

# Recombinant probiotics for allergen immunotherapy

## Abstract

Research efforts to improve the efficacy of Allergen Immunotherapy (AIT) involve the discovery of new adjuvant. Some strains of probiotic bacteria are able to induce up-regulation of co-stimulatory molecules in DCs, promote TH1 cytokine production and trigger Treg differentiation and activity. In mouse models, they have been shown to prevent or suppress the harmful TH2 response and to potentiate allergen- or allergoid-based immunotherapy of type I allergic diseases. These observations could explain the effectiveness of the prolonged administration of probiotics in the prevention of allergic disorders in infants. Consequently, the probiotics could be used as adjuvant for AIT. However, the simultaneous administration of probiotics and AIT might not come to specific responses because of degradation and not optimal presentation to APC. Moreover, the lack of specificity of the immunological response might be overcome by using probiotic bacteria expressing the sensitizing allergen. This would allow the stimulation of the intestinal immune system by both immunomodulating factors at the same site. Only a few studies noticed the development of AIT strategies based on recombinant bacteria expressing the allergen. However, most of them reported basic favorable immunomodulatory properties, rather than showing the development of immune tolerance or symptoms reduction. The allergen sequestration inside the bacterial cell may represent limits for these preventive and therapeutic approaches. Recently, our research group developed a novel AIT strategy based on a food-grade bacterium *Streptococcus Thermophilus* (ST) with TH1 skewing property, able to produce the allergen intracellularly and to release it at the gut-associated lymphoid tissue (GALT) level thanks to its intrinsic autolytic behavior with tolerance induction, and readdressing the pre-existing allergen-elicited TH2 response. Hence, the present knowledge on the subject will be critically considered in this review, underlying the most recent experimental achievements.

**Keywords:** recombinant probiotics, allergen immunotherapy, streptococcus, prevention, therapy

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**Abbreviations:** SIT, specific immunotherapy; ST, streptococcus thermophilus; GALT, gut-associated lymphoid tissue; Treg, T-regulatory; AIT, allergen immunotherapy; Ig, immunoglobulin; APC, antigen-expressing cells; DC, dendritic cells; MAMPs, molecular pattern associated with microorganisms; TLR, toll-like receptors

## Introduction

Currently, AIT represents the sole allergen-specific therapeutic approach dealing with IgE-mediated allergic disease able to control not only its symptoms, as other treatments do, but also to rebalance the immunological profile of allergic subjects. In fact, they develop detrimental immune responses towards allergens, exogenous proteins that should be otherwise harmless, entering the body from the external environment by feeding, breathing or by cutaneous absorption. The effectiveness of AIT is based on its immunological mechanisms which are complex and still not completely clarified. In this scenario, a central role seems to be played by the antigen-expressing cells (APCs), typically dendritic cells (DCs) and macrophages.<sup>1</sup> They are induced by the AIT to mature into a phenotype able to guide differentiation of naïve T lymphocytes (TH0) into T helper type-1 (TH1) or T regulatory (Treg) cells.<sup>1</sup> The APCs promote these latter effects by activating two alternative cytokine patterns, IL-2 and IL-12 in the first case and IL-10 in the second. In turn, TH1 cells produce IFN- $\gamma$  which prompt the immunoglobulin (Ig) isotype switch towards the production of allergen-specific IgG4 (IgG2 in mice), that compete with IgE for the binding to the allergen. The cytokines produced by Treg (IL-10 and TGF- $\beta$ ) exert an inhibitory activity inducing energy or apoptosis of TH2 cells. In this way, they

suppress the production of their characteristic cytokine IL-4, which represents the main input for B lymphocytes to produce IgE. As a result, basophils and eosinophils cannot be activated to release mediators of the allergic inflammation which, as it is well known, fuel and amplify pre-existing immunological response and associated symptoms. In addition, the IgE-mediated antigen presentation by APCs and the stimulation of specific TH2 clones are inhibited. All these immunological variations counteract the allergic responses and contribute to restore the non-pathological ones towards exogenous proteins. The ultimate effects are desensitization and attenuation, or even complete disappearance, of allergic symptoms. However, the AIT, which involves the administration of an allergen in native form, could trigger anaphylaxis, in particular when high doses are necessary for an improved clinical outcome. Hence, AIT could be ameliorated in terms of safety and efficacy by developing hypoallergenic antigens,<sup>2-5</sup> or adjuvant molecules<sup>6-9</sup> able to stimulate Treg and TH1 cells function. A more recent approach involves the production of carrier substances promoting the optimal presentation of the therapeutic allergen to the immune system. An example of translational research into clinical practice is represented by the development of allergoids, chemically modified allergenic proteins with reduced binding affinity for IgE, presenting lower or no risk of side effects.<sup>10</sup>

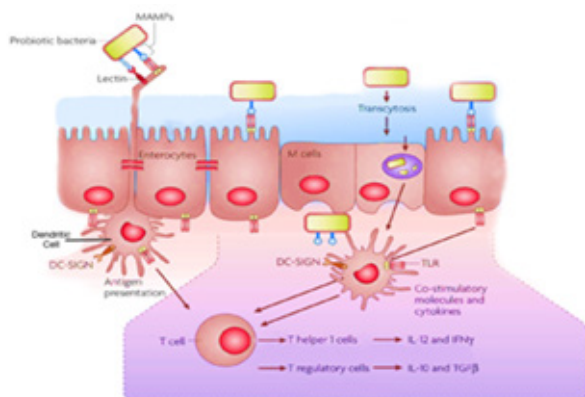
## Probiotic bacteria, the immune system and allergy

Probiotics are generally commensal and/or symbiotic gram-positive bacteria, resistant to the gastric environment and able to settle transiently the small intestine. Acting together, they create the microbiota, a complex system contributing to the intestine functional

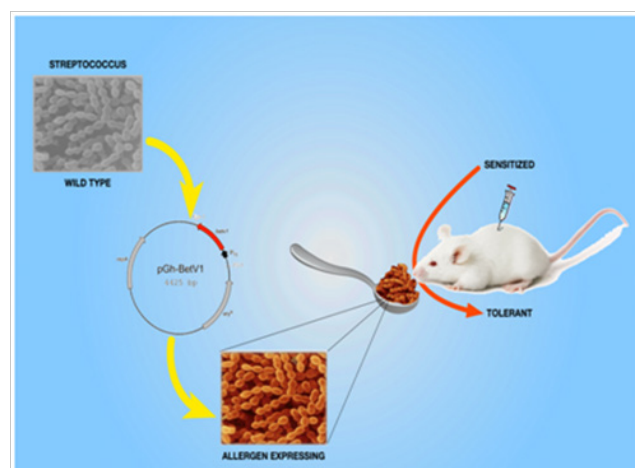
activity and favoring its normal physiology also considering its intimate association with the immune system. For this reason, this system may represent a possible target for the treatment of allergic diseases, being able to develop regulatory immune responses through IL-10 induction<sup>11</sup> providing as well adequate stimuli for the acquisition of antigen tolerance. Probiotic bacteria could be suited to this purpose. It has been demonstrated that certain strains of probiotics regulate the immunological homeostasis of the intestinal mucosa,<sup>12–14</sup> and partially affect the development of allergic diseases.<sup>15–17</sup> Furthermore, epidemiological studies revealed the correlation between the composition of the intestinal microflora and the prevalence of atopy.<sup>18</sup>

Lactobacilli (LAB) are the main probiotic bacteria employed for the preparation of dairy products and of probiotics supplements for human use; actually, they are not pathogenic, do not damage the mucous membranes, do not own genes for antibiotic resistance and are not degraded by bile acids.<sup>10,19</sup> Therefore, the use of LAB as adjuvant factor in AIT represents an innovative approach for desensitization. For instance, some strains are able to suppress the TH2 harmful response which characterizes allergy.<sup>20–22</sup> The mechanism through which LAB trigger the suppression of the TH2 harmful response is not fully clarified so far. Several in vitro and in vivo studies demonstrated that most - but not all - lactobacilli strains own immune system adjuvant properties, such as the ability to activate DC and to induce the TH1 cytokines, inhibit the TH2 cytokines and reduce the IgE production.<sup>23–28</sup>

In murine models of allergy, it has been proved that several LAB strains are able to induce Treg cells<sup>29,30</sup> and their inhibitory cytokines IL-10<sup>31,32</sup> and TGF- $\beta$ ,<sup>32,33</sup> enhancing the protection from an inflammatory response of the airways.<sup>34</sup> Behind these immunological changes there is a complex interaction between probiotics and the host cells, i.e. intestinal epithelial cells (IEC)<sup>35</sup> and intestinal dendritic cells (DC).<sup>36</sup> A closer approach between probiotics and the DCs inside the lamina propria may take place in the intestinal lumen. This is possible thanks to the protrusion of dendritic extensions through the IECs layer or through the lymphoid tissue “dome region” associated with GALT, where probiotics are transferred through specialized epithelial cells named M cells. The contact may occur through the interaction of molecules exposed on the surface of the different cell types involved, such as the bacterial Molecular Pattern Associated with Microorganisms (MAMPs) and the Toll-like receptors (TLRs) expressed on IECs and DCs, as well as the DC-SIGN also expressed on DCs. The link between ligand and receptor induces DCs maturation so that to produce two possible alternative types of cytokines, IL-12 or IL-10 (and TGF- $\beta$ ), essential for polarization of naïve CD4<sup>+</sup>T cells (TH0) towards TH1<sup>37</sup> effector cells or T regulatory cells<sup>35–38</sup> (Figure 1).



**Figure 1** Cellular and molecular interactions between lactobacillus and intestinal epithelium and GALT.



**Figure 2** Vaccine production and probiotic AIT administration.

These data stimulate the studying of new lactobacilli strains, in vitro and in vivo, that can improve the therapeutic efficacy of AIT. Lactobacilli present immunomodulatory activity on T effector cells and T regulatory cells in humans. However, the clinical application of lactobacilli for allergic diseases is still controversial. On the one hand, some clinical trials demonstrated that the administration of lactobacilli prevent the occurrence of allergic diseases in childhood<sup>39–42</sup> and, more recently, the co administration of a probiotic and peanut-OIT produced sustained unresponsiveness in children with peanut allergy, but the relative contribution of the probiotic to this outcome is to be clarified.<sup>43</sup> On the other hand, other studies did not show any positive effect nor benefits were observed just in the short term.<sup>41,44</sup> In some clinical studies on adults affected by atopic dermatitis or allergic rhinitis, lactobacilli produced a modulation of the immune response<sup>45,46</sup> and, just in one case, a reduction of nasal symptoms.<sup>46</sup> On the contrary, they revealed to be ineffective for allergic asthma.<sup>47</sup> Even in patients affected by allergic rhinitis and treated with lactobacilli, it was not observed any evidence of symptoms reduction despite a clear shift of TH1-TH2 and an induction of Treg.<sup>48</sup> Probably, the absence of a clinical remission is related to the fact that probiotics evoke immunological responses that are independent from the allergen and, thus, not specific for it.<sup>49</sup> This might limit the clinical efficacy of the treatment in the long term, which could be achieved with an immunological tolerance established by Treg cells specific for the antigen.<sup>50,51</sup> For this reason, it has been argued that the immunomodulatory effect of probiotics could be enhanced and made more specific thanks to a joint administration with the allergen. However, a simple “probiotic-vaccine” association may result ineffective after the enzymatic degradation of the allergen administered orally. In this case, the association of a probiotic with an allergoid, rather than with an allergen, may result more appropriate. In support of this theory, in a previous study, our research group demonstrated that the AIT effectiveness based on the allergoid Amb a 1 of *Ambrosia* is enhanced by the co-administration of *Lactobacillus paracasei* LP6.<sup>52</sup> Recombinant probiotics may represent the proper instrument for the development of a safer and more effective anti-allergic vaccine, administrable orally, is given by recombinant probiotics producing the allergen.

## Probiotic bacteria expressing an allergenic protein for vaccination

Probiotic bacteria are microorganisms that can be modified in order to produce inside, or in a secreted form, exogenous proteins. These latter generate recombinant probiotics and, when expressing an

allergenic protein, could be used as innovative vaccines with enhanced or upgradeable characteristics for the application in AIT protocols. Thanks to recombinant DNA technology, it is now feasible to produce large quantities of pure proteins either in native or mutated form, using the bacteria, including those probiotic, as natural “incubators” for their production. For example, by using this technology any DNA sequence could be inserted inside a bacterial expression vector

introduced into the bacterium; this latter transcribe and translate it into the polypeptide chain inside its cell or secrete it in the culture medium. Furthermore, through the site-specific mutagenesis, it is possible to modify particular stretches of gene sequence in order to obtain proteins with altered activity. For the allergens, can be obtained hypoallergenic molecules able to maintain their immunogenicity.

**Table I** Overview of studies focusing on recombinant probiotics in experimental allergy

Protocol	Probiotic	Recombinant Allergen	Site of Vaccination	Model of Allergic Sensitization	Immunological Effects (and Symptomatological)	References
Prophylaxis in Adult Mice	Lactococcus lactis	BLG	Intragastric	BALB/c Mice	↓ IgE specific ↑ IgG2a specific ↑ IFN- $\gamma$ Effects correlate with the levels of BLG expressed by <i>L. lactis</i>	53
	Lactobacillus plantarum; Lactococcus lactis	Bet v I	Intranasal	BALB/c Mice	↓ IgE specific ↑ IgG2a specific ↓ IgG1/IgG2a ↓ Eo in BALF e ↓ IL-5 in BALF ↑ sIgA specifiche in polmone e intestino	63
	Lactococcus lactis	OVA	Intragastric	BALB/c mice (TCR specific for OVA)	↓ local and systemic response; ↑ lymphocytes T CD4(+)CD25(-); ↑ IL-10 ↓ IFN- $\gamma$ in splenocytes reactivated in vitro; ↑ Foxp3 e CTLA-4 in Treg	55
	Lactobacillus plantarum	Der p I	Intranasal	BALB/c mice	↑ IgG2a specific ↓ Eo in BALF e ↓ IL-5 in BALF	69
Prenatal and Neonatal Prophylaxis	Lactobacillus plantarum NCIMB8826	Bet v I	Settlement of the mothers	BALB/c mice (mothers in pregnancy)	Newborn  Pre- sensitization: Th1 profile (IFN- $\gamma$ from splenocytes stimulated in vitro with Ag). Post sensitization: ↓ IL-4 e IL-5 in SP cells and MLN reactivated in vitro with Bet v I; ↓ IgE, IgG1, IgG2a specific in serum; ↑ Foxp3 mRNA in splenocytes	56
	Lactobacillus paracasei NCC 2461	Bet v I	Oral	BALB/c mice (pregnant or lactating mothers)	In newborns:  ↓ Eo in lungs; ↓ IL-5 in BAL, lungs e mediastinal lymph nodes in vitro; : IgE, IgG; ↓ IL-4 e IL-5 in splenocytes in vitro reactivated in vitro with Bet v I or ConA; ↑ Foxp3 mRNA lung; ↑ TGF- $\beta$ in serum; ↓ peribronchial inflammation and mucus	57
Therapy in Adult Mice	Lactobacillus plantarum	Der p I (peptide)	Mucosal	Mice C57BL/6 J	↓ IFN- $\gamma$ (not specific) ↓ IL-5 (specific)	58
	Lactobacillus acidophilus	Der p 5	Oral	BALB/c	↓ IgE specific	59

Table Continued...

Protocol	Probiotic	Recombinant Allergen	Site of Vaccination	Model of Allergic Sensitization	Immunological Effects (and Symptomatology)	References
				Mice	↑ IgG specific ?? ↓ Eo in BALF e ↓ Hyperactive response of the airways (AHR)	
	Lactococcus lactis	Bovine Beta-lactoglobulin (BLG)	Intranasal	Mice	↓ IgG1 in BAL;  ↓ IL-4 ↑ IFN-γ in splenocytes reactivated in vitro; ↓ local response to nasal challenge	<sup>60</sup>
	Lactococcus lactis	Ara h 2	Oral	Mice	↓ TH2, ↑ TH1 ↑ SIgA, Treg (local response)	<sup>66,67</sup>

**Table abbreviations:** BAL: Bronchoalveolar Lavage; Der p 5: Dermatophagoides Pteronyssinus Group-5 Allergen; BLG: Bovine Beta-Lactoglobulin; OVA: Ovalbumin; Bet v 1: Major Allergen of Birch Pollen; ConA: Concanavalin A; TCR: T-cell Receptor; Cry j 1: Major Allergen of Japanese Cedar Pollen; Der p 1: Immunodominant T Epitope of D. Pteronyssinus

A recombinant probiotic expressing the allergen could be employed as antiallergic vaccine, thanks to its features which are innovative not only in comparison with conventional treatments but also compared to the use of the sole probiotic or probiotic-allergen association. In the first instance, recombinant probiotics can be easily administered orally. Secondly, the probiotic is likely to protect the intracellular allergen from proteolytic degradation and to release it in situ. Moreover, by doing so, it is possible to convey the allergen in the immunological districts (mesenteric lymph nodes and Peyer's patches) where it could promote the immunological tolerance in a specific manner.

A very interesting aspect of this approach is the chance to obtain high local concentrations of allergen thanks to its accumulation inside the probiotic cell; therefore, this may result in a more effective vaccine employing lower cumulative doses of allergen with a decreased risk of anaphylactic secondary effects.

So far, just a limited number of studies reported data on the development of AIT strategies based on the use of recombinant bacteria expressing the allergen<sup>53–60</sup> and no one of them has found application on humans. Pre-clinical studies in mouse models of allergy demonstrated that recombinant probiotics are able to counteract the allergic sensitization process with appropriate immunological variations, both in newborn and adult mice, when the treatment is administered as prophylaxis.<sup>61–66</sup> Peanut allergen-producing *L. lactis* strains modulated allergic immune responses redirecting TH2-polarized to non-allergic TH1 immune responses with involvement of sIgA and regulatory T cells.<sup>67</sup> However, for what concerns adult mice, no data are provided regarding the induction of Treg and the local inflammatory response after airways challenge.

By contrast, the two studies on newborns detected an increase of Foxp3, the transcription factor that identifies Treg cells, and a reduction of bronchial reactivity within the offspring of mothers treated during pregnancy and lactation. In these two cases, the instauration of tolerance implies the education of the immune system during its maturation, with effects on innate immunity and on natural Treg cells; moreover, despite not being driven by the antigen, they could have a non-specific effect also on responses to a specific allergen. A very different process from the previous one must be established in adult and pre-sensitized mice, which requires the intervention of adaptive immunity and inducible Treg cells with antigen specificity. Finally, in adult sensitized mice, the use of recombinant probiotics administered according to a therapeutic setting, in two out of three studies, is associated with decreasing symptoms of allergic inflammation, even

if it is not fully clarified the involvement of Treg cells in the observed effect (Table 1).

The preclinical studies described so far confirm the adjuvant effect of recombinant probiotics in the prevention and treatment of allergies, but do not clarify whether they are vaccinal tools able to stimulate long-lasting immunological tolerance mediated by Treg towards the allergen. For this reason, our group has recently conducted a research in vivo using a probiotic strain with peculiar characteristics, evaluating the Treg and pulmonary response.<sup>51</sup>

## Our study with streptococcus thermophilus expressing the allergen rBetV1

In this study, it was generated a strain of *Streptococcus Thermophilus* (ST) expressing Bet v 1, the major allergen of *Betula verrucosa*, in order to verify its possible adjuvant or therapeutic effect in BALB/c mice made IgE-responsive to this same allergen.<sup>51</sup> The choice of this strain stems from different considerations. First, it has been taken into account the large presence of peptidoglycan on the cellular wall and of (lipo)teichoic acids, owning immunomodulatory activities. Secondly, it has been considered its autolytic character provided by the presence of lysogenic bacteriophage expressing an enzyme able to degrade the bacterial cell wall; finally, it represents a prokaryotic expression system suitable for integrating and expressing permanently foreign genes.

In particular, the impossibility of such recombinant probiotic strain to colonize stably the intestine due to its autolytic character is a rather captivating feature for human application because the risk of transferring the antibiotic resistance is null.

The immunomodulation level and therapeutic efficacy of the ST recombinant probiotic was assessed and compared to the effects of only ST and ST+rBet v 1 association by measuring various immunological and histopathological parameters (cytokines in serum and produced by immune cells in vitro, Treg cells, inflammatory cells and cytokines in the lung tissue). This study showed that the therapeutic treatment of pre-sensitized mice with the recombinant probiotic expressing the allergen produces, upon recall airway challenge, specific local and systemic anti-inflammatory and inhibitory response along with clearance of lung eosinophilia,<sup>51</sup> which characterizes the inflammatory response of allergic asthma in mice.<sup>64</sup> Our findings suggest that the recombinant probiotic releases the allergen in an immunologically active form into the intestine and stimulates the resident immune cells determining the observed allergen-specific and favorable immunomodulant effects.



## Concluding remarks

In the present review, we have shown the most recent experimental findings supporting the use of recombinant probiotics in the prevention of allergic disorders, suggesting as well their potential usability for the allergen immunotherapy (AIT). We showed that the probiotic lactobacillus *Streptococcus Thermophilus* expressing the major allergen of *Betula verrucosa* administered orally is able to restrict the TH2 allergic inflammatory reaction in sensitized mice, with a shift towards TH1 and Treg allergen-specific immune responses.<sup>51</sup> Our study confirmed the immunomodulating properties of probiotics and showed that allergen-expressing probiotics can represent vaccines to induce tolerance within an experimental allergen immunotherapy setting (Figure 2). The contribution of our approach, which highlights how recombinant probiotics may act, at once, as adjuvant and antigenic stimuli, contributes to figure them not only as valid players in preventive protocols, as most studies over the past years demonstrated, but also as tools for specific immunotherapy applications.

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## Conflicts of interest

Author declares there are no conflicts of interest.

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## References

1. Frati F, Moingeon P, Marcucci F, et al. Mucosal immunization application to allergic disease: sublingual immunotherapy. *Allergy Asthma Proc.* 2007;28(1):35–39.
2. Di Gioacchino M, Cavallucci E, Ballone E, et al. Dose-dependent clinical and immunological efficacy of sublingual immunotherapy with mite monomeric allergoid. *Int J Immunopathol Pharmacol.* 2012;25(3):671–679.
3. Di Gioacchino M, Perrone A, Petrarca C, et al. Early cytokine modulation after the rapid induction phase of sublingual immunotherapy with mite monomeric allergoids. *Int J Immunopathol Pharmacol.* 2008;21(4):969–976.
4. Quercia O, Bruno ME, Compalati E, et al. Efficacy and safety of sublingual immunotherapy with grass monomeric allergoid: comparison between two different treatment regimens. *Eur Ann Allergy Clin Immunol.* 2011;43(6):176–183.
5. Passalacqua G, Albano M, Fregonese L, et al. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. *Lancet.* 1998;351(9103):629–632.
6. Baris S, Kiykim A, Ozen A, et al. Vitamin D as an adjunct to subcutaneous allergen immunotherapy in asthmatic children sensitized to house dust mite. *Allergy.* 2014;69(2):246–253.
7. Krishna MT, Huissoon AP. Clinical immunology review series: an approach to desensitization. *Clin Exp Immunol.* 2011;163(2):131–146.
8. Kendall M, Mitchell TJ, Costigan G, et al. Downregulation of IgE antibody and allergic responses in the lung by epidermal biolistic microparticle delivery. *J Allergy Clin Immunol.* 2006;117(2):275–282.
9. Fili L, Cardilicchia E, Maggi E, et al. Perspectives in vaccine adjuvants for allergen-specific immunotherapy. *Immunol Lett.* 2014;161(2):207–210.
10. Bagnasco M, Passalacqua G, Villa G, et al. Pharmacokinetics of an allergen and a monomeric allergoid for oromucosal immunotherapy in allergic volunteers. *Clin Exp Allergy.* 2001;31(1):54–60.
11. Veenbergen S, Samsom JN. Maintenance of small intestinal and colonic tolerance by IL-10-producing regulatory T cell subsets. *Curr Opin Immunol.* 2012;24(3):269–276.
12. Artis D. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat Rev Immunol.* 2008;8(6):411–420.
13. Bron PA, van Baaren P, Kleerebezem M. Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. *Nat Rev Microbiol.* 2012;10: 66–78.
14. Looijer-van Langen MAC, Dieleman LA. Prebiotics in chronic intestinal inflammation. *Inflamm Bowel Dis.* 2009;15(3):454–462.
15. Penders J, Stobberingh EE, van den Brandt PA, et al. The role of the intestinal microbiota in the development of atopic disorders. *Allergy.* 2007;62(11):1223–1236.
16. Prescott SL, Wiltschut J, Taylor A, et al. Early markers of allergic disease in a primary prevention study using probiotics: 2.5-year follow-up phase. *Allergy.* 2008;63(11):1481–1490.
17. Prescott SL, Dunstan JA, Hale J, et al. Clinical effects of probiotics are associated with increased interferon-gamma responses in very young children with atopic dermatitis. *Clin Exp Allergy.* 2005;35(12):1557–1564.
18. Kalliomäki M, Kirjavainen P, Eerola E, et al. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol.* 2001;107(1):129–134.
19. Borchers AT, Selmi C, Meyers FJ, et al. Probiotics and immunity. *J Gastroenterol.* 2009;44(1):26–46.
20. Smelt MJ, de Haan BJ, Bron PA, et al. L. plantarum, L. salivarius, and L. lactis attenuate Th2 responses and increase Treg frequencies in healthy mice in a strain dependent manner. *PLoS One.* 2012;7(10):e47244.
21. Torii A, Torii S, Fujiwara S, et al. Lactobacillus Acidophilus strain L-92 regulates the production of Th1 cytokine as well as Th2 cytokines. *Allergol Int.* 2007;56(3):293–301.
22. Niers LEM, Timmerman HM, Rijkers GT, et al. Identification of strong interleukin-10 inducing lactic acid bacteria which down-regulate T helper type 2 cytokines. *Clin Exp Allergy.* 2005;35(11):1481–1489.
23. Hisbergues M, Magi M, Rigaux P, et al. In vivo and in vitro immunomodulation of Der p 1 allergen-specific response by Lactobacillus plantarum bacteria. *Clin Exp Allergy.* 2007;37:1286–1295.
24. Kekkonen RA, Kajasto E, Miettinen M, et al. Probiotic *Leuconostoc mesenteroides* ssp. *cremoris* and *Streptococcus Thermophilus* induce IL-12 and IFN-gamma production. *World J Gastroenterol.* 2008;14(8):1192–1203.
25. Kato I, Tanaka K, Yokokura T. Lactic acid bacterium potently induces the production of interleukin-12 and interferon-gamma by mouse splenocytes. *Int J Immunopharmacol.* 1999;21(2):121–131.
26. Peluso I, Fina D, Caruso R, et al. Lactobacillus paracasei subsp. paracasei B21060 suppresses human T-cell proliferation. *Infect Immun.* 2007;75(4):1730–1737.
27. Ménard S, Candalh C, Bambou JC, et al. Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. *Gut.* 2004;53(6):821–828.
28. Enomoto M, Noguchi S, Hattori M, et al. Oral administration of Lactobacillus plantarum NRIC0380 suppresses IgE production and induces CD4(+)CD25(+)Foxp3(+) cells in vivo. *Biosci Biotechnol Biochem.* 2009;73(2):457–460.

29. Lyons A, O'Mahony D, O'Brien F, et al. Bacterial strain-specific induction of Foxp3+ T regulatory cells is protective in murine allergy models. *Clin Exp Allergy*. 2010;40(5):811–819.
30. Karimi K, Inman MD, Bienenstock J, et al. Lactobacillus reuteri-induced regulatory T cells protect against an allergic airway response in mice. *Am J Respir Crit Care Med*. 2009;179(3):186–193.
31. Nonaka Y, Izumo T, Izumi F, et al. Antiallergic effects of Lactobacillus pentosus strain S-PT84 mediated by modulation of Th1/Th2 immunobalance and induction of IL-10 production. *Int Arch Allergy Immunol*. 2008;145(3):249–257.
32. Von der Weid T, Bulliard C, Schiffrin EJ. Induction by a lactic acid bacterium of a population of CD4(+) T cells with low proliferative capacity that produce transforming growth factor beta and interleukin-10. *Clin Diagn Lab Immunol*. 2001;8(4):695–701.
33. Feleszko W, Jaworska J, Rha RD, et al. Probiotic-induced suppression of allergic sensitization and airway inflammation is associated with an increase of T regulatory-dependent mechanisms in a murine model of asthma. *Clin Exp Allergy*. 2007;37(4):498–505.
34. Ivory K, Chambers SJ, Pin C, et al. Oral delivery of Lactobacillus casei Shirota modifies allergen-induced immune responses in allergic rhinitis. *Clin Exp Allergy*. 2008;38(8):1282–1289.
35. Mohamadzadeh M, Olson S, Kalina WV, et al. Lactobacilli activate human dendritic cells that skew T cells toward T helper 1 polarization. *PNAS, USA*. 2005;102(8):2880–2885.
36. Mann ER, Landy JD, Bernardo D, et al. Intestinal dendritic cells: their role in intestinal inflammation, manipulation by the gut microbiota and differences between mice and men. *Immunol Lett*. 2013;150(1–2):30–40.
37. Berenson LS, Ota N, Murphy KM. Issues in T-helper 1 development--resolved and unresolved. *Immunol Rev*. 2004;202:157–174.
38. Levings MK, Bacchetta R, Schulz U, et al. The role of IL-10 and TGF-beta in the differentiation and effector function of T regulatory cells. *Int Arch Allergy Immunol*. 2002;129(4):263–276.
39. West CE, Hammarström ML, Hernell O. Probiotics in primary prevention of allergic disease--follow-up at 8-9 years of age. *Allergy*. 2013;68(8):1015–1020.
40. Wickens K, Black P, Stanley T V, et al. A protective effect of Lactobacillus rhamnosus HN001 against eczema in the first 2 years of life persists to age 4 years. *Clin Exp Allergy*. 2012;42(7):1071–1079.
41. Kalliomäki M, Salminen S, Poussa T, et al. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet*. 2003;361(9372):1869–1871.
42. Rautava S, Kainonen E, Salminen S, et al. Maternal probiotic supplementation during pregnancy and breast-feeding reduces the risk of eczema in the infant. *J Allergy Clin Immunol*. 2012;130(6):1355–1360.
43. Tang MLK, Ponsonby AL, Orsini F, et al. Administration of a probiotic with peanut oral immunotherapy: A randomized trial. *J Allergy Clin Immunol*. 2015.
44. Gore C, Custovic A, Tannock GW, et al. Treatment and secondary prevention effects of the probiotics Lactobacillus paracasei or Bifidobacterium lactis on early infant eczema: randomized controlled trial with follow-up until age 3 years. *Clin Exp Allergy*. 2012;42(1):112–122.
45. Drago L, Toscano M, De Vecchi E, et al. Changing of fecal flora and clinical effect of L. salivarius LS01 in adults with atopic dermatitis. *J Clin Gastroenterol*. 2012;46 Suppl: S56–63.
46. Wassenberg J, Nutten S, Audran R, et al. Effect of Lactobacillus paracasei ST11 on a nasal provocation test with grass pollen in allergic rhinitis. *Clin Exp Allergy*. 2011;41(4):565–573.
47. Vandenplas Y. No effect of oral treatment with an intestinal bacterial strain, Lactobacillus rhamnosus (ATCC 53103), on birch-pollen allergy: a placebo-controlled double-blind study. *J Pediatr Gastroenterol Nutr*. 2002;35:587–588.
48. Perrin Y, Nutten S, Audran R, et al. Comparison of two oral probiotic preparations in a randomized crossover trial highlights a potentially beneficial effect of Lactobacillus paracasei NCC2461 in patients with allergic rhinitis. *Clin Transl Allergy*. 2014;4(1): 1.
49. Taylor A, Verhagen J, Akdis CA, et al. T regulatory cells in allergy and health: a question of allergen specificity and balance. *Int Arch Allergy Immunol*. 2004;135(1):73–82.
50. Ahangarani RR, Janssens W, VanderElst L, et al. In vivo induction of type 1-like regulatory T cells using genetically modified B cells confers long-term IL-10-dependent antigen-specific unresponsiveness. *J Immunol*. 2009;183(12):8232–8243.
51. Petrarca C, Clemente E, Toto V, et al. rBet v 1 immunotherapy of sensitized mice with Streptococcus thermophilus as vehicle and adjuvant. *Hum Vaccin Immunother*. 2014;10(5):1228–1237.
52. Petrarca C, Lazzarin F, Lanuti P, et al. Lactobacillus paracasei Lp6 favors immune modulation induced by allergoid treatment in ragweed sensitized mice. *Int J Immunopathol Pharmacol*. 2011;24(4):881–893.
53. Adel-Patient K, Ah-Leung S, Creminon C, et al. Oral administration of recombinant Lactococcus lactis expressing bovine beta-lactoglobulin partially prevents mice from sensitization. *Clin Exp Allergy*. 2005;35(4):539–546.
54. Daniel C, Repa A, Wild C, et al. Modulation of allergic immune responses by mucosal application of recombinant lactic acid bacteria producing the major birch pollen allergen Bet v 1. *Allergy*. 2006;61(7):812–819.
55. Huibregtse IL, Snoeck V, de Creus A, et al. Induction of ovalbumin-specific tolerance by oral administration of Lactococcus lactis secreting ovalbumin. *Gastroenterology*. 2007;133(2):517–528.
56. Schwarzer M, Repa A, Daniel C, et al. Neonatal colonization of mice with Lactobacillus plantarum producing the aeroallergen Bet v 1 biases towards Th1 and T-regulatory responses upon systemic sensitization. *Allergy*. 2011;66(3):368–375.
57. Schabussova I, Hufnagl K, Tang MLK, et al. Perinatal maternal administration of lactobacillus paracasei ncc 2461 prevents allergic inflammation in a mouse model of birch pollen allergy. *PLoS One*. 2012;7(7):e40271.
58. Kruisselbrink A, Heijne Den Bak-Glashouwer MJ, Havenith CE, et al. Recombinant Lactobacillus plantarum inhibits house dust mite-specific T-cell responses. *Clin Exp Immunol*. 2001;126(1):2–8.
59. Charng Y, Lin C, Hsu C. Inhibition of allergen-induced airway inflammation and hyperreactivity by recombinant lactic-acid bacteria. 2006;24(33-34):5931–5936.
60. Cortes-Perez NG, Ah-Leung S, Bermúdez-Humarán LG, et al. Allergy therapy by intranasal administration with recombinant Lactococcus lactis Producing bovine beta-lactoglobulin. *Int Arch Allergy Immunol*. 2009;150(1):25–31.
61. Ogita T, Nakashima M, Morita H, et al. Streptococcus Thermophilus ST28 ameliorates colitis in mice partially by suppression of inflammatory Th17 cells. *J Biomed Biotechnol*. 2011;2011:378417.
62. Shimosato T, Tohno M, Sato T, et al. Identification of a potent immunostimulatory oligodeoxynucleotide from Streptococcus Thermophilus lacZ. *Anim Sci J*. 2009;80(5):597–604.
63. Repa A, Granette C, Daniel C, et al. Mucosal co-application of lactic acid bacteria and allergen induces counter-regulatory immune responses in a murine model of birch pollen allergy. *Vaccine*. 2003;22(1):87–95.
64. Jacobsen EA, Lesuer WE, Willetts L, et al. Eosinophil activities modulate the immune/inflammatory character of allergic respiratory responses in mice. *Allergy*. 2014;69(3):315–327.

65. Ohkouchi K, Kawamoto S, Tatsugawa K, et al. Prophylactic effect of *Lactobacillus* oral vaccine expressing a Japanese cedar pollen allergen. *J Biosci Bioeng*. 2012;113(4):536–541.
66. Ai C, Zhang Q, Ren C, et al. Genetically engineered *Lactococcus lactis* protect against house dust mite allergy in a BALB/c mouse model. *PLoS One*. 2014;9(10):e109461.
67. Ren C, Zhang Q, Wang G, et al. Modulation of peanut-induced allergic immune responses by oral lactic acid bacteria-based vaccines in mice. *Appl Microbiol Biotechnol*. 2014; 98(14):6353–6364.
68. Ozdemir O. Various effects of different probiotic strains in allergic disorders: an update from laboratory and clinical data. *Clin Exp Immunol*. 2010;160(3):295–304.
69. Rigaux P, Daniel C, Hisbergues M, et al. Immunomodulatory properties of *Lactobacillus plantarum* and its use as a recombinant vaccine against mite allergy. *Allergy*. 2009;64(3):406–414.