

Probiotics for the Prevention and Treatment of Allergies, with an Emphasis on Mode of Delivery and Mechanism of Action

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Abstract: Allergy, also termed type I hypersensitivity, is defined as a “disease following a response by the immune system to an otherwise innocuous antigen”. The prevalence of allergies is high and escalating, with almost half the populations of North America and Europe having allergies to one or more common environmental antigens. Although rarely life-threatening allergies cause much distress and pose an important economic burden. Recent studies demonstrate the importance of the commensal bacteria of the gastrointestinal tract, termed the microbiota, in stimulating and modulating the immune system. This goes hand-in-hand with the hygiene hypothesis, proposed by Strachan in 1989. With this in mind, the use of pre- and probiotics has gained interest to prevent and treat allergies through modulation of the gut microbiota and the immune system. Probiotics, namely Lactobacilli and Bifidobacteria, are live microorganisms that can be incorporated in the diet in the form of functional foods or dietary supplements to beneficially influence the host. In recent studies, probiotic formulations demonstrated the capability to successfully modulate allergic rhinitis, atopic disorders and food-related allergies. A number of probiotic mechanisms of action are involved in controlling hypersensitivity responses, many of which are still not yet understood. Microencapsulation has gained importance as a device for the oral delivery of probiotic cells and may play an important role in the development of a successful probiotic formulation to treat and prevent allergies. Despite the promising research on probiotic biotherapeutics, further investigations are required to develop a successful therapeutic to treat and prevent allergies.

Keywords: Allergy, hypersensitivity, lactic acid bacteria, microcapsule, microbiota, probiotic.

INTRODUCTION

According to the World Health Organization 20% of the world's population is affected by allergies, specifically Type I hypersensitivity reactions [1]. An allergy is an uncontrolled and misdirected immune response towards an innocuous antigen, termed allergen [2]. Allergies are usually minor inconveniences but may result in fatality if uncontrolled. Several treatments currently exist, but target only the alleviation of allergy-associated symptoms. Because of the role of the gut microbiota in the etiology and pathogenesis of allergies, probiotic bacteria have gained interest as allergy therapeutics. In order to improve the viability and metabolic activity of orally-delivered probiotic bacteria, microencapsulation technology has been investigated. Microencapsulation, specifically the entrapment of viable bacterial cells in a polymeric matrix, has proven to be successful for the treatment of a number of disorders, described in another review [3]. With these successes, microencapsulated probiotics can easily be envisioned as a successful therapeutic for the treatment and prevention of allergies.

The goal of this review is to describe and compile the latest research investigating the use of probiotic formulations for the treatment and prevention of allergies. This review will first highlight the importance of the human gastrointestinal tract (GIT) and its associated immune system, termed the gut associated lymphoid tissues (GALT). It will then emphasize the role of the intestinal microbiota in the etiology and pathogenesis of allergies. The focus of the review will then lay on the capability of probiotics to modulate the immune response, specifically with respect to the

deregulated immune system present in hypersensitivity reactions. Furthermore, probiotic modes of delivery of biotherapeutics for the treatment and prevention of allergies will be discussed.

TYPE I HYPERSENSITIVITY REACTIONS

An allergen is a substance that is usually harmless but in certain cases could be recognized as an antigen by the host, to which the body mounts a hypersensitive reaction. Common allergens include dust mites, pollen, drugs/antibiotics and foods such as peanuts. Allergens can be introduced via the respiratory tract, the GIT and the skin, leading to a series of hypersensitivity reactions and disease conditions, as detailed later [4-6]. Briefly, hypersensitivity reactions can be antibody- or cell-mediated and are further classified into categories, namely Type I, Type II, Type III and Type IV [2,7]. Types I to III are antibody-mediated whereas Type IV is cell-mediated. Type I hypersensitivity, the focus of this review, is also termed immediate hypersensitivity and is mediated by mast cell activation and IgE production, leading to the release of histamine, lipids and chemotactic factors. Disease conditions such as allergic rhinitis, asthma, conjunctivitis, dermatitis, food allergy and drug allergy are associated with Type I hypersensitivity reactions [7]. Type I hypersensitivity symptoms are usually mild but can escalate to be life-threatening, especially if not identified/treated promptly and effectively [8]. Symptoms include sneezing, wheezing, sinus pain, runny nose, coughing, hives, swelling of throat and mouth, itchy eyes/lips/throat, difficulty swallowing/speaking, nausea, vomiting, diarrhoea, loss of consciousness and even death [9]. Current treatments focus on the symptomatic treatment of allergies and include antihistamines, decongestants, leukotriene receptor blockers and corticosteroids. As aforementioned, histamine release contributes to vasodilation and vascular permeability, for which antihistamines, such as bilastine and cetirizine, are commonly used to block [10-11]. Leukotrienes contribute to nasal congestion and bronchoconstriction, and can be countered using antagonists such as

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montelukast, zafirlukast and pranlukast [12]. Corticosteroids have pronounced anti-inflammatory effects and can also be used to treat allergic symptoms [13]. As all the aforementioned therapies target symptomatic relief, specific immunotherapy has been proposed for immune-modulation [14,15]. Specific immunotherapy consists of the introduction of a large bolus of allergen with the goal of rendering an individual immuno-tolerant [16].

An allergen introduced in the GIT can contribute to important allergic conditions, arising due to activity of the immune cells of the GIT. It is also important to note that the gut microbiota plays a part in immunomodulation and in maintaining homeostasis of the commensal gut microbiota. The importance of gut-associated immunomodulation in allergic disorders is discussed in later sections.

THE HUMAN GASTROINTESTINAL TRACT

The human GIT consists of all the structures from the mouth to the anus, although this review will focus on the intestines. The GIT begins at the mouth and is followed by the esophagus and pharynx, which provide a pathway to the stomach. The functions of the stomach are to store, dissolve, partially digest and regulate the intake of food to the small intestine. Most of the absorption and digestion occur in the small intestine. The small intestine is followed by the large intestine which is subdivided into the ascending, transverse and descending colon. The large intestine is responsible for the storage and concentration of undigested matter and salt and water absorption, as well as being inhabited by a large microbial population, termed the gut microbiota. The luminal side of the intestinal wall has a highly convoluted structure made up of villi and microvilli, termed the brush border [17]. The intestinal wall tissue can be subdivided into four concentric layers. 1) The mucosa, the layer of the GIT wall that comes into direct contact with the bolus and is responsible for absorption, digestion and secretion. The mucosal layer is made up of the epithelium, the lamina propria and the muscularis mucosa. The epithelial layer is made up of a single layer of cells linked at the luminal surface by tight junctions. The cells at the epithelium include exocrine cells that secrete mucus into the GIT lumen, endocrine cells that release hormones into the blood and M (multi-fenestrated) cells found in the follicle-associated epithelium of the Peyer's patches [2]. Peyer's patches are important for immune surveillance, with microorganisms and antigens encountering macrophages, dendritic cells, B cells and T cells in these aggregated lymphoid nodules. 2) The submucosa, connective tissue below the mucosa, contains nerves (submucosal plexus), blood vessels and lymphatic vessels. 3) Below the submucosa there is a smooth muscle layer termed the muscularis externa, responsible for the movement and mixing of GIT contents. 4) The serosa, a layer of connective tissue supports the GIT in the abdominal cavity [17]. The localization and structure of the GIT is closely associated with its immune system.

THE INTESTINAL IMMUNE SYSTEM IN ALLERGIES, THE ROLE OF THE INTESTINAL MICROBIOTA AND PROBIOTIC BIOTHERAPEUTICS

The immune system, under normal conditions, has evolved to avoid responses to benign food and bacterial antigens, while maintaining the capability to detect and kill pathogenic organisms attempting to gain entry through the gut. Part of this immune modulation arises from the gut microbiota, made up of approximately 10^{14} commensal microorganisms, detailed in the next section [318]. Figure 1a provides a schematic representation of the GIT's immune system, also termed GALT. GALT belongs to the mucosa-associated lymphoid tissue (MALT). The importance of the GALT is highlighted by the fact that the intestine holds the largest mass of lymphoid tissue in the human body, containing 10^6 lymphocytes/g of tissue [19]. Furthermore, as much as 60% of the total immunoglobulins produced by the immune system are secreted into the GIT [19]. The tonsils, adenoids and Peyer's patches are the major com-

ponents of the GALT. A review by McKay and Perdue [20] describes the organization and localization of the immune cells of the GIT. The GALT is made up of organized lymphoid tissues, such as the mesenteric lymph nodes and Peyer's patches [2]. On the other hand, the GALT is also comprised of scattered lymphocytes in the intestinal lamina propria and gut epithelium, with the presence of IgA^+ plasmablasts for protection against environmental microbes [21]. Both CD4^+ and CD8^+ T-lymphocytes are present in the GALT, each with their own cytokine profiles and functions. The lamina propria T-lymphocytes are mainly of the CD4^+ phenotype with inducer functions [22]. Conversely, the intra-epithelial T-lymphocytes are mainly of the CD8^+ phenotype with cytotoxic functions [22]. The lamina propria of the GALT also houses IgA producing B-lymphocytes, specifically memory and plasmocytes [19]. Finally, the lamina propria also harbors granulocytes and mast cells, the main players of the immune system in allergies.

The primary cells associated with hypersensitivity reactions, leading to an immune response, are the mast cells and basophils. The amplified response is mediated by platelets, neutrophils and eosinophils [2]. The immune response to an allergen is mediated primarily by IgE , which is preferentially produced by some individuals when exposed to certain antigens, termed allergens. IgE is a dimeric antibody important in mounting an immune response to parasitic organisms, but also plays an essential role in type I hypersensitivity, as it is tightly bound to mast-cell surfaces through $\text{Fc}\epsilon\text{RI}$, a high-affinity IgE receptor [2]. It is to be noted that epidermal Langerhans cells, basophils and eosinophils also express $\text{Fc}\epsilon\text{RI}$ on their surfaces and, hence, may also contribute to type I hypersensitivity responses [23]. Two main components contribute to the over-production of IgE : 1) signals that lead to the differentiation of naive T helper ($\text{T}_\text{H}0$) cells to Type 2 ($\text{T}_\text{H}2$) cells and 2) $\text{T}_\text{H}2$ activity and cytokine action on B cells to switch to IgE production [224]. Interleukin-4 (IL-4) is directly associated with allergies, contributing to the differentiation of CD4^+ T-cells to a $\text{T}_\text{H}2$ phenotype and the induction of B-cell class-switching to IgE production [25]. Along with an increased number of $\text{T}_\text{H}2$ cells, there is evidence of a lower number of Type 1 helper ($\text{T}_\text{H}1$) cells producing interferon- γ ($\text{IFN-}\gamma$) to decrease IgE production. Not surprisingly, it has been shown, by a number of research groups, that a deficiency in $\text{IFN-}\gamma$ production is a predictor of atopic diseases [26-30].

T regulatory (Treg) cells also play a role in suppressing the pro-allergic $\text{T}_\text{H}2$ cell responses. The T cell responses in allergic diseases are described in depth in a recent review by Islam and Luster [31]. The secretion of IL-5 and IL-13 , secreted primarily by $\text{T}_\text{H}2$ cells also favor IgE class-switching and proallergic responses [32]. The binding of allergens to IgE leads to cross-linking of the $\text{Fc}\epsilon\text{RI}$ receptor, triggering allergic responses and the release of histamines, leukotrienes, prostaglandins and interleukins (ILs). On mast cells, cross-linking of the $\text{Fc}\epsilon\text{RI}$ receptor leads to mast cell activation, initialized by a Ca^{2+} cytoplasmic influx that promotes degranulation and release of hypersensitivity mediators, as described in a recent review by Galli and Tsai [33]. The immune reaction is further propagated by the platelet activation factor that leads to the aggregation of platelets and release of heparin, vasoactive amines and histamine [34]. Neutrophil and eosinophil chemotactic factors are also secreted to attract neutrophils and eosinophils that release necrotic enzymes. The responses triggered by the immune cascade can result in mucus secretion, bronchoconstriction, vasodilation, vascular permeability, edema and pain and may involve the skin, eyes, nasopharynx, bronchopulmonary tissues and the GIT.

Keeping the characteristics of the allergic immune response in mind, it is clear that the nature of an individual's reaction to an innocuous antigen is directly correlated with the T-cell response balance. This balance can be linked to both genetic predisposition and environmental factors encompassing previous exposure to antigens. At birth, due to high levels of $\text{T}_\text{H}2$ cytokines at the fetomaternal interface, the T population is skewed towards the $\text{T}_\text{H}2$

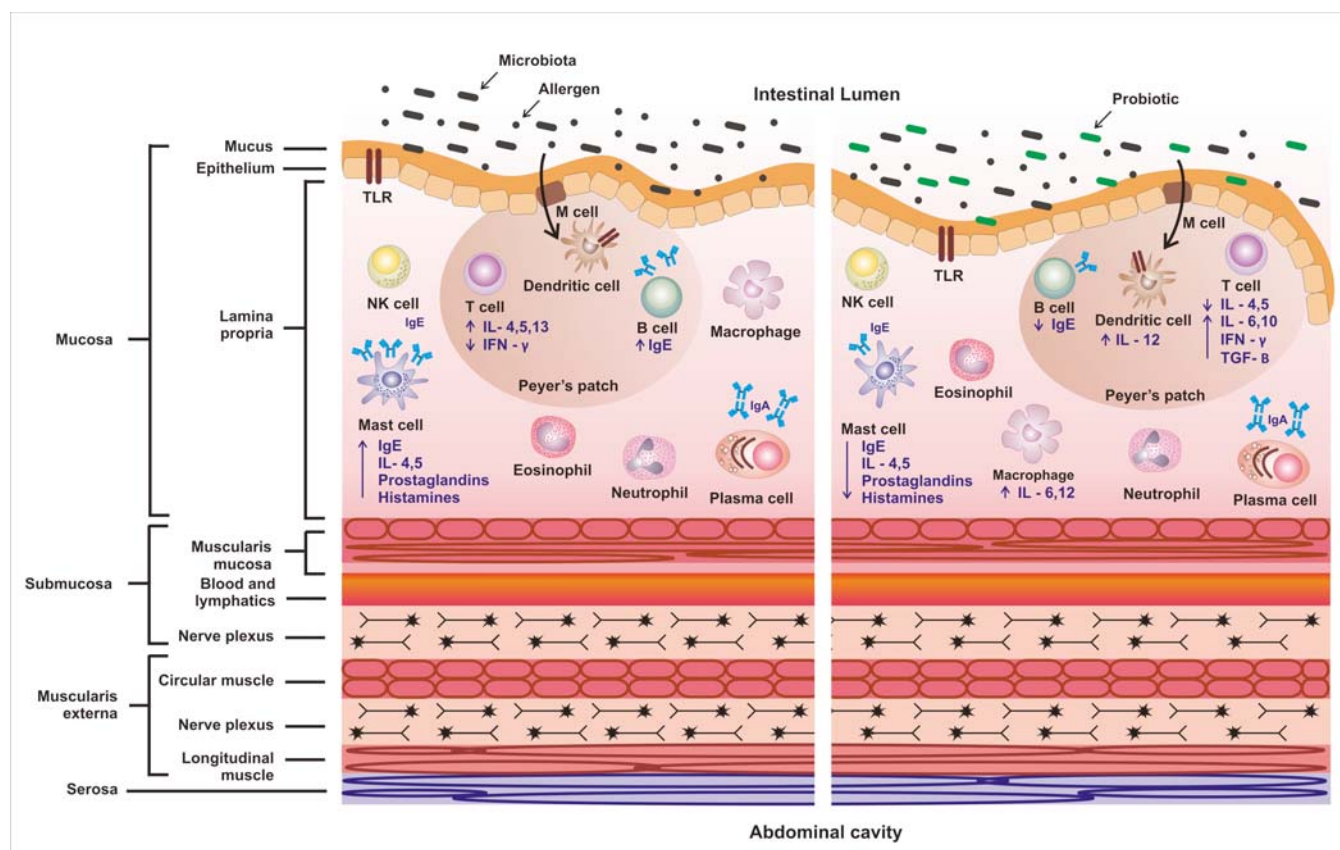


Fig. (1). Localization of the components of the GALT and the interaction of commensal intestinal bacteria with the immune system of the GIT. **(A)** The interaction of allergens with the GALT leads to the secretion of pro- T_H2 cytokines and the release of hypersensitivity mediators, notably prostaglandins and histamines by the mast cells of the lamina propria. **(B)** The oral delivery of probiotic bacterial cells GIT acts to mediate the responses elicited by allergens, leading to a shift away from T_H2 cytokines and a decrease in the secretion of hypersensitivity mediators.

phenotype, promoting the development of hypersensitivity responses [35]. Throughout early childhood, proper immune/antigenic stimulation can contribute to the development of an immune response that is in homeostasis, with decreased hypersensitivity. An important contributor to antigenic stimulation is provided by the bacteria that colonize the GIT of humans shortly after birth, termed the gut microbiota. It is estimated that the human gut microbiota harbors 10^{14} bacterial cells, ten-fold the number of human cells composing the body [36]. The majority of those microorganisms reside in the colon, with a density of 10^{11} - 10^{12} cells per gram of luminal content [37]. The GIT is sterile at birth with microbial colonization taking place by a succession of bacterial populations [38-39]. Most of the intestinal bacteria belong to the *Bacteroidetes*, *Firmicutes* and *Actinobacteria* phyla, with an estimated 1000-1150 different species [38-41]. The adult gut microbiota composition is relatively stable over time and highly variable between individuals [40]. In addition, it differs along the length (e.g., intestinal compartments) and width (e.g., luminal vs. mucosal microhabitats) of the GIT [40]. A number of factors shape the gut microbiota, including host factors (pH, bile salts, and genetics), non-host factors (diet, medication, route of delivery, and environmental factors), and bacterial factors (genetics, cell-adhesion, stress resistance and metabolic capacity) [37-39].

There is important evidence demonstrating that allergic patients have an abnormal gut microbiota. As early as the 1980s, investigators suggested that infants with dermatological syndrome caused by food allergy harboured an abnormal gut microbiota, characterized with a deficiency in *Bifidobacteria* and *Lactobacilli* and an increase

of *Enterobacteriaceae* [42]. Several studies show a lower prevalence of *Bifidobacterium* and *Bacteroides* sp. and higher counts of coliforms, *Staphylococcus aureus* and *Clostridia* in allergic infants [43-47]. Another study reported lower levels of *Bifidobacterium adolescentis* and *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, and *Lactobacillus casei* in fecal samples from allergic children [48]. However, there remains skepticism regarding the association between members of the gut microbiota and allergy, with large European prospective studies reporting no association [49-50]. On the other hand, lower microbial diversity has been observed in allergic patients, as well as a change in metabolic end-products such as short-chain fatty acids originating from the gut microbiota [51-54].

Procedures using gnotobiotic, germ-free (GF), depleted microbiota and conventionalized animals, allow for the exploration of the interactions between the gut microbiota, the GALT and the systemic immune system. GF animals display poorly developed Peyer's patches, lymph nodes and lymphoid follicles and reduced Treg activity [55-58]. Interestingly, immunological abnormalities observed in GF animals can be partly reversed with microbial antigenic stimulation in early life. In the early 1970s, investigators observed that the lymph nodes, spleen and Peyer's patches became more developed in GF mice exposed to antigenic stimuli, coincident with blood antibody levels [55]. Peyer's patches were activated if antigen was absorbed from the intestinal lumen into the circulation, but not if the antigen was administered parenterally. Other researchers demonstrated that specific members of the commensal gut microbiota such as, *Bacteroides fragilis* and *Bacillus subtilis*, drive GALT development and antibody somatic diversification

[59]. Another study, using conventionalized mice, demonstrated that *B. fragilis* stimulates the development of secondary lymphoid structures and modulates the T_H1/T_H2 balance [60]. Furthermore, it was recently demonstrated that the colonization of neonatal-mice with a conventional microbiota protected the animals from developing experimental allergy [61]. Similarly, a recent study using GF mice colonized by oral gavage of caecal content or by co-housing with specific pathogen-free mice confirmed the importance of gut microbiota colonization timing, immediately after birth, for directing the future host immune profile [62]. Macpherson and Harris [63] have postulated that soluble breakdown products from microorganisms are responsible for the development of the immune system. Molecular mechanisms responsible for the development of the immune system involve numerous microbe-associated molecular patterns (MAMPs) including bacterial cell wall conserved elements, such as lipopolysaccharides, lipoteichoic acid and peptidoglycan, and unmethylated CpG motifs characteristic of bacterial DNA [64–67]. Immune recognition of MAMPs is achieved predominantly by Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain molecules. This triggers the production of inflammatory cytokines and chemokines, and the expression of co-stimulatory molecules involved in innate and adaptive immunity, described by Lavelle *et al.* [68]. Detailed molecular mechanisms are discussed in a recent paper [69]. It appears that neonate colonization of the gut microbiota is necessary for the normal development of the GALT and systemic immune system. However, altered colonization of the gut microbiota in early life may disrupt the development of the immune system and initiate the development of allergic diseases.

This is highly consistent with the hygiene hypothesis which proposes that antigen exposure during early life shapes the development of the immune system, lowering the incidence of allergic disease [70]. The hygiene hypothesis further predicts that increased hygienic conditions, the use of antibiotics and sterile food preparation result in the constant isolation of the immune system from positive microbial exposure, thus favouring an increased susceptibility to immune-mediated disorders. This is supported by a large body of epidemiological evidence which demonstrates a direct correlation between the decreasing level of infectious burden and the rising incidence of allergic disorders [71–73]. Taken together, it is clear that an imbalanced gut microbiota—characterized by abnormal bacterial communities and metabolic end products—may contribute to the development of allergic diseases.

The interest in modulating the human gut microbiota with probiotic biotherapeutics has received much attention for the prevention and/or treatment of allergic diseases. Probiotics are “live microorganisms which when consumed in adequate amounts confer a health benefit on the host” [74–75]. Probiotic bacteria are orally ingested and transit through the GI tract to the colon. The most popular probiotic bacteria include Lactobacilli and Bifidobacteria, natural commensals of the gut microbiota [39–41]. A number of clinical trials have documented the change induced by probiotic bacteria on the composition of the gut microbiota in allergic patients [76–78]. It is hypothesised that probiotic modulation of the gut microbiota composition is achieved by diverse mechanisms such as, the production of pathogen inhibitory substances, blocking of pathogenic bacteria cell adhesion sites, and nutrient competition and production, as previously described [3]. However, other studies have reported no effect of probiotics on the gut microbiota composition in allergic patients [79–80]. Probiotic bacterial strain-specific effects and age-sensitivity of the patients may account for these discrepancies.

PROBIOTICS AS ALLERGY BIOTHERAPEUTICS AND THEIR ROLE IN IMMUNOREGULATION

As described, probiotic bacteria can colonize the GIT and modulate the composition of the intestinal microbiota. The commensal intestinal bacteria are in constant interaction with the im-

mune system, as described by Macpherson and Harris [63]. In terms of allergies, the immunoregulatory properties of probiotic bacteria, specifically lactic acid bacteria (LAB) can prove beneficial, restoring the immune balance. The mechanism(s) of action of probiotics are numerous, with each strain potentially affecting the host differently [81]. Current evidence demonstrates that probiotic bacteria can, indeed, act to rebalance the immune response away from pro-allergic T_H2 cell-driven reactions. Probiotic microorganisms can achieve this by promoting the action of two different T cell subsets: T_H1 and Treg [82]. LAB can also influence the production of a number of markers associated with allergy, including IgE and IL-4. The immunoregulatory properties of probiotic bacteria are described here.

A number of probiotic LAB and yogurt-derived bacteria have demonstrated the capacity to enhance IFN- γ and IFN- α production. IFN- γ , in high enough levels, can limit the over-expression of T_H2 responses including IL-4 secretion, in turn decreasing immunoglobulin class-switching to IgE production and allergic responses. Hence, low levels of IFN- γ are a predictor of atopic diseases [26–30]. Early research demonstrated that live yogurt bacteria can increase the production of IFN- γ in mice [83–84]. Shida *et al.* [85], also demonstrated that *Lactobacillus casei* could induce the secretion of IFN- γ in stimulated splenocytes. In addition, mouse intraperitoneal injection with yogurt-derived *Lactobacillus bulgaricus* stimulated the production of IFN- α and IFN- γ [86]. Early research also demonstrated that the consumption of *L. bulgaricus* by humans increased the activity of blood mononuclear cell pro-interferon enzyme, 2-5A synthetase [87–88]. Research by Pohjavuori *et al.* [89] demonstrated that *L. rhamnosus* GG, orally-administered to infants, increased IFN- γ production. It is to be noted that this result appeared to be strain specific, as other strains tested in the trial influenced the immune response differently [89]. Kishi *et al.* [90] also demonstrated an increased IFN- α production in humans, following administration of live *Lactobacillus brevis* subsp. *coagulans*, especially in subjects with low initial levels of IFN- α . Similarly, Arunachalam *et al.* [91] demonstrated that *Bifidobacterium lactis* consumption, in humans, enhanced IFN- α production by peripheral blood mononuclear cells (PBMCs). In more recent research, *Lactococcus lactis* and *Lactobacillus plantarum* were shown to increase IFN- γ production by murine spleen cells, in culture, and following *in vivo* intranasal application [92]. Kim *et al.* [93] demonstrated an increase in IFN- γ production by ovalbumin-induced mice administered *Lactobacillus acidophilus* and *B. lactis*. The exact mechanism(s) of action by which interferon levels are up-regulated following administration of LAB remains unclear. However, as aforementioned, yogurt-derived Lactobacilli can increase 2-5A synthetase activity originating from PBMCs [87]. This would imply the presence of bacterial-leukocyte interactions.

Probiotic bacterial strains have also been shown to induce the secretion of IL-12, a pro-interferon monokine produced primarily by dendritic cells and macrophages. IL-12 promotes the differentiation of T_H0 to T_H1 cells and increases natural killer cell activity [94]. This, in turn, promotes the secretion of IFN- γ , further promoting a T_H1 over a proallergic T_H2 response. Early research, by Miettinen *et al.* [95], demonstrated that LAB can induce the secretion of IL-12 by human PBMCs. Similarly, *L. casei* was shown, by Shida *et al.*, to inhibit IgE production by inducing IL-12 secretion [85]. More recently, Niers *et al.* [96] identified LAB strains capable of inducing the production of IL-12 by PBMCs. Moreover, research by Meijerink *et al.* screened 28 strains of Lactobacilli and Bifidobacterium, *in vitro*, using PBMCs [97]. A number of the LAB strains were shown to greatly induce the secretion of IL-12, but in a strain dependent manner [97]. Despite results demonstrating an increase in IL-12 secretion by LAB strains, different Lactobacilli appear to modulate dendritic cell markers, such as IL-12, in a strain-dependent fashion, as observed by Christensen *et al.* [98].

LAB have also shown the capability to enhance the secretion of IL-10, an anti-inflammatory cytokine. The potential role of IL-10 in allergies has been described by Hawrylowicz and O'Garra [99]. Specifically, IL-10 has been shown to inhibit the activation and cytokine secretion of mast cells and eosinophils [100-101]. It can also inhibit the activation of T_H2 cells [102] and the secretion of IgE [103] while promoting the induction of IL-10-secreting Tregs [104]. Hence, increased IL-10 secretion, induced by LAB, can shift away from an allergic response mediated by mast cells and T_H2 cells. Indeed, Di Giacinto *et al.* [105] demonstrated that administration of the probiotic formulation, VSL#3, induced IL-10 secretion in mice. In another study, Lammers *et al.* [106] investigated the use of Bifidobacterium genomic DNA on PBMCs isolated from healthy humans. Interestingly, probiotic genomic DNA, alone, induced the secretion of IL-10 by PBMCs [106]. In addition, *Lactobacillus reuteri* administration to pregnant women elevated the levels of IL-10 in colostrum [107]. Research previously described, also demonstrated a LAB induction of IL-10 secretion by PBMCs [96]. Similarly, research has also identified a number of LAB strains capable of inducing IL-10, in a strain dependent manner, *in vitro* using PBMCs [97]. An increase in IL-10 production in ovalbumin-induced mice administered *L. acidophilus* and *B. lactis* has also been demonstrated [93]. In addition, recent research has highlighted IL-10 induction by *L. casei*, associated with significant anti-inflammatory properties [108-109]. VSL#3 was also shown to be a potent inducer of dendritic cell IL-10 secretion [110]. Kwon *et al.* [111] demonstrated that increased IL-10 secretion by probiotic bacteria (*L. acidophilus*, *L. casei*, *L. reuteri*, *Bifidobacterium bifidum* and *Streptococcus thermophilus*) suppresses experimental immune disorders, including atopic dermatitis, in mice.

Certain LAB have also been shown to reduce the levels of the T_H2 cytokines IL-4 and IL-5. IL-4 is a cytokine that influences the differentiation of T_H0 cells to the pro-allergic T_H2 cells which then further secrete IL-4 [112]. The initial IL-4 secretion is postulated to come from basophils and mast cells [113]. IL-5, often co-expressed with IL-4, is produced by T_H2 , mast cells and eosinophils. Its role is to stimulate B cell growth and immunoglobulin secretion. IL-4 and IL-5 both promote IgE production by B-cells and, so, probiotic reduction of IL-4 and IL-5 levels could reduce the T_H2 response and control Type I hypersensitivity reactions. Indeed, early research by Shida *et al.* demonstrated the ability of *L. casei* to inhibit IL-4 and IL-5 secretion by splenocytes [85]. This inhibition was shown to be IL-12 dependent, and resulted in a decreased IgE production [85]. Similarly, Pochard *et al.* [114] demonstrated that LAB (*L. plantarum*, *L. lactis*, *L. casei* and *L. rhamnosus*) can inhibit the secretion of IL-4 and IL-5 using PBMCs from patients allergic to house dust mite. In addition, Sutas *et al.* [115] demonstrated that *L. rhamnosus*-degraded casein could down-regulate IL-4 production by PBMCs. Furthermore, significantly lower levels of IL-4 in ovalbumin-induced mice treated with *L. acidophilus* and *B. lactis* have also been demonstrated [93]. In a study previously described concerning the modulation of IL-12, a decrease in the production of IL-4 and IL-5 by splenocytes exposed to *L. casei* has been observed [97]. As well, Ohno *et al.* [116] demonstrated a lower production of IL-4 and IL-5 from splenocytes of mice given *B. bifidum*, suppressing IgE production.

Researchers have also hypothesized that probiotics may alleviate allergic reactions by stimulating TLRs. Stimulation of TLRs leads to the production of IL-6, which further induces B cell immunoglobulin class-switching to IgA production, as well as IL-12 production by dendritic cells [117]. Research has already demonstrated that the administration of TLR agonists may prevent the onset of allergies [118-120]. Hence, probiotic stimulation of TLRs could prove beneficial for the modulation of Type I hypersensitivity. Indeed, Hoarau *et al.* [121] investigated the effects of *Bifidobacterium breve* supernatant on human dendritic cells. *B. breve* was shown to activate TLR-2 and induce IL-10 secretion, skewing

the immune response towards a regulatory phenotype [121]. Research by Forsythe *et al.* [122] demonstrated that *L. reuteri* attenuated asthmatic responses in a mouse model of allergic airway inflammation. The effect of *L. reuteri* was shown to be dependent on TLR-9, for mediation of the anti-inflammatory responses [122]. Similarly, anti-inflammatory effects of VSL#3 via TLR-9 were observed by Rachimlewitz *et al.* [123]. Koizumi *et al.* [124] demonstrated IFN- γ and IL-12 production by *Lactobacillus pentosus* by spleen cells in a TLR-2/TLR-4-dependent manner. Likewise, in a dust mite allergy mouse model, *L. plantarum* reduced the allergic response through a TLR-2-dependent pathway which led to the production of IL-12 and IL-10 [125].

The probiotic modulation of the above markers plays a key role in maintaining a balance between T_H1 and T_H2 responses, a determinant in the prevention and treatment of allergic disorders, summarized in (Fig. 1b) [126]. Many more markers are involved in this immunomodulation, as this is a complex cascade of interdependent events. It is also important to note that the immunomodulation by probiotic bacteria is strain specific, with some LAB strains having completely opposing effects on immune responses [98].

Probiotic treatment for allergic rhinitis has proven successful in a number of published studies, using a number of different probiotic bacterial strains, as seen in Table 1 [127]. For example, many research groups have demonstrated that the long-term consumption of milk containing *L. casei* can improve the health status of patients with allergic rhinitis [128-130]. A study with the same probiotic, however, showed no effect on asthma [128]. In a similar clinical trial, Ciprandi *et al.* [131] concluded that the consumption of *Bacillus clausii* can modulate allergic rhinitis, reduce eosinophil activity and relieve nasal symptoms. In addition, Ishida *et al.* [132] demonstrated that *L. acidophilus* can relieve perennial allergic rhinitis and pollen allergies. Research by different groups also demonstrated that *L. paracasei* can improve the quality of life of patients with allergic rhinitis [133-135]. *B. bifidum*, in guinea pigs, was also shown to alleviate nasal reactions associated with allergic rhinitis [136]. In terms of a cocktail formulation of probiotic bacteria, Ouwehand *et al.* [76] demonstrated that a combination of *L. acidophilus* and *B. lactis*, administered to children, could alleviate allergic rhinitis symptoms. On the other hand, research by Lue *et al.* investigated the administration of a probiotic bacterium, *Lactobacillus johnsonii*, in combination with standard levocetirizine antihistamine therapy in children [137]. The probiotic combination therapy with the antihistamine was shown to be far more effective than treatment with antihistamine alone [137].

Probiotic bacteria have also shown great interest for the treatment and prevention of atopic diseases, as summarized in Table 2. The first study to report an effect of probiotics on atopic disease was by Kalliomaki *et al.* [138]. *L. rhamnosus*, administered prenatally to mothers and postnatally to infants decreased the frequency of atopic eczema compared to the control group [138]. This research was supported by Wickens *et al.* who demonstrated that *L. rhamnosus* reduced the prevalence of eczema [139]. Similarly, research by Isolauri *et al.* [140] demonstrated that administration of *L. rhamnosus* or *B. lactis*, to infants with atopic disease, greatly alleviated allergic inflammation and the signs and symptoms of atopic eczema. In addition, *B. bifidum* and *B. lactis*, administered to high-risk infants, had preventive effects on eczema but not on atopic eczema [141]. However, another research group performed a similar trial and saw no effect on atopic disease frequency, suggesting that *L. rhamnosus* may only be effective in some children [142]. Interestingly, administration of a probiotic mixture of *L. rhamnosus*, *B. breve* and *Propionibacterium freudenreichii* to pregnant women, and, subsequently, their infants, resulted in allergy prevention in caesarean-delivered children only [143]. These results suggest that infants with an absent microbial load, due to caesarean delivery, may benefit from probiotic supplementation to prevent the development of atopic diseases. *L. fermentum* and *L. reuteri* were

Table 1. Existing probiotic formulations for allergic rhinitis.

Probiotic	Treatment Duration	Carrier	Reference
<i>L. rhamnosus</i>	30 days	Whey formula	[170]
<i>L. paracasei</i>	30 days	Fermented milk	[134]
<i>L. casei</i>	360 days	Fermented milk	[128]
<i>L. paracasei</i>	30 days	Capsule	[133]
<i>L. acidophilus</i>	56 days	Fermented milk	[132]
<i>L. casei</i>	150 days	Milk	[130]
<i>B. longum</i>	91 days	Yoghurt	[171]
<i>L. reuteri</i>	9 days	MRS broth	[122]
<i>L. paracasei</i>	28 days	Fermented milk	[135]
<i>B. bifidum</i>	49 days	Saline	[136]
<i>L. johnsonii</i>	84 days	Capsule	[137]
<i>L. casei</i>	84 days	Fermented milk	[129]
<i>L. acidophilus</i>	120 days	Capsule	[76]
<i>B. clausii</i>	21 days	-	[131]

Table 2. Existing probiotic formulations for atopic disorders.

Probiotic	Treatment Duration	Carrier	Reference
<i>L. rhamnosus</i>	30 days	Whey formula	[170]
	14-28 days	Capsule	[138]
	60 days	Whey formula	[140]
	118 days	-	[172]
	28 days	Capsule	[173]
	7 days	Saline	[174]
	Mother -28 days Newborn-180 days	Capsule	[175]
	52 days	Whey formula	[154]
	208 days	Capsule	[176]
	208 days	Capsule	[177]
	28-42 days	Capsule	[178]
	Mother-215-229 days Newborn- 720 days	Capsule	[139]
<i>B. lactis</i>	60 days	Whey formula	[140]
<i>L. reuteri</i>	28-42 days	Oil	[179]
<i>Escherichia coli</i>	7-28 days	-	[145]
<i>L. fermentum</i>	56 days	Capsule	[144]
<i>L. paracasei</i>	120-390 days	Cereal	[180]

(Table 2) Contd....

Probiotic	Treatment Duration	Carrier	Reference
<i>L. rhamnosus</i> <i>B. breve</i> <i>P. freudenreichii</i>	Mother-28-42 days Newborn- 182 days	Capsule	[143]
<i>L. rhamnosus</i> <i>B. lactis</i>	126 days	Powder/Capsule	[181]
<i>L. rhamnosus</i> <i>L. reuteri</i>	140 days	Powder	[182]
<i>B. bifidum</i> <i>B. lactis</i> <i>L. lactis</i>	Mother -180 days Newborn - 360days	Freeze-dried powder	[141]
<i>L. casei</i> <i>L. paracasei</i>	-	Heat inactivated	[148]

also shown to decrease the severity of atopic disease when administered to young children with moderate and severe disease [144-145].

Probiotic therapeutics have also shown interest for the management of food allergies, as seen in Table 3. Meijerink *et al.* [97] investigated 28 probiotic strains for their capability to modulate food, specifically peanut, allergies using human PBMCs. As aforementioned, a number of probiotic bacteria, in strain-dependent manner, modulated inflammatory markers associated with allergies. Three probiotic bacteria—*Lactobacillus salivarius*, *L. plantarum* and *L. casei*—were tested *in vivo* in a peanut-sensitized mouse model [97]. *L. casei* and *L. salivarius* demonstrated partial protection in a food allergy mouse model [97]. Kim *et al.* administered *B. lactis* and *L. acidophilus* to an ovalbumin-induced allergic mouse model [93]. The results indicated that these two probiotic bacteria may be useful for the prevention of food allergy, with decreased levels of allergy-associated inflammatory markers [93]. Schiavi *et al.* [146] demonstrated that probiotic treatment with VSL#3 could reduce anaphylaxis symptoms in mice sensitized with peanut extract, using both prevention and treatment approaches. The same research group also demonstrated that VSL#3 could protect against anaphylactic reactions induced by the allergen, shrimp tropomyosin, in a mouse model of food allergy [147].

It is clear, from the presented results, that probiotic formulations show great potential for the treatment and prevention of a number of allergic disorders. Unfortunately, these results remain preliminary, with strain-specific results and results that are not necessarily reproduced by other research groups in differing conditions. Further optimization of the probiotic therapeutics is, hence, necessary. We propose, in the following section, one possibility for the development of more efficient probiotic formulations for the treatment and prevention of allergies.

MICROENCAPSULATED PROBIOTICS FOR THE TREATMENT AND PREVENTION OF ALLERGIES

Probiotic formulations are proving promising for the treatment and prevention of allergies. Research by a number of groups has demonstrated the use of dead bacterial cells or bacterial cell components for the treatment and prevention of allergies [106-148-150]. Inactivated bacteria are often favorable due to their longer shelf-life and lower safety risks [151]. However, as the definition confirms, the term probiotic implies the use of viable microorganisms and viability is a criteria that needs to be met to be termed a probiotic [74-75-152]. Furthermore, research demonstrates that some cells can

only inflict their effects if viable. For example, research by Kaila *et al.* demonstrated that the administration of viable *L. casei* GG promoted a greater immune response to rotavirus than inactivated bacteria [153]. In terms of anti-allergy therapeutics, research by Kirjavainen *et al.* [154] investigated the efficacy of heat-inactivated and viable *L. rhamnosus* for the management of atopic disease. They demonstrated that treatment with the heat-inactivated probiotic was inefficient compared to the viable probiotic for management of atopic disease [154]. Forsythe *et al.* [122] also demonstrated the need for viable bacteria as killed organisms were not as effective in attenuating airway hyper-responsiveness as live *L. reuteri*. The requirement for bacterial viability may depend on the mechanism of action of the probiotic and the intended target and application. Adams addresses the paradox of live and dead cells by proposing that live probiotic cells may influence the gastrointestinal microbiota and have an immunomodulatory effect, whereas dead cells can exert anti-inflammatory responses [155].

Keeping the need for viability in mind, probiotic bacteria must be delivered to the target sites in sufficient number and metabolically active to be effective. The target site of orally administered probiotic bacteria is usually the colon, the site of bacterial biomass, described previously. One of the major limitations for the oral delivery of viable bacteria to the lower GIT is the presence of acids and bile, specifically the acidic environment of the stomach. It has been estimated that only 1% of bacteria delivered orally survive the gastric transit, greatly limiting the overall therapeutic effect of any orally-delivered probiotic formulation [156]. Microencapsulation has been proposed as a delivery method to ensure viable probiotic delivery. It is a method defined as the “entrapment of a compound or a system inside a dispersed material for its immobilization, protection, controlled release, structuration and functionalisation” [157]. There exists a number of microcapsule delivery systems that have been proposed for the oral delivery of live bacterial cells, as described extensively in a review by Prakash *et al.* [3]. A number of encapsulation methods involve the use of calcium alginate, a natural polymer. For example, one of the most characterized and commonly used methods of microencapsulation is the alginate-polylysine (PLL)-alginate (APA) microcapsule which has been used for drug, stem cell and bacterial cell delivery [158]. APA microencapsulation, based on a polyelectrolyte complexation reaction, has been shown as capable of delivering viable and metabolically active probiotic bacteria to the lower GIT [159-161]. More recently, genipin-crosslinked-alginate-chitosan GCAC microencapsulation has been developed, with promising results in terms of mechanical

Table 3. Existing probiotic formulations for food allergies.

Probiotic	Treatment Duration	Carrier	Reference
<i>Bifidobacterium animalis</i> <i>B. lactis</i> <i>B. longum</i> <i>L. acidophilus</i> <i>L. casei</i> <i>L. fermentum</i> <i>Lactobacillus gasseri</i> <i>L. johnsonii</i> <i>L. plantarum</i> <i>L. reuteri</i> <i>L. rhamnosus</i> <i>L. salivarius</i>	31 days	Free in sodium bicarbonate solution	[97]
<i>B. lactis</i> <i>L. acidophilus</i>	49 days	Freeze-dried powder	[93]
<i>L. casei</i> <i>L. plantarum</i> <i>L. acidophilus</i> <i>Lactobacillus delbrueckii</i> <i>B. longum</i> <i>B. breve</i> <i>B. infantis</i> <i>S. salivarius</i>	20 days	Freeze-dried powder	[147]

stability and pH resistance [162]. Work is ongoing with respect to the development and optimization of microcapsule methods capable of delivering viable and metabolically active probiotic bacteria to desired target sites.

Although no research currently exists on the use of microencapsulated probiotic bacteria in the context of allergies, successes already exist for the use of these formulations in other disease applications. These successes include applications for the treatment and prevention of renal diseases [163-165], colon cancer [166], hypercholesterolemia [161-167] and metabolic syndrome [168]. Microencapsulation has great potential for other disease applications, including for the management of hypersensitivity reactions. With the current success demonstrated by probiotic bacteria in the treatment and prevention of allergies, described above, microencapsulation could provide an important improvement to current bacterial formulations, enhancing their therapeutic effects.

CONCLUSIONS AND FUTURE DIRECTIONS

An allergy involves deregulation of the immune system. A thorough understanding of the pathophysiology of allergies can enable for the development of an effective therapeutic. As described, various methods are available for the symptomatic relief of allergies/hypersensitivity reactions; however, there is limited success in treating the etiology. Specific immunotherapy is the only approach, to date, which targets the deregulation of the immune system with an aim to establish immune tolerance to innocuous substances. As aforementioned, the human gut houses the largest lymphoid tissues, the GALT, which plays a crucial role in the development of hypersensitivity reactions. The gut microbiota is also

involved in the development and progression of allergies with lactic acid bacteria showing great potential for the modulation of hypersensitivity responses. Probiotic bacteria have demonstrated the potential to up-regulate T_H1 responses while down-regulating T_H2 responses, pivotal in the development of hypersensitivity reactions. In addition, an increase in $IFN-\gamma$ and $IFN-\alpha$ production, needed for T_H1 expression to rebalance the immune system, can be elicited by probiotic bacteria. Many other markers of the immune system may also be modulated by probiotics. A number of trials involving probiotics have also proven successful for the treatment of hypersensitivity reactions, as described in this review.

Despite the great potential of probiotic formulations, there remain a number of obstacles to surpass for probiotics to be effective therapeutics for allergies. A recent review by Jeurink *et al.* highlights the many challenges associated with the use of prebiotics and probiotics as therapeutics in allergic diseases [169]. As highlighted in this paper, there is a requirement for a method to deliver a high number of viable cells to the colon. Microencapsulation, having proven successful in the treatment of a number of disorders, is proposed as a carrier system for the development of an allergy biotherapeutic. In addition, studies have demonstrated that the anti-allergic effects are species- and strain-specific, suggesting the need for proper screening and strain selection methods. Future work needs to rely on proper animal and clinical trials investigating safety, mechanism of actions and efficacy for the different allergic phenotypes. Despite the obstacles that still remain, it is clear that probiotic bacteria show great potential for the development of a successful allergy biotherapeutic.

CONFLICT OF INTEREST

The authors report no financial or other conflict of interest relevant to the subject of this article.

ACKNOWLEDGEMENTS

The authors would like to acknowledge a Canadian Institute of Health Research (CIHR) Grant (MPO 64308) and grants from Micropharma Ltd. to Dr. S. Prakash, a Doctoral Alexander Graham Bell Canada Graduate Scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC) to C. Tomaro-Duchesneau and Fonds de recherche du Québec-Santé (FRSQ) Doctoral awards to L. Rodes and M. Malhotra.

LIST OF ABBREVIATIONS

APA	=	Alginate-polylysine-alginate
GALT	=	Gut-associated lympho reticular tissue
GF	=	Germ-free
GIT	=	Gastrointestinal tract
IFN- γ	=	Interferon- γ
ILs	=	Interleukins
IL-4	=	Interleukin-4
LAB	=	Lactic acid bacteria
M	=	Multi-fenestrated
MALT	=	Mucosa-associated lymphoid tissue
MAMPs	=	Microbe-associated molecular patterns
PBMCs	=	Peripheral blood mononuclear cells
PLL	=	Polylysine
T _H 0	=	naïve T helper cells
T _H 2	=	Type 2 helper T cells
T _H 1	=	Type 1 helper T cells
Treg	=	Regulatory T cells
TLRs	=	Toll-like receptors

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