

Probiotics and Mucosal Immunity:

Strain-specific effects on Th1/Th2 cell modulation

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ABSTRACT

Commensal bacteria in the gastrointestinal tract play an integral role in both innate and humoral immunity. It is well established that this protective role can be maintained or modulated by the ingestion of probiotics. More recently, it has been shown that specific probiotic strains can influence the secretion of cytokines to help direct naïve helper T cells towards either a Th1 dominant, cell-mediated immune response or towards a Th2 dominant, humoral immune response. This paper will review current knowledge of the Th1: Th2: Th3/ Treg model of humoral immunity as well as introduce how strain-specific probiotics can be used therapeutically to help balance this immune response and therefore, help prevent and treat disease.

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It is now well established that commensal intestinal microflora influence the physiology and pathology of the host. Moreover, it has been shown that the microbiological content of the gastrointestinal tract can be altered by the oral administration of health promoting microorganisms, namely probiotics. As a result, there is increased interest in the role of probiotics in maintaining optimal human health by preventing and treating disease. Probiotics are defined as living microorganisms that, upon ingestion in sufficient numbers, exert health benefits beyond basic nutrition.¹ Probiotic bacteria, like certain *Lactobacillus* and *Bifidobacterium* species, influence human health in a number of different ways.

One of the most accepted and researched effects of supplementation with probiotics is immune system modulation.² Probiotics influence several components of an immune response including humoral, cellular or innate immunity. A specific component of the humoral immune response involves the secretion of specific cytokines to influence naïve T-helper (Th) cells to become either Th1 or Th2 dominant and therefore, promote cellular or humoral immunity, respectively. Probiotics can help influence the release of specific cytokines by unique T cell subsets and therefore, play a fundamental role in mucosal immunity.³

In a healthy immune system, there is a balance between Th1 and Th2 cell activity. This balance is transiently shifted when necessary to support cell-mediated immunity or humoral immunity but usually is rebalanced quickly once the specific immune challenge is removed. However, if a cytokine imbalance persists, a specific T-cell pathway is maintained and immunopathological diseases like atopy, hypersensitivity reactions and chronic inflammation can occur.³

A recent hypothesis, termed the hygiene hypothesis, postulates that the increased prevalence of allergy in industrialized nations is due to inadequate microbial stimulation and therefore, development of a robust Th-1 response during childhood. This poorly developed Th-1 response occurs because of excessive hygiene, intensive food sterilization and modification of commensal gut flora in newborns from persistent feeding with artificial formulas. Without adequate feedback from Th-1 cell dependent cytokines, Th-2 cell dependent cytokines, like IL-4, IL-5, IL-9 and IL-13, predominate to promote the development of the allergic response.^{4,5}

It recently has been observed that specific probiotic strains stimulate the secretion of specific cytokines and,

therefore, facilitate the development of naïve T-cells towards a particular immune pathway. This paper, which is part of the proceedings of a symposium, will summarize the Th1/Th2 model of the immune system, the cytokines that regulate T cell development and the effects of specific probiotic strains on the modulation of mucosal immunity. An enhanced understanding of how specific strains of bacteria influence the immune system can be useful in selecting probiotics that can help correct the imbalances postulated by the hygiene hypothesis. Restoring the balance between Th-1 and Th-2 cells would help prevent and treat many diseases.

THE MUCOSAL BARRIER

The intestinal mucosa has an extensive surface area (>300m²) that provides a physical and immunological barrier to infection.¹ The physical barrier includes the mucous layer, the epithelial cells and the tight junctions between these cells. It acts as a structural separation between the lumen of the gastrointestinal tract (essentially part of the external environment) and the internal environment. The immunological barrier is part of the mucosal-associated lymphoid tissue (MALT) and is termed gut-associated lymphoid tissue (GALT). GALT contains one of the largest pools of immunocompetent cells in the body, with over 60-80% of total immunoglobulins circulating at some point in their life through this tissue. GALT also has more than 10⁶ lymphocytes/g tissue, making it a more concentrated source of lymphocytes than all of the other immune organs combined. The GALT can be separated into organized lymphoid tissue and diffuse lymphoid tissue. Organized lymphoid tissue includes mesenteric lymph nodes and Peyer's patches, containing microfold (M) cells, dendritic cells (DC) and B cells. Dendritic cells are antigen-presenting cells (APC) that sample antigen, process it, modify it and present it to other immune cells, like naïve T cells, to initiate specific immune functions. Diffuse lymphoid tissue is found primarily in the connective tissue of the lamina propria of the gastrointestinal tract layers.⁶ It contains lymphocytes like CD4+ T cells in the lamina propria, CD8+ T cells between the epithelial cells (intraepithelial lymphocytes (IEL)), B lymphocytes (memory and plasma cells that produce type A immunoglobins (IgA)), and natural killer (NK) cells.¹

The intestinal mucosal barrier is a highly selective, intelligent system that protects us from pathogens.⁶ It must selectively exclude potentially toxic and infectious material from entering systemic circulation. It also must

tolerate commensal bacteria and beneficial nutrients. Hypo-responsiveness towards ingested substances is the predominate response of the GALT. This response is termed oral tolerance and can be both T- and B- cell mediated. Normally, oral tolerance prevents an immunogenic response, but if suboptimal oral tolerance is present, hypersensitivity responses to oral antigens can occur. For example, if a person does not tolerate milk proteins, a hypersensitivity reaction to dairy products can occur.⁶ This hypersensitivity response often begins with either Th1 or Th2 cell polarization.

TH1/TH2 POLARIZATION

The initial step in the polarization of naïve T cells is the interaction of APCs, mostly DCs, with a non-self or “danger signaling” antigen. The physical nature of the antigen and the cytokines subsequently released by the APC encountering this antigen determines whether a Th1 or Th2 skewed immune response occurs.³ For example, when APCs are challenged with intracellular pathogens, like viruses, a common cytokine secreted is IL-12. This cytokine promotes the differentiation of naïve T cells into Th1 cells. Maturing Th1 cells then produce the cytokine IFN- γ , which feeds back in an autocrine loop to trigger the development of more APCs as well as further promote the maturation of naïve T cells into Th1 cells.⁷ Additional cytokines, like IL-18, also can be secreted to further influence Th1 development by enhancing IL-12 dependent Th1 cell differentiation and effector function.³ This self-perpetuating cycle of Th1 dominant cytokines persists until the immune challenge is sufficiently reduced.

If APCs are challenged with blood-borne pathogens, cytokines like IL-4 are released to promote Th2 cell differentiation. Additional cytokines like IL-13 also are secreted under certain conditions, and further promote Th2 polarization and development in an IL-4 independent manner.³ Maturing Th2 cells continue to release IL-4, generating an autocrine feedback loop that further increases the differentiation of naïve T cells to Th2 cells.⁷ Th2 cell dominant cytokines are continuously secreted until the blood-borne pathogens are removed or sufficiently reduced.

When naïve T cells become Th1 or Th2 polarized they are committed to that specific pathway and cannot be reversed. Cytokines secreted by Th2 cells activate specific B cells for antigen clearance as well as for antibody class switching. Cytokines secreted by the Th1 cells primarily promote

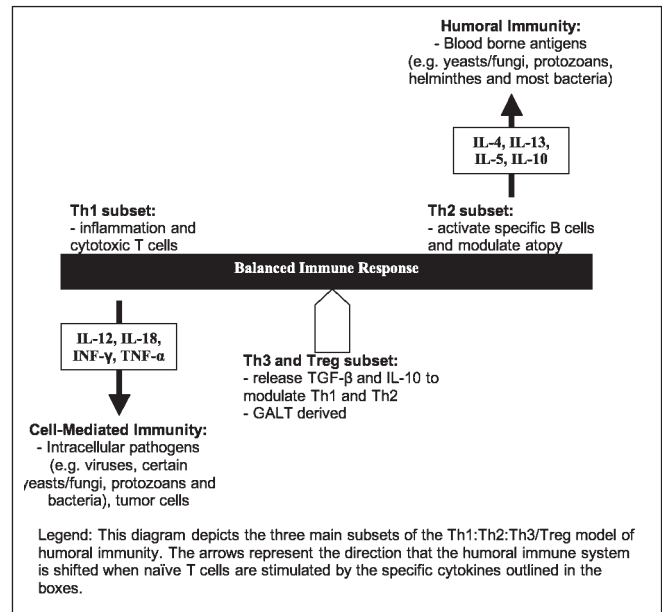


FIGURE 1 Healthy Immune System

inflammation and the activation of cytotoxic T cells.⁶ These Th1 and Th2 specific cytokines interact with each other to antagonize their respective maturation and actions.^{3,7} For example, Th1 cells secrete IFN- γ which inhibits proliferation of Th2 cells, whereas Th2 cells secrete the cytokine IL-4 that prevents Th1 cell differentiation.⁷ Further regulation of Th1 and Th2 cells occurs with additional subsets of T cells, termed regulatory T cells (Treg) or Th3 cells.⁷ Specific regulation of T cells by Th1, Th2 or Treg/Th3 cells will be discussed in the next sections.

TH1 SUBSET

The Th1 cell pathway supports cell-mediated immunity, preventing disease from intracellular pathogens like viruses, certain bacteria, yeast, fungi and protozoans. Th1 cell-associated immunity also has a major role in preventing tumor cell development. However, if naïve T cells are chronically Th1 polarized, an overactive cell-mediated immune response can result. For example, persistently high secretion of IL-12 will cause Th1 cells to produce large amounts of pro-inflammatory cytokines like IFN- γ and TNF- α . These cytokines further activate macrophages to produce additional pro-inflammatory mediators (i.e. IL-12 and IL-18) in a positive feedback loop that has potential pathological consequences (see figure 1).

Persistent Th1-mediated inflammation of the gastrointestinal tract is associated with pathologies like Crohn's disease,³ *H. pylori* gastritis, cellular autoimmunity, chronic recurrent inflammation and possibly rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus. This Th1 rigidity and the potential pathological consequences can be moderated by up-regulating the production of Th2 dominant cytokines, like IL-4, IL-10 and IL-13. These cytokines inhibit the development of Th1 cells and macrophage activation and, therefore, can prevent inflammatory tissue damage resulting from an overabundance of Th1 cell stimulation.⁸ Furthermore, the immunosuppressive cytokines IL-10 and TGF- β , released by regulatory T cells, also down-regulate Th1 rigidity and help control colitis and other inflammatory diseases.⁹

Certain probiotic strains can help down-regulate these Th1 dominant disorders and return balance to the immune response. For example, TNF- α plays a key role in the pathogenesis of intestinal inflammation in Crohn's disease, a disease associated with Th1 polarization. Borruel et al. (2002) studied the effect of probiotic bacteria on TNF- α production by obtaining ileal specimens from ten patients with Crohn's disease. They observed a significant reduction in the production of TNF- α by inflamed Crohn's disease mucosa when cultured with *L. casei* or *L. bulgaricus*, but not with *L. crispatus* or *E. coli*.¹⁰ The authors concluded that these probiotic strains had the ability to attenuate the release of the pro-inflammatory cytokines that promote Th1 rigidity by interacting with the intestinal mucosal immunocompetent cells.

Furthermore, in mouse studies it was found that a probiotic mixture of nine different bacterial strains ameliorated recurrent Th1-mediated murine colitis by inducing IL-10 secretion and promoting development of IL-10 dependent TGF- β bearing regulatory cells.⁴ Daily administration of 2 mg (approximately 3 billion bacteria) of this probiotic mixture for three weeks to mice during a remission period induced an immunoregulatory response involving TGF- β secreting cells and, therefore, resulted in a milder form of recurrent colitis. These two studies support the therapeutic use of certain strains of probiotics in different animal species to prevent and moderate diseases associated with chronic Th1 cell dominance.

TH2 SUBSET

The Th2 cell pathway supports humoral immunity by

activating specific B cells and promoting antibody class switching. Th2 cells release the cytokines IL-4, IL-13, IL-5 and IL-10 that activate antibody formation or release from immune cells like B cells, mast cells or eosinophils⁷ (see figure 1). Persistent, uncontrolled Th2 activation is associated with diseases like atopy (including allergies, eczema, asthma, and allergic rhinitis), chronic fatigue and immunodeficiency syndrome, eosinophilic rhinosinusitis, ulcerative colitis and possibly certain cancers.

Many studies in the past decade have shown that specific probiotic strains can benefit allergic diseases characterized by this chronic expression of Th2 cell-related cytokines. For example, Pochard et al. (2002) in an in vitro study demonstrated that different *Lactobacillus* strains reduce IL-4 levels (a Th2 cytokine) and enhance production of Th2 cell-related cytokines, supporting a more balanced Th1/Th2 response.¹¹ More specifically, a three-hour incubation of *L. plantarum*, *L. lactis*, *L. casei* and *L. rhamnosus* GG (at a concentration of ten bacteria per purified CD4+T cell) inhibited the production of IL-4 and IL-5 in a dose-dependent manner from human polymorphonuclear cells and stimulated the production of the pro Th1 cell cytokines, IFN- γ and IL-12. In the same experiment, no significant inhibition of IL-4 and IL-5 secretion occurred when the cells were incubated with *Escherichia coli* TG1, demonstrating that the inhibition of cytokine secretion was specific to certain bacterial strains. It was postulated that supplementation with these specific *Lactobacillus* strains could benefit patients with allergic diseases by reducing Th2 cytokine production.

Other studies have shown that *L. rhamnosus* GG can reduce allergic disease symptoms in humans. Ingestion of therapeutic levels of *L. rhamnosus* GG by breast-feeding mothers and newborn babies resulted in a 50% inhibition of the risk of developing atopic eczema in babies.^{12,13} These studies provide an example of how administration of strain-specific probiotics can modulate the immune response and down-regulate the Th2 dominance associated with the development of allergies.

REGULATORY T CELLS

There are other subsets of T cells, termed type 1 T regulatory cells (Treg) or Type 3 T regulatory cells (Th3) that help regulate T-helper cell functions and maintain intestinal homeostasis. For example, Treg cells predominantly secrete IL-10, a cytokine that down-regulates Th1 activity and, therefore, reduces Th1-associated inflammation. Adequately

primed Th3 cells primarily secrete TGF- β , which helps modulate both Th1 and Th2 activity⁷ (see figure 1). There is increased understanding that Treg and Th3 cells are influential in the maintenance of mucosal immunity and, therefore, the prevention of pathology. Furthermore, certain probiotic strains can moderate these regulatory responses. For example, *L. paracasei* (NCC2461) stimulates in vitro regulatory T cells to produce TGF- β and IL-10, cytokines implicated in the oral tolerance response to bovine beta-lactoglobulin in mice.^{14,15} Artificial induction of the oral tolerance response, via the administration of strain-specific probiotics, would help modulate hypersensitivity reactions.

CONCLUSIONS AND FUTURE DIRECTIONS

There are convincing initial studies with animal models and humans to demonstrate that probiotics can reduce Th1 or Th2 skewed disorders, like gastrointestinal inflammation and allergic diseases. These initial studies are encouraging but future studies must be conducted to more clearly determine the impact that specific probiotic strains have on T cells and immunopathology. Further studies also are needed to determine the therapeutic dose and timing of probiotic administration needed to produce these immune responses in humans.

This increased knowledge of the effect of specific strains of probiotics on immune-system modulation has widespread medical implications. For example, when a patient has a Th1-cell dominant disorder, such as Crohn's disease, then administration of specific probiotic strains can be given to promote secretion of IL-10 and TGF- β cytokines and, therefore, down-regulate chronic Th1 cell-associated inflammation and promote a return to balanced immunity.

Alternately, if a person has a Th2 cell dominant disorder, like allergies, specific probiotic strains can be given to help release TGF- β , reduce IL-4 and IL-5 or increase IL-12 and, therefore, shift the immune system back to a balanced Th1:Th2 response.

An increased understanding of the unique effects of strain-specific probiotics on the immune system will help healthcare professionals be more specific with their therapeutic intent and select certain probiotic supplements based on the diagnosed condition and the desired direction needed to positively influence the immune system. Nutraceutical companies also can formulate safe, effective probiotic supplements with unique effects on the immune system that can be more effectively used to reduce pathogenesis and maintain intestinal homeostasis. Currently, there are some multistrain probiotics that are known to affect Th1- or Th2-cell associated immunity. For example, NFH ProBio SAP-90 contains a blend of probiotic strains that will cause a more balanced Th1:Th2 response, whereas HMF forte more specifically activates the Th1 pathway and thus down-regulates overactive Th2 disorders. Conversely, the probiotic mixture VSL#3 promotes secretion of cytokines that drive a Th2 response and, therefore, will prevent and treat diseases associated with Th1 cell rigidity. With future clinical trials, there will be an increased understanding of how probiotic strains can regulate the production of T cell cytokines to produce a balanced T helper cell response (Th1=Th2=Th3/Tr1) and prevent imbalance (Th1>Th2 or Th2>Th1). Ideally, a more selective choice of probiotics by healthcare professionals can be used to prevent and treat certain immunopathologies and, therefore, maintain optimal health.

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