

Can Immunoregulatory Lactic Acid Bacteria Be Used as Dietary Supplements to Limit Allergies?

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Key Words

Dietary supplements · Allergy · Intestinal microflora · Lactic acid bacteria · Immunoregulation · Interferon · Interleukin-12

Abstract

Studies in gnotobiotic animals have suggested that the intestinal bacterial flora may play an important role in priming the immune system during ontogeny to limit dysfunctional responses, including allergy. Prospective clinical studies have identified a higher incidence of allergy expression in early childhood among children who have low enteric populations of lactic acid bacteria (LAB), such as lactobacilli and bifidobacteria, further supporting a role for gut-colonizing bacteria in regulating immunological atopy. There is some evidence to suggest that supplementing the human diet with probiotic LAB might combat both allergy development and expression of atopy in allergy sufferers; however, definitive information, in the form of controlled intervention trials, remains scant. Recent immunological evidence has indicated that certain strains of LAB can stimulate the production of type I and II interferons and pro-interferon monokines (IL-12 and IL-18), following contact with the immune sys-

tem; therefore, probiotic forms of immunoregulatory LAB could be used as dietary supplements to modify the gut microflora and provide pro-T helper cell 1 (Th1) STAT-activating signals sufficient to deviate the immune phenotype and correct the Th2-type bias which promotes allergy. This review outlines the clinical and laboratory evidence of a role for LAB in combating allergies, and attempts to explain this phenomenon in terms of our current understanding of immunoregulatory signals produced by gut-colonizing microbes.

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Introduction: Role of the Intestinal Microflora in Systemic Immunoregulation

Over the last decade, increasing research attention has been paid to the role of the intestinal microflora in health. The normal human gut can contain up to 10^{11} commensal bacteria per gram of lower intestinal contents [1], comprising a large microbial ecosystem which is predominated by gram-positive genera, such as the lactic acid bacteria (LAB) lactobacilli and bifidobacteria. Under normal circumstances, the resident gut flora causes neither pathogenesis nor inflammation and instead contributes to gut

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homeostasis and function. Important physiological processes attributed to a properly functioning gut microflora include maintenance of a barrier layer against colonization by pathogens, aiding in nutrient digestion and assimilation and providing immunological surveillance signals at the gut mucosa-lumen interface [2]. This latter mechanism is essential to the maintenance of tolerance to food-borne proteins and in providing down-regulatory cytokine signals (such as TGF β and IL-10) to prevent intestinal inflammation; indeed, elimination of such regulatory control has been shown experimentally to promote uncontrolled gastrointestinal (GI) tract inflammation and lethal colitis, as demonstrated by Kulkarni and Karlsson [3] using transforming growth factor- β (TGF β)-deleted mice. This suggests that the resident intestinal flora is far from being a passive entity in immunological terms.

It has recently been demonstrated that epithelial cells of the gut mucosa, the front line cells that establish intimate contact with intestinal microbes, participate in an intercommunicating cytokine network with leucocytes in response to gut bacteria [4–6]. The interaction of gut-colonizing microbes with the host at this tissue level may be important in the ontogeny of immunological sensitization, and consequently in control of immune development systemically. Studies with gnotobiotic models have indicated that animals raised in a germ-free environment establish poor immunoregulatory control mechanisms, and are prone to diseases associated with immune dysfunctions [7–9]. Experimentally, germ-free mice have been shown to lack tolerance to oral delivery of a T helper (Th) cell-dependent antigen (ovalbumin, OVA), and consequently to develop over-expression of Th2-type immunoresponses to systemic antigen challenge, such as production of IgE/IgG1 class antibodies and elevated production of interleukin-4 (IL-4) [10]. Interestingly, reconstitution of the gnotobiotic GI tract with the LAB strain *Bifidobacterium infantis* was able to restore oral tolerance induction to OVA in mice [10], raising the possibility that microbial signals important in priming the immune system for appropriate immunoregulation could be derived from external (dietary) sources.

Clinical Evidence for a Role of the GI Flora in Combating Allergy

For over a decade now, it has been theorized that appropriate early-life exposure to environmental microbes, including gut-colonizing microbes, is important for preventing the over-expression of allergic-type immu-

noresponses. The central tenet of this paradigm is the so-called ‘Hygiene Hypothesis’, which states that as a consequence of increasingly sterile living environments, antibiotic use and ultra-hygienic food preparation, humans are no longer exposed to appropriate microbial signalling mechanisms in early life, which can lead to poorly developed immunoregulatory mechanisms and over-expression of Th2-type allergic responses [11]. Studies in Scandinavia have drawn comparisons between children raised in comparatively sterile ‘westernized’ environments and those raised in more traditional regions, which report low antibiotic use and consumption of a large proportion of lactobacilli-containing fermented foods. The incidences of skin-test-positive allergies and clinical atopy were found to be lower among children from families who followed a traditional lifestyle [12]. Moreover, the GI tract flora was found to be more frequently colonized by lactobacilli in non-allergic children than in allergic children [13], supporting the notion that appropriate microbial colonization of the gut can lower the risk of developing allergy [14, 15].

Aside from limiting the development of allergies, there is also some evidence that appropriate microbial colonization of the gut can control the over-expression of atopy in allergen-sensitized subjects. Studies by Trapp et al. [16] and van de Water et al. [17] indicated that long-term consumption of live yogurt cultures could reduce circulating levels of IgE and lower the subjective indices of clinical rhinitis among young and elderly volunteer subjects. Controlled intervention studies by Wheeler et al. [18] further indicated a trend toward reduced eosinophilia among adult asthmatics who consumed yogurt containing *Lactobacillus acidophilus*, although this effect was not statistically significant. Majamaa and Isolauri [19] demonstrated that clinical symptoms of atopic eczema could be reduced in nursing infants whose mothers were given the probiotic LAB strain *Lb. rhamnosus* GG, while milk-hypersensitive infants who consumed a hydrolyzed whey formula supplemented with GG exhibited reduced ex vivo indices of GI inflammation; Peltó et al. [20] also detected a decrease in blood cell inflammatory markers among adult food-hypersensitive subjects who consumed milk containing GG.

In general, however, conclusive evidence that gut-colonizing bacteria play an active role in reducing allergy expression among sensitized subjects remains scant. In part, this has been due to a paucity of well-controlled and interpreted clinical studies aimed at determining the clinical impact of dietary LAB [21]. However, there has been considerable interest in this area using animal models,

and in many cases an ability of orally delivered LAB to combat allergies has been linked to the type of immunoregulation exerted by these bacteria.

Probiotic Lactic Acid Bacteria and Immunoregulation

A probiotic can be defined as an orally delivered micro-organism that is able to colonize the human gut, transiently, and exert an influence on host physiology, such that this influence may benefit health. In the context of the foregoing discussion of immunoregulatory signals derived from gut-colonizing bacteria and their potential role in combating allergies, it should be borne in mind that several strains of probiotic LAB have been shown in experimental models, as well as human clinical studies, to stimulate interferon production. Early studies had indicated that spleen cells from mice which had been fed live yogurt cultures exhibited enhanced capacity to secrete interferon- γ (IFN γ) in in vitro culture [22–25]. Furthermore, mice injected with yogurt-derived lactobacilli showed enhanced systemic levels of type I and II interferons (IFN α and IFN γ , respectively) [26]. In humans, circulating levels of the pro-interferon enzyme 2'–5' A synthetase were found to be elevated following consumption of yogurt bacteria [27–29], while the capacity of isolated blood mononuclear cells to secrete IFN α has been shown to be elevated following consumption of defined probiotic *Lactobacillus* or *Bifidobacterium* strains of LAB [30, 31].

Both type I and II interferons have been shown experimentally to limit the over-expression of Th2-type lymphocyte responses. In particular, IFN γ acts antagonistically to the major pro-Th2 cytokine IL-4 by down-regulating IL-4 expression and reducing B cell immunoglobulin isotype switching [32, 33]; IFN α can act as an augmentative signal for IFN γ production, can promote Th1-type immune responses and reduce IgE production [34, 35]. Evidence from animal model studies had indicated that feeding mice with defined strains of LAB can reduce circulating IgE levels and IgE-mediated cutaneous hypersensitivity [36, 37]. Furthermore, oral delivery of some strains of LAB has been shown to enhance the capacity of mitogen-stimulated murine splenic lymphocytes to secrete IFN γ but not IL-4 or IL-5 [37–41], suggesting a pro-Th1 type of immunoregulation.

Further elucidation of the mechanisms by which defined strains of LAB are able to up-regulate interferon production has come from in vitro culture studies. Early reports had indicated that the addition of yogurt-derived

lactobacilli to in vitro cultures of murine splenic leucocytes or human peripheral blood mononuclear cells could increase interferon production and 2'–5' A synthetase activity in response to mitogenic stimulation [27, 42], suggesting that the regulatory mechanisms involved might reflect direct bacterial-leucocyte interactions. Further studies, utilizing splenocytes derived from allergen-primed mice, have demonstrated that the inclusion of LAB cells in allergen-stimulated cell cultures can increase production of IFN γ , but reduces levels of allergen-driven IL-4, IL-5 and IgE [43, 44]. Interestingly, while the LAB-mediated down-regulation of Th2-type responses in allergen-stimulated cell cultures was shown to be dependent on the induction of IFN γ , this effect was found to be entirely dependent on signals generated by adherent accessory cells: in the absence of accessory cells, isolated lymphocytes would not produce IFN γ in the presence of LAB strains, even if stimulated with T cell mitogen [38]. This latter result suggests that pro-interferon factors, induced by microbial contact with accessory cells, might be responsible for anti-allergy immunoregulation induced by LAB.

Evidence of a Role for Pro-Interferon Monokines in Anti-Allergy Immunoregulation

Recent studies have indicated that some food-borne, as well as human gut-derived, LAB strains are potent inducers of pro-interferon monokines, principally IL-12 and IL-18 [45–48]. Co-culture of human or murine leucocytes with defined strains of LAB has been shown to potently induce production of IL-12 and IL-18 [38, 41, 43–47]. In oral feeding studies, strains of *Lactobacillus* have been shown to potentiate the capacity for ex vivo secretion of IL-12 by isolated murine leucocytes [41, 48]; however, to date, there have been no studies on the ability of orally delivered LAB to potentiate IL-12 production in humans.

Pro-interferon factors, particularly IL-12, have the potential to combat allergy expression in two ways. First, by enhancing host capacity to produce IFN γ [49], and second by providing both an effective deviation signal for Th1 cell phenotype expression during the early differentiation of Th0 cells, and by regulating IL-4 production in a manner analogous to IFN γ [50, 51]. Thus, IL-12 is thought to play a critical role in limiting both the establishment and maintenance of Th2-type responses. It is quite possible, therefore, that the potential of dietary sources of LAB to regulate anti-allergy Th2-type immune responses is large-

ly dependent on the ability of these strains to induce expression of IL-12 in host leucocytes. This has been shown experimentally in in vitro studies by Shida et al. [44], who demonstrated that *Lactobacillus casei* cells were able to reduce the capacity for OVA-primed murine splenocytes to secrete allergen-driven IgE, IL-4 and IL-5, but that the inclusion of a neutralizing anti-IL-12 monoclonal antibody could ablate the immunoregulatory action of *Lb. casei* and restore production of the allergenic molecules.

However, it should be realized that not all strains of immunoregulatory probiotic LAB are likely to fulfill the role of pro-Th1/anti-Th2 biological mediators. Some strains of *Bifidobacterium* have been shown to enhance antibody responses at mucosal surfaces following oral delivery [52, 53], while strains of *Lactobacillus* have been shown to potentiate the production of IL-5 in in vitro cell culture [54] and are therefore unlikely to prove effective anti-allergy immunoregulators. Recent studies have suggested that the ability of immunoregulatory strains of LAB to induce pro-Th1-type responses is unlikely to be an exclusive feature; Hesse et al. [46, 47] showed that while certain strains of lactobacilli could induce high levels of pro-Th1 cytokines, even strong stimulators of IL-12 induced some production of the cytokine synthesis inhibitory factor IL-10. But in the context of gut-dwelling microbes that do not initiate pathogenesis, this is a necessary action: excessive production of IL-12 can promote localized cellular infiltration and tissue inflammation in the intestinal epithelium [55, 56], thus down-regulatory LAB-induced factors also play an essential role in regulating host responses.

Signalling Mechanisms of Anti-Allergy Immunoregulatory LAB

Aside from interferons and pro-interferon factors, LAB are also capable of inducing expression of other regulatory cytokines, such as IL-1, IL-6, IL-8 and tumor necrosis factor- α (TNF α) [57–59], but, as previously outlined, excessive tissue responses are ordinarily controlled by the co-induction of auto-regulatory anti-inflammatory signals, such as IL-10 and TGF β . Recent research has indicated that both mononuclear leucocytes and immunocompetent gut epithelial cells are capable of transducing these multiple cytokine signals from contacting LAB [4–6], yet research to identify the factors involved in this signalling has made only limited progress. Initial studies indicated that both live and heat-killed forms of lactoba-

cilli were effective at stimulating interferon production in in vitro cell culture systems [29], suggesting that cell wall structures were probably integral in this mechanism. Indeed, cell wall preparations of LAB have been shown to induce cytokine expression [60–62], although this does not discount a role for further modulation of immune signalling by secretory products. Among the active molecules from gram-positive bacterial walls, lipoteichoic acid, peptidoglycan and muramyl dipeptide have been shown to bind surface pattern recognition receptors (including CD14, Toll-like and scavenger receptors) and to induce activation signals in leucocytes [28, 63–67]. Recent research by Miettinen et al. [68] has indicated that contact between LAB and leucocytes activates the phosphorylation of DNA-binding transcription factors. The probiotic strain *Lb. rhamnosus* GG was shown to be capable of initiating rapid nuclear translocation of NF- κ B, while a more delayed cytokine-mediated activation of STAT 1, STAT 3 and IFN regulatory factor 1 was observed following co-culture of GG with CD14-expressing human mononuclear cells [68]. Thus, immunoregulatory strains of LAB appear capable of modulating cytokine gene expression at the transcriptional level.

But the ability of gene transcriptional modulation to promote anti-allergy immunoregulation, at a systemic level, has yet to be proven as the sole mechanism by which LAB can potentially control allergic immunoresponses. Other mechanisms of action have been demonstrated in in vitro studies, which in the case of food allergy include an ability of LAB to enzymatically hydrolyze food constituent proteins that might ordinarily prove allergenic. This mechanism has been shown to have a dual effect: to reduce the allergenicity of food proteins first, by eliminating B cell epitopes present on macromolecules [69]; second by liberating immunoregulatory peptides from food-borne proteins, which can effectively down-regulate Th2-driven responses to the intact protein [70, 71]. In addition, the role of gut-colonizing LAB in stabilizing the intestinal mucosal barrier has been postulated to prevent food-borne macromolecules passing into direct contact with the immune surveillance system [72], again serving to prevent allergic sensitization to food components.

Summary: Potential Use of Immunoregulatory LAB in Anti-Allergy Dietary Supplements

Despite the proposal of alternative modes of LAB anti-allergy control, the notion of pro-Th1 STAT activation by immunoregulatory LAB at the gut mucosal interface, as

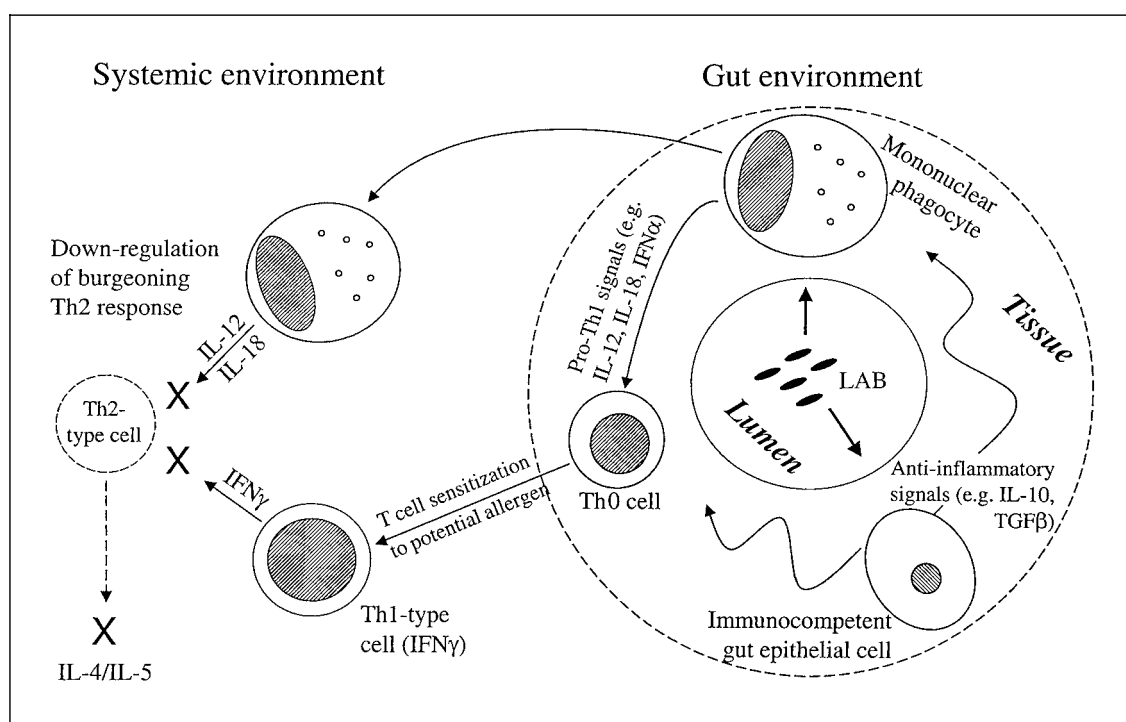


Fig. 1. Theoretical model by which gut-dwelling LAB might provide immunoregulatory signals for pro-Th1 immune deviation, and down-regulation of Th2-type allergic responses. Gram-positive lactic acid bacteria encounter immuno-competent cells in the gut-associated lymphoid tissue regions. Biologically active components of the bacteria (e.g. lipoteichoic acid, peptidoglycan) bind pattern recognition receptors of accessory leucocytes (e.g. mononuclear phagocytes) and induce pro-Th1 cytokine expression (IL-12, IL-18, IFN α) (excessive tissue reactions are controlled by cross-regulatory anti-inflammatory factors, such as IL-10 and TGF β). T lymphocytes encountering potential allergens in the draining lymphatics receive these pro-Th1 signals, and the resulting immune recognition is deviated away from an over-active Th2 phenotype.

depicted in figure 1, represents an attractive model for the potential development of food-based intervention strategies to control or limit allergy. Many of the LAB strains that have been shown to increase systemic level expression of interferons are derived from common dietary foodstuffs (such as yogurt, cheese and fermented vegetables [27–31, 36–44]) and are thus likely to be safe for consumption as probiotic dietary supplements. Nevertheless, safety testing of such strains is still warranted, particularly in light of the evidence from animal models that some strains of LAB can promote strong IL-12 expression, since excessive localized production of this cytokine has been associated with GI inflammatory disease [55, 56].

As to proving clinical efficacy, there is still the need for well-designed and appropriately controlled intervention trials to identify remedial action of LAB against a number of atopic conditions, and moreover to correlate potential improvements with systemic indices of immune devia-

tion (interferon, IL-4 and IL-12 expression and IgE levels) to verify the model of pro-Th1 immunoregulation. In addition, intervention trials to test the 'Hygiene Hypothesis' have not been conducted, but could involve using dietary LAB supplements in 'westernized' communities which report a high incidence of childhood allergy, in order to prove or disprove that colonization of the gut with exogenous bacterial strains can provide the appropriate microbial signals necessary for the ontogeny of the immune system.

Overall, the compelling evidence suggests that appropriate microbial colonization of the gut is important in providing priming signals to prevent the over-expression of Th2-dominated atopic responses. Recent studies have begun to elucidate the differences in signal transduction between 'appropriate' gut-colonizing bacteria and potential pathogens in terms of their induction of inter-commu- nication signals between gut epithelial cells and leucocytes

[6 68]. Quite how the immune surveillance system distinguishes between 'friendly' luminal micro-organisms and potential pathogens remains undefined, but from the perspective of generating appropriate immunoregulatory signals, the next key step will be defining precisely how these signals are able to influence T cell receptor-mediated antigen recognition and immune deviation. By understanding the molecular mechanisms by which gut-colonizing microbes are able to induce pro-Th1 responses, without initiating tissue inflammation, we should be able to make further progress toward manipulating the gut microflora as a safe and effective means of combating allergy.

Addendum

Since writing the manuscript (late 2000/early 2001), the authors have become aware of a series of clinical reports from a Finnish research group involving the use of dietary LAB as anti-allergy agents [73–75]. These recent reports (published in November/December 2000 and April 2001) attest to the ability of orally delivered *Lb. rhamnosus* strain GG to modulate clinical symptoms in atopic infants and children with dermatitis/eczema, and

to reduce the development of allergic atopy in at-risk infants during the first 2 years of life. Consumption of *Lactobacillus* GG by atopic infants [73], or by expectant mothers pre partum and/or their babies post partum [75], was shown to reduce clinical indices of atopy and the incidence of atopic eczema, respectively. In atopic children given *Lactobacillus* GG, there was a transient increase in IFN γ secretion by anti-CD3 antibody-stimulated lymphocytes in vitro in 3 of 4 reported subjects, but no appreciable effect of LAB on serum concentration of IL-12 [74]. Most interestingly, however, LAB treatment increased both in vivo (serum) and ex vivo (mitogen-stimulated) levels of IL-10 [74], raising the suggestion that induction of anti-inflammatory cytokine production by LAB may be just as important in limiting atopic disease as pro-Th1 immune signalling [75]. Nevertheless, it has been reported previously that *Lactobacillus* GG can induce secretion of IL-12, IL-18 and IFN γ in cultures of human mononuclear leucocytes [45] and can activate NF- κ B, STATs 1 and 3 and IFN-regulatory factor 1 in human primary macrophage cultures [68], and so its mode of action could still be reliant (wholly or partially) on the pro-Th1 immune deviation model proposed in this review.

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