

Lactic acid bacteria as novel adjuvant systems for prevention and treatment of atopic diseases

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Purpose of review

Allergic diseases have become the new epidemic in westernized countries. Several factors including reduction of microbial contact early in life may have contributed to this dramatic development. As conventional immunotherapy has obvious limitations, there is a need for the development of new prophylactic and therapeutic treatment approaches. In this respect, lactic acid bacteria have received increasing attention as potential treatment tools against allergy. In this review, different treatment strategies with probiotics will be discussed in light of the recent literature.

Recent findings

Supplementation trials with probiotics have shown to reduce allergic manifestations in children. New treatment approaches use selected probiotic strains as mucosal antigen delivery vehicles for recombinant allergens. The most recent studies focus on the optimal intervention time to enhance the treatment efficacy with probiotics for primary disease prevention.

Summary

Probiotics may serve as attractive adjuvant systems for improved allergy treatment. Recent work supports the value of well conducted clinical and experimental studies that compare different treatment regimes and identify the best candidate probiotic strains for therapeutic immunomodulation. Such data, as well as understanding the mechanisms of immune modulation in pathogenic and therapeutic settings, are fundamental to be able to recommend probiotic use in allergy treatment.

Keywords

adjuvant, allergic disease, lactic acid bacteria, mucosal, prevention/therapy, recombinant bacteria

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Introduction

The increasing prevalence of allergic diseases in westernized countries has recently prompted a search for possible causative factors for this epidemiological change as well as the development of new, safe and effective treatment strategies against allergic diseases.

The hygiene theory postulates that a reduced microbial contact early in life, and changes in nutrition, might have contributed to an imbalance in immunological responses to different antigens including allergens [1]. New treatment approaches for allergies utilize novel adjuvant systems aimed at counteracting immune dysregulations via induction of Th1-like and/or immunosuppressive responses. Lactic acid bacteria (LAB), a group of Gram-positive bacteria of which some are part of the human indigenous flora and/or are involved in food fermentation processes, have awakened scientific interest for the development of new mucosal delivery

vehicles and adjuvant systems with immunomodulating properties.

This review shall give an overview of recent knowledge on the relevance of probiotics in allergy development and prevention. We discuss different prophylactic and therapeutic treatment strategies with selected probiotic strains while attempting to explain the underlying mechanisms of induced immunomodulation.

State of the art

In this part of the review, we summarize the current understanding of development and increase of allergic diseases as well as of the standard treatment procedures. On the basis of epidemiological studies, we will discuss the potential role of LAB in allergy development and prevention. Furthermore, recently published data will be presented here explaining how the interaction of certain probiotic strains with the host immune

system may lead to their defined immunomodulatory properties.

Type I allergy

Allergic diseases, such as allergic rhinoconjunctivitis, allergic asthma or atopic dermatitis, are chronic inflammatory disorders that are characterized by excessive Th2-biased immune responses against normally innocuous antigens such as tree or grass pollen, house dust mite and animal dander antigens. The prevalence of allergic diseases has been steadily increasing, so that currently more than 25% of the population in industrialized countries suffer from atopic disorders. Although allergic diseases clearly have a heritable component, this factor alone cannot explain the recent epidemiological changes [2]. There is a strong body of evidence suggesting that the absence of certain infections during early childhood due to high hygienic standards and 'sterile' living conditions may significantly contribute to the increasing prevalence of allergy. This phenomenon, called the 'hygiene hypothesis', is supported by several epidemiological studies showing a higher prevalence of allergic diseases in urban than in rural areas of industrialized as well as developing countries [3–7]. Consistent with this hypothesis, a reduced risk of atopy is associated with increasing numbers of siblings [8], vaccination with *Bacillus Calmette-Guerin* containing attenuated mycobacteria [9] or exposure to certain orofecal pathogens such as hepatitis A, *Toxoplasma gondii* and *Helicobacter pylori* [10]. Moreover, changes (discussed below) in the composition and homeostasis of the commensal gut flora have been linked to increased allergy development [11]. However, the immunological background of the 'hygiene hypothesis' has not been solved yet. Initially, a reduced production of particularly IL-12 and IFN- γ by immune cells due to a lack of microbial contact early in life was suggested to drive the excessive expansion of Th2 cells. More recently, regulatory T (Treg) cell production of immunosuppressive cytokines, such as IL-10 or TGF- β , for the prevention of immunological disorders has been implicated [12–14]. These apparently opposing mechanisms may cooperate in a complex network with a fine balance between allergen-specific Treg, Th2 and/or Th1 cells. Together, these interactions seem to determine whether a healthy or an allergic immune status develops in response to allergen contact [15].

Currently approved treatment strategies

Allergen-specific immunotherapy is so far the only curative treatment approach against type I allergy [16]. Conventional immunotherapy (SIT) is performed by injecting increasing amounts of allergen extracts in order to induce immunological hyporesponsiveness toward the respective allergen. Although SIT has been used for almost a century, the underlying immunological mechanisms are still not entirely solved [17]. Clearly, several immunological pathways are involved, ranging from

induction of allergen-specific 'blocking' IgG4 to the generation of allergen-specific Treg cells [15,18].

Although SIT is an effective treatment in many cases of venom and inhalant allergies, the current protocols also present some disadvantages, such as long treatment duration, anaphylactic side reactions or reduced patient compliance due to frequent injections. Approaches to improve the conventional treatment protocols have therefore focused on three main aspects, that is, changing from a subcutaneous to mucosal route of allergen application; the use of new adjuvants with strong immunomodulating properties; and prophylactic rather than therapeutic treatment protocols.

With respect to mucosal allergen application, sublingual immunotherapy has become an attractive alternative to conventional immunotherapy [19–21]. Mucosal treatment protocols and the prevention of allergy by oral tolerance induction have been extensively studied in experimental animal models [22,23]. Recently, human studies have indicated that immune deviation and upregulation of IL-10-producing Treg cells may occur upon administration of allergens via the oral mucosa [24,25].

Adjuvant systems that can shift allergic Th2 responses toward a Th1-biased profile or control the expression of Th2 cytokines by inducing the production of IL-10 and TGF- β are considered essential tools for therapeutic interventions. New adjuvant systems that are not based on aluminum salts, which are known to promote Th2-biased immune responses [26,27], are being screened for improved treatment efficacy. As discussed below, there is increasing interest in the use of LAB both as mucosal delivery vehicles for allergens as well as adjuvant systems in their own right.

Lactic acid bacteria and their influence on allergic diseases

LAB are a noninvasive and nonpathogenic group of Gram-positive bacteria that are defined by their ability to produce lactic acid from the fermentation of sugars. They have been used for food processing and preservation for centuries. Specific species are also members of the indigenous microflora present in different mucosal compartments of the body. Changes in the composition of the gut bacteria have been linked to several diseases, including diabetes and inflammatory bowel disease [28–30]. Currently, there is much interest in the role of the intestinal microbiota, and specially LAB, in the development and prevention of allergic diseases [11,31,32]. Several studies indicated that the composition of the commensal gut flora, and particularly the presence of certain LAB species, is different in atopic than in non-atopic children [33–36]. More recently, the KOALA Birth Cohort Study conducted in the Netherlands

demonstrated that differences in gut microbiota composition precede the development of atopic sensitization during infancy [37]. Moreover, lactobacilli have been found to be present in the breast milk of healthy mothers and may have a positive effect on the colonization of the infant gut [38]. Accordingly, it has been shown that treatment with antibiotics in the neonatal period is a risk factor for wheezing [39]. These data suggest that substitution with selected LAB may have a positive influence on allergy development.

Role of lactic acid bacteria in allergy prevention: human intervention trials

There is growing interest in supplementing the human diet with LAB for allergy prevention. Being generally regarded as well tolerated, LAB might be appropriate for early protective immune regulation. Indeed, there have been several clinical studies that show a benefit in prevention of atopic eczema in infants when given to pregnant mothers and to their infants [40,41,42[•],43^{••}]. However, it is not yet clear whether the protection to the infant is mediated by direct contact with the probiotics or whether it is provided via the placenta or breast milk. In one report, administration of *Lactobacillus rhamnosus* GG (LGG) to pregnant and lactating mothers significantly reduced the risk of developing atopic eczema in infants and was associated with increased levels of TGF- β 2 in the breast milk [44]. In another study, supplementation of pregnant mothers with *Lactobacillus reuteri*, which also led to reduced atopy in their infants, induced low breast milk TGF- β 2 [45].

However, two contradictory reports on the protective effect of probiotic treatment warrant caution: Kopp *et al.* [46] reported that administration of LGG to pregnant mothers followed by postnatal treatment did not prevent from development of atopic dermatitis in infants, but rather significantly increased the risk of episodes with wheezing bronchitis in the probiotic group. Similarly, exclusive postnatal administration of *Lactobacillus acidophilus* to high-risk infants during the first 6 months of life increased frequency of atopic dermatitis and increased rate of sensitization to common allergens [47].

Although the conclusions from the different studies are still controversial with respect to the beneficial effects of probiotic supplementation on allergy development, these studies provide a valuable lesson for the therapeutic use of LAB. Careful strain selection and characterization of the immunomodulatory properties will be essential prior to application in prophylactic and therapeutic settings.

Studies on lactic acid bacteria and their interaction with the host immune system

LAB are known to influence both mucosal and systemic innate and adaptive immune function. Of particular

importance is their potential to elicit antigen-specific secretory IgA responses at mucosal surfaces [48,49[•]]. In murine models, oral administration of *L. acidophilus* led to significant increase in IgA production in Peyer's patches [50] and administration of *Lactobacillus casei* increased induction of IgA⁺ cells in the gut [51]. In human trials, probiotic administration was associated with higher levels of fecal IgA [52] and increased plasma levels of total IgA [43^{••}].

LAB have intrinsic adjuvant properties to induce proinflammatory or regulatory cytokines *in vitro* and *in vivo*. Our own data show that certain *Lactococcus lactis* and *Lactobacillus plantarum* strains induce the production of IL-12 and consequently the production of IFN- γ in naïve murine splenocytes in a dose-dependent manner [53]. Consistent with this, Sashihara *et al.* [54] also demonstrated lactobacilli strain-dependent induction of high levels of IL-12 by murine splenocytes. Kekkonen *et al.* [55] tested 11 different probiotic strains for cytokine production in human peripheral blood mononuclear cells (PBMCs) and showed that there were strain-specific differences in the production of IL-12 and IFN- γ , but that all bacteria tested did induce TNF- α . In another study, different strains were shown to drive different immune responses either toward a proinflammatory or a regulatory profile [56]. Among Lactobacilli spp., *L. reuteri* and *Lactobacillus casei*, but not *L. plantarum*, were shown to induce IL-10-producing regulatory T cells *in vitro* [57]. These studies imply that LAB induce cytokine signals that can counterbalance allergic Th2 responses.

Dendritic cells play a key role in regulating T-cell responses. LAB have been shown to interact with dendritic cells and to differentially modulate dendritic cells maturation in a strain-dependent manner [58,59]. For instance, interaction of human dendritic cells with probiotic bacteria triggered their full maturation program and instructed dendritic cells to convert naïve CD4⁺ T cell into hyporesponsive T cells that display suppressor function [60].

It is now well established that different microbes or microbial structures can interact with the immune system via Toll-like receptor (TLR) signaling [61]. Recently, the essential role of TLR in activation of type 1 immune responses by *Lactobacillus pentosus* has been shown in a murine model [62] and feeding of mice with *L. casei* increased the number of TLR2-positive cells in the gut [63]. In the human system, *L. rhamnosus* enhanced the expression of TLR2 in macrophages [64^{••}] and this same receptor was found to be responsible for the recognition of *L. plantarum* by PBMCs [65].

Apart from TLR, bacterial antigens can be also recognized through molecules like nucleotide-binding

oligomerization domain receptors (NODs) or lectins. Indeed, *L. lactis* activates cells through NOD1 and NOD2 [66] and *L. reuteri* and *L. casei* have been shown to bind the C-type lectin dendritic cells specific intracellular adhesion molecule 3-grabbing nonintegrin (dendritic cells-SIGN) [57]. Moreover, oral application of *L. casei* increased the expression of mannose receptor CD206 on the gut immune cells in mice [63].

The immunogenicity of certain LAB does not require their viability and survival in the gut. Sawada *et al.* [67] showed that heat-treated *L. rhamnosus* is able to modulate the development of atopic skin lesions and oral administration of heat-killed *L. brevis* or *L. gasseri* to OVA-sensitized mice reduced total and antigen-specific IgE production [54,68]. Recently, many studies have focused on the identification of different bacterial components that elicit immunomodulatory effects [69,70].

Lactic acid bacteria-based strategies for prophylaxis and therapy of allergy

In this part of the review, we will discuss the most recent experimental studies using LAB either as mucosal adjuvants or as delivery and expression systems for recombinant allergens to prevent or treat allergic immune responses. Finally, we will present the latest studies on the optimal time window of intervention for primary prevention with selected LAB.

Use of lactic acid bacteria as mucosal adjuvant

To enhance the immunogenicity of antigens applied via the mucosa, co-administration of adjuvants is usually required. Using a mouse model of type I allergy to birch pollen [71], we have previously tested several mucosal adjuvants for their capacity to modulate allergic immune responses. We demonstrated that immune modulation can be achieved by the use of cholera toxin [72], cholera toxin subunit B [73,74] and certain bacterial components (CpG motifs) [75].

To investigate the potency of immune modulation by LAB strains, we studied antigen-specific immune responses induced by *L. lactis* (MG1363) and *L. plantarum* (NCIMB8826). Both strains induced high levels of IL-12 and IFN- γ *in vitro*, which was positively correlated with the modulation of allergic immune responses *in vivo* [53]. Intranasal co-application of the major birch pollen allergen Bet v 1 and LAB prior to or after sensitization of mice induced a shift toward Th1 immune responses along with a reduction of IgE-dependent basophil degranulation. However, pretreatment with *L. plantarum* or *L. lactis* alone did not result in allergen-specific modulation. Similar results were recently obtained in a mouse model of house dust mite allergy, showing that *L. plantarum* intranasally applied together with the major house mite

allergen Der p 1 before sensitization led to a shift toward allergen-specific Th1-responses. Also in this study, mice pretreated with probiotic alone were not protected and developed allergic responses [65].

Polysensitized patients suffering from allergic multisensitivities are difficult to treat. In order to study new treatment approaches against these syndromes, we established a mouse model of polysensitization to major birch and grass pollen allergens [76]. Intranasal treatment with selected probiotics (*Bifidobacterium longum* or *Lactobacillus paracasei*) at the time of polysensitization and allergen challenge, rather than prior to sensitization, significantly reduced allergic polysensitization and airway inflammation (I. Schabussova *et al.*, unpublished data). These data emphasize the notion that the timing of probiotics intervention is crucial for the development of a protective response and suggest that amelioration of allergic symptoms in polysensitized individuals could be achieved using certain probiotics for seasonal treatment.

Use of recombinant lactic acid bacteria as vaccine vehicle

Active delivery of recombinant molecules to mucosal surfaces by genetically modified LAB represents a novel and attractive vaccination approach. Of great utility is that recombinant bacteria can safely transport the expressed antigen through the stomach to the inductive sites of the gut immune system. Moreover, local delivery of the vaccine antigen in close vicinity with the immunomodulating LAB could serve to improve the efficacy of the treatment. The effect of improved adjuvanticity is, in part, attributed to enhanced antigen uptake and presentation at the site of antigen/allergen encounter [49[•]].

One of the first recombinant LAB constructs was a *L. lactis* strain that had been transfected with a gene coding for murine IL-10 and was used for treatment of colitis in two different mouse models [77]. This recombinant strain was used to prevent sensitization to the major cow's milk allergen β -lactoglobulin (BLG) in a mouse model of food hypersensitivity [78[•]]. The reduction of anaphylaxis was associated with diminished BLG-specific IgE and IgG1 antibodies along with enhanced antigen-specific secretory IgA. Similar to this study, recombinant strains expressing also other cytokines, such as IL-12, have been shown to modulate allergic immune responses [79]. Nevertheless, cytokine-based treatment could induce unexpected side effects due to unspecific and broad interference with the immune system. Thus, LAB have also been explored for production and mucosal delivery of defined antigens [80,81], including allergens.

Recombinant *L. lactis* producing different amounts of BLG were tested in a mouse model of BLG sensitization [82]. Oral administration of recombinant *L. lactis* before

sensitization induced a Th1 type immune response to BLG and also decreased the levels of BLG-specific IgE. The most effective strains to induce this phenomenon were those that produced the highest amount of BLG. With respect to aero-allergens, intranasal treatment with *L. plantarum* that express a peptide of the allergen Der p 1 reduced allergen-specific Th2 responses [83]. More recently, *L. acidophilus* was engineered to produce another dust mite allergen, Der p 5 [84]. Oral application of these recombinant bacteria inhibited allergen-induced airway inflammation and hyper-reactivity and significantly decreased allergen-specific IgE production. Suppression of local and systemic OVA-specific T-cell responses has been observed in OVA T-cell receptor (TCR) transgenic mice (DO11-10) that were fed with OVA-secreting *L. lactis* [85]. This suppression was mediated by induction of regulatory cells that function through a TGF- β -dependent mechanism. Tolerance induction by *L. lactis* OVA delivery was superior to both low-dose and high-dose feeding of soluble antigen.

In our study, which was part of a European commission funded project (LABDEL) to develop and test products based on the oral delivery of vaccines and therapeutic agents using LAB, we evaluated the potential of recombinant *L. lactis* and *L. plantarum* to modulate allergic responses in a murine model of birch pollen allergy. Both strains were engineered to produce the major birch pollen allergen Bet v 1, one of the clinically most relevant airborne allergens. Intranasal pretreatment with recombinant strains was successful in significantly reducing allergen-specific IgE and increasing IgG2a levels in sensitized mice, indicating a shift toward nonallergic Th1 immune responses [86]. Moreover, intranasal application of these bacteria induced allergen-specific IgA both in lungs and gut, indicating that immunomodulation could be achieved via the oral route. Both wild-type and recombinant strains led to reduction of eosinophils and IL-5 in bronchoalveolar lavages, possibly due to the intrinsic immunosuppressive properties of these bacterial vectors [86]. Comparing different mucosal routes of application, it was found that the intranasal application of recombinant bacteria seemed more effective than the intragastric route, which might in case of *L. lactis* be explained by a lower acidic resistance compared to *L. plantarum* [87]. These data are in agreement with recent work showing that the intestinal transit time of *L. plantarum* NCIMB8826 is significantly longer than that of *L. lactis* MG1363 [88,89].

We also sought to determine if secreted or intracellularly produced Bet v 1 has an influence on the treatment efficacy. Particularly after intranasal pretreatment, the Bet v 1 secreting strain induced a stronger reduction of allergen-specific IgE compared to the strain producing intracellular Bet v 1 [87]. Similar results were obtained by

others who compared pretreatment with strains producing intracellular and secreted BLG [82]. Studies to evaluate why secretion of antigen into the local environment is advantageous for immunosuppression of allergic responses are ongoing.

The studies discussed with recombinant LAB, herein, have so far only been conducted in animal models. Notably, the first pilot trial in volunteers with recombinant bacteria expressing human IL-10 has been recently completed, indicating that recombinant LAB as vehicles for mucosal delivery of vaccine antigens and other therapeutic molecules represent attractive candidates for well tolerated and effective treatment of allergic diseases in humans [90].

Use of lactic acid bacteria for prenatal, perinatal, and postnatal intervention

Data from clinical and experimental studies indicate that prophylactic rather than therapeutic strategies are more effective in allergy treatment. Along these lines, a recent meta-analysis of six prevention and four treatment clinical trials suggests that prenatal and postnatal probiotic intervention is an effective defense against pediatric dermatitis [91].

Numerous studies have aimed at defining the optimal intervention time – so-called ‘window of opportunity’ to prevent allergy by the use probiotic strains. Using a mouse model of bronchial asthma, oral administration of LGG or *Bifidobacterium lactis* from birth for 8 weeks suppressed all aspects of the asthmatic phenotype such as antigen-specific IgE production, airway inflammation and airway hyper-responsiveness [92]. Oral administration of LGG resulted in a significant inhibition of both allergen-specific IgE and IgG2a, suggesting general immunosuppressive effects by the bacterial treatment. The immunosuppression was associated with TGF- β producing Treg cells. In a murine model of OVA allergy, mice received intragastric applications of LGG either before conception, during pregnancy and lactation (perinatal supplementation) or before conception and during pregnancy only (prenatal supplementation) [93]. In both treatment regimens, allergic airway and peribronchial inflammation were significantly reduced compared with that in the control group. The authors suggested that the effects are mediated, at least in part, via the placenta, probably by the induction of a proinflammatory environment with high levels of TNF- α .

So far, only few studies demonstrated the longevity of protective effects. Moreover, it is not yet clear which prenatal treatment, be it alone or a combination of prenatal, perinatal, and/or postnatal interventions, leads to the most successful outcome of primary prevention.

In our recent murine study on primary prevention of birch pollen allergy, we compared different treatment windows of pregnant mothers during gestation and/or lactation and of the offspring during the neonatal phase (I. Schabussova *et al.*, unpublished data). Preliminary data indicate that oral application of live probiotic bacteria to pregnant mice before allergic sensitization and aerosol challenge suppressed eosinophilic airway inflammation. Consistent with this, allergen-induced cytokine production was suppressed in restimulated cultures of spleens, lungs and mediastinal lymph nodes. Moreover, administration of the probiotic strains to mice during gestation and lactation had a suppressive effect on allergen-specific immune responses in the sensitized offspring.

As mentioned earlier, recent reports may challenge the beneficial effects of early probiotic intervention on allergy development. In a study by Ezendam and van Loveren [94], administration of *L. casei* Shirota during lactation enhanced lung inflammation in a mouse model of OVA-induced respiratory allergy. However, this study showed that the time of intervention was crucial, as the application of the same bacteria later in life did not show any effect. These data suggest that the timing of intervention seems decisive for either causing exacerbation of disease or allergy protection.

The results from the clinical and experimental primary prevention trials reviewed here appear inconclusive, probably as a result of variable treatment regimes, use of different bacterial strains, different target population and different experimental models. In addition, this field would benefit from a careful examination of the predictive value of allergy animal models, for example, by establishing a correlation between the data collected from clinical and animal trials.

Conclusion

Growing evidence from epidemiological, clinical and experimental studies supports the idea that probiotic bacteria may have an important role in programming the immune system during ontogeny. In this respect, primary prevention of allergy with probiotics represents a new and exciting field in allergy research. In several studies, the supplementation of the human diet with certain LAB such as lactobacilli and bifidobacteria early in life was demonstrably beneficial for allergy prevention, even though several other studies have failed to support this notion. Important insights have been made, indicating that the beneficial effects of probiotics are strain dependent, as well as dependent on the mode and timing of treatment. More studies, particularly on the underlying mechanisms of protective immunity in allergy prevention, are needed, though, before general recommendations for probiotic supplementation in early infancy can be made.

Further improvements may come from genetically modified LAB strains that are particularly suited to the delivery of recombinant allergens in the form of mucosal vaccine vehicles. The translation of prophylactic and therapeutic studies in animal models into clinical trials, including dose-finding studies, remains a major challenge of forthcoming allergy research. Nevertheless, the fact that the first human trial with a recombinant LAB strain has recently been completed gives hope that mucosal vaccination with LAB can be a successful treatment for allergy in humans.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 594).

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