strain specific, thus not all probiotics will have similar effects on each signaling pathway. A detailed summary of the current research on various probiotics and the specific signaling pathway they affect in different model systems has been published elsewhere [11] and will not be included in this paper. In sum, the binding of probiotics and commensal microflora to innate immune systems receptors such as TLRs and NLRs would help regulate major intracellular pathways that help maintain the homeostatic balance between pro-inflammatory and anti-inflammatory responses at mucosal surfaces.

It is important to note that some probiotics (e.g., *Bifidobacterium lactis* BB12, *Bacteroides vulgatus*) also stimulate NF- $\kappa$ B controlled signaling by a number of different mechanisms [11], suggesting they have pro-inflammatory effects. This pro-inflammatory effect of some probiotics could be beneficial, as suggested by current scientific evidence that a certain low level of inflammation could have physiological benefits including maintenance of epithelial barriers and ‘priming’ of immune system responses. Furthermore, the immunostimulatory phenotypes of commensal bacteria and probiotics might serve an important purpose of promoting host defense against pathogens..

### ***Direct effects: 2) Influence maturation and development of important immune cells such as dendritic cells and T cells***

In addition to their ability to affect intestinal epithelial cells and macrophages, probiotics also help influence the differentiation and function of a number of other immune cells associated with an inflammatory response, including dendritic cells as well as T cells (refer to [11, 12] for a diagrammatic representation). The ability of probiotics to affect the maturation, survival and function of dendritic cells [11] has widespread implications, because these professional antigen-presenting cells play key roles at mucosal surfaces in maintaining tolerance to commensal microbiota and innocuous food antigens. They also are an important source of cytokines that help promote differentiation of naive T cells into Th1, Th2, Th17 or T regulatory cells, thus the appropriate cell-mediated or humoral immune response [11]. Finally, different probiotic strains and species of beneficial microbes also influence the development and survival of different types

of regulatory T cells, including Th3, Tr1, CD4+CD25+ regulatory, CD8+ suppressor and  $\gamma\delta$ T cells [11], suggesting they can have a widespread role in maintaining intestinal homeostasis and the balance between oral tolerance and reactivity to ingested food antigens as well as to commensal microbes.

**Direct effects: 3) Influence synthesis of the important regulatory cytokines IL-10 and TGF- $\beta$**

Different populations of regulatory T cells exert their inflammation-modulating effect by secreting specific cytokines, including IL-10 (which strongly suppresses a Th-1 dominant pro-inflammatory response) or more regulatory cytokines such as TGF- $\beta$  (which promotes development of regulatory T cells). Certain probiotic strains help regulate inflammation via their enhanced ability to influence the secretion of these important cytokines [11]; refer to [11, 12] for a diagrammatic representation. This effect has been demonstrated, *in vitro* [52,53] and *in vivo*, in various animal models [54, 55, 56, 57] and clinical studies [58].

**Clinically meaningful effects on inflammation?**

There also are a limited number of studies demonstrating that supplementation with certain probiotics significantly reduced hs-CRP levels in adults that were healthy [59], pregnant [60], critically ill [61] or had inflammatory bowel disease [62] as well as other inflammatory disorders (e.g., ulcerative colitis, chronic fatigue syndrome and psoriasis) [63] and type 2 diabetes [64], suggesting that they could have a widespread anti-inflammatory effect that is clinically relevant.

Considerable heterogeneity exists in the clinical trial design published to date making it difficult to determine the exact dosing and strains of probiotics needed to ensure maximal therapeutic benefits in specific clinical conditions including inflammatory disease. For example, in the trials listed above there were major differences in study populations, types and daily dose of probiotics used, type of placebo, length of study as well as the delivery medium (Table 1).

**Table 1:** Summary of Clinical trials measuring effects of Probiotics on the inflammatory marker CRP

Reference	Study design	Study population & characteristics	Probiotic strains used	Daily dose provided	Delivery medium	Placebo used	Length of study
[59] Kekkonen RA, Lummela N et al. 2008	Randomized, double-blind and placebo-controlled parallel group intervention; Single strains given to different groups and compared to placebo	Healthy adults (n=68) Mean age 44 years; mean BMI 24kg/m <sup>2</sup>	<i>Lactobacillus rhamnosus</i> GG ATCC 53103 (n=13) <i>Bifobacterium animalis</i> ssp. lactis Bb12 (n=16) <i>Probiobacterium freudenreichii</i> spp shermanii JS (n=17)	1.6 X 10 <sup>10</sup> cfu 3.5 X 10 <sup>10</sup> cfu 3.3 X 10 <sup>10</sup> cfu	250 mL milk-based fruit drink	Milk-based fruit drink without probiotics (n=16)	3 weeks
[60] Asemi Z, Jazayeri S et al. 2011	Randomized clinical trial; 2 probiotic species given and compared to	Healthy pregnant women in 3 <sup>rd</sup> trimester (n=70)	<i>Bifidobacterium animalis</i> ssp lactis Bb-12 <i>Lactobacillus acidophilus</i> LA-5 (n=37)	200 g of probiotic yogurt containing 10 <sup>7</sup> CFU/ g of each (i.e. 2X 10 <sup>9</sup> cfu)	Yogurt prepared with starter culture of <i>Streptococcus</i>	Yogurt without probiotics (n=33)	9 weeks

	placebo				<i>thermophilus and Lactobacillus bulgaricus</i>		
[61] Sanaie S, Ebrahimi-Mameghani M et al., 2013	Double-blind randomized control; given standard treatment with or without multistrain probiotic VSL#3	Critically ill patients admitted to ICU, receiving enteral nutrition (Fresenius original fiber) (n=40)	VSL#3: Strains not given because “*VSL Pharmaceuticals consider the strain number proprietary” <a href="http://www.vsl3.com/discover/aboutprobiotics.asp">http://www.vsl3.com/discover/aboutprobiotics.asp</a> <i>Lactobacillus casei</i> , <i>Lactobacillus plantarum</i> , <i>L acidophilus</i> , <i>Lactobacillus delbrueckii subsp. Bulgaricus</i> <i>Bifidobacterium longum</i> , <i>Bifidobacterium breve</i> , <i>Bifidobacterium infantis</i> <i>Streptococcus salvarius subsp. Thermophilus</i> (n=20)	2X 900 billion (i.e. 1.8 X 10 <sup>12</sup> cfu)	Standard treatment without placebo (n=20)	None	7 days
[62] Shadnoush M, Shaker Hosseini R et al., 2013	Randomized, comparative study; 2 probiotic species given and compared to healthy controls (n=84)	IBD patients in remission; mean age 38 years; mean BM 24.17 kg/m <sup>2</sup> ; randomized into two groups (with probiotics (n=86) or without probiotics (n=90))	NO species or strain information given <i>Bifidobacterium Lactobacillus</i> (n=86)	250 g yogurt containing 10 <sup>6</sup> cfu/ g (i.e. 2.5 X 10 <sup>8</sup> cfu)	1.5% fat yogurt (NO starter culture strains given)	Plain yogurt without probiotics (n=90)	8 weeks
[63] Groeger D, O’Mahony L et al., 2013	Randomized double-blind, placebo-controlled; single strain given to 3 different groups and compared to placebo	Ulcerative colitis patients (n=22) Psoriasis patients (n=26) CFS patients (n= 48) Healthy controls (n=22)	<i>Bifidobacterium infantis</i> 35264	1X 10 <sup>10</sup> cfu	Oral sachets	Maltodextran (5g)	6-8 weeks
[64] Asemi Z, Zare Z et al. 2013	Randomized double-blind placebo controlled; 7 strain multispecies probiotic supplement given	Diabetic patients (n=54); age 35-70 years	NO strain information given <i>L. acidophilus</i> <i>L. casei</i> <i>L. rhamnosus</i> <i>L. bulgaricus</i> <i>Bifidobacterium breve</i> <i>B. longum</i> <i>Streptococcus thermophilus</i> plus 100 mg fructo-oligosaccharide (n=27)	2X 10 <sup>9</sup> cfu 7X 10 <sup>9</sup> cfu 1.5X 10 <sup>9</sup> cfu 2X 10 <sup>8</sup> cfu 2X 10 <sup>10</sup> cfu 7X10 <sup>9</sup> cfu 1.5X10 <sup>9</sup> cfu	Oral capsules	Similar capsule without probiotics (n=27)	8 weeks

Furthermore, many studies do not list the strains of probiotics used in the intervention, even though it is becoming increasingly clear that not all strains have similar effects even if they are from the same species. All of these factors (and others) could potentially influence the ability of probiotics to control inflammation and therefore, it is difficult at this time to make definitive

clinical recommendations. However, in general the range of doses used to alleviate symptoms of disease ranges from 100 million cfu/d for *Bifidobacterium infantis* 35624 to 300 to 450 billion cfu 3 times daily for VSL#3 (a mixture of 1 strain of *Streptococcus thermophilus*, 4 *Lactobacillus* species, and 3 *Bifidobacterium* species strains) in various clinical trials [65].

## CONCLUSION

There is sufficient current scientific literature to support supplementation with probiotics as a beneficial addition to comprehensive treatment of inflammatory disease. Specific probiotic strains can help repair hyperpermeable epithelial barriers and therefore, indirectly regulate inflammation by shutting down potentially important sources of stimuli of chronic inflammation, including LPS. They also are important inducers of other molecules that play widespread roles in modulating inflammation, including butyrate and AMPs. Furthermore, specific strains and/or their components bind TLRs or NLRs to directly influence the activity of various important signaling pathways, influencing inflammation by promoting production of more suppressive and/or regulatory cytokines such as IL-10 and TGF- $\beta$ . Using a combination of different well-researched probiotic strains to simultaneously modulate multiple signaling pathways could have a profound synergistic or additive effect on controlling and resolving inflammation in a timely fashion.

There is a need for additional preclinical and clinical research to further differentiate the optimum dose, posology, and exact strains of probiotics that have the most profound long-term effect on modulating the inflammatory response. Additional complexity in developing individualized inflammation regulating therapies using probiotics is created by the knowledge that different persons have unique endotypes of commensal microbiota as well as the recognition that a numerous factors, including age, genetics, use of certain medications, diet, geography and even psychological stress, can cause lasting shifts in commensal microbial ecology. Furthermore, the different types of diseases and even the phase the patient is in (especially in chronic relapsing diseases) will influence the quantity and quality of pro-inflammatory cytokines that need to be modulated by probiotics to produce clinically meaningful effects. Finally, it is unlikely that probiotics can act as a stand-alone therapy for treating inflammatory disease; therefore, it will be important to further delineate which therapies and medications can be used concomitantly to produce the safest and most effective clinical outcome.

### List of abbreviations:

AMPs: antimicrobial peptides

$\gamma\delta$ T: gamma delta T cells

hsCRP: high-sensitivity C-reactive protein

IgA: immunoglobulin A

IL-10: interleukin-10

LPS: lipopolysaccharide (also termed endotoxin)

NO: nitric oxide

NF- $\kappa$ B: nuclear factor kappa B

NLRs: nucleotide-binding oligomerization domain receptors

MAPK: mitogen-activated protein kinase



PPAR- $\gamma$ : peroxisome proliferator-activated receptor gamma  
PI3K/PKB/Akt: phosphoinositide-3-kinase-protein kinase B  
TGF- $\beta$ : transforming growth factor beta  
TLR: Toll-like receptor  
TNF- $\alpha$ : tumor necrosis factor alpha

**Competing interest:** Dr. Lescheid is a member of Medical Consultancy Group for Nutritional Fundamentals of Health, a company that formulates and sells Natural Health Products including probiotics. He also has lectured on this topic in the past, including lectures for this company.

**Authors' contributions:** DWL was the sole contributor to the conception, writing, revision and editing of this manuscript.

**Author's information:** DWL's academic interest in probiotics was fostered by a Ph.D. in molecular biology (i.e. where microbes were used as vectors), a degree in Naturopathic medicine (i.e., where probiotics were taught as a potential part of a treatment plan for certain diseases) and by a five year position as an Assistant Professor teaching Microbiology and Infectious Disease. He also has seven years of clinical experience, using probiotics as safe and effective treatments for a variety of different conditions.

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