

Anti-allergy properties of fermented foods: an important immunoregulatory mechanism of lactic acid bacteria?

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Abstract

Clinical reports have suggested that dietary consumption of fermented foods, such as yogurt, can alleviate some of the symptoms of atopy and might also reduce the development of allergies, possibly via a mechanism of immune regulation. Controlled studies have indicated that consumption of fermented milk cultures containing lactic acid bacteria (LAB) can enhance production of Type I and Type II interferons at the systemic level. In animal models, LAB have been shown to promote interferon expression, and to reduce allergen-stimulated production of IL-4 and IL-5 in some cases. Recent results have shown that LAB are potent inducers of pro-interferon monokines (IL-12 and IL-18), and that cytokine secretion is stimulated by the interaction of Gram-positive cell wall components with surface receptors of mononuclear phagocytes, via NF- κ B and STAT signalling pathways. However, it is clear that the extent and quality of LAB-induced immunoregulation is strain-dependent. This review discusses the clinical and laboratory evidence for anti-allergy properties of fermented foods, and proposes a model for the mechanism by which some well-defined strains of immunoregulatory LAB might down-regulate a Th2 allergic phenotype. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

At the beginning of the last century, Metchnikoff [1] observed that the consumption of fermented foods could be beneficial to health. He further proposed that the health-promoting properties of such foods were imparted by fermentative microbes. It is now

understood that lactic acid bacteria (LAB) present in fermented foods are primarily responsible for imparting health benefits, and various strains of dietary LAB have been shown to benefit a number of host physiological responses, including immune function [2–4].

Most recently, it has emerged that the mode in which LAB influence the immune system may be utilized to combat human pathologies that have an immunological component [5,6]. In his original thesis, it is unlikely that Metchnikoff had considered a role for fermented foods in combating allergies. Yet over the last decade there has been accumulating evidence that the consumption of fermented foods,

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particularly milk-based dairy foods, can alleviate some of the symptoms of atopy and may limit allergy development. While such a paradigm remains controversial, this review seeks to explain the purported anti-allergy mechanisms of fermented foods in terms of our current understanding of the immunoregulatory properties of LAB.

2. Clinical evidence for fermented foods and LAB in combating allergies

Dietary studies have suggested that long-term consumption of yogurt can reduce some of the clinical symptoms of allergy in adults with atopic rhinitis or nasal allergies, and can lower serum levels of IgE, particularly among the elderly [7–9]. In contrast, Wheeler et al. [10,11] reported that normal yogurt had no effect on atopy or immune parameters, although adult asthmatics who consumed *Lactobacillus*-supplemented yogurt demonstrated a longitudinal trend toward reduced eosinophilia and increased production of interferon-gamma (IFN γ). In addition to studies on adults, a recent report [12] indicated that milk whey formula supplemented with *L. rhamnosus* (strain L. GG) could alleviate some aspects of atopy and intestinal inflammation among infants with allergic symptoms (atopic eczema or food allergy). Furthermore, clinical scores of eczema were reduced in breast-fed infants whose mothers consumed L. GG.

The mode by which allergies might be reduced by consuming fermented foods is uncertain. Several theories exist, including the ability of LAB to enzymatically hydrolyze allergenic food molecules, or to stabilize the gut mucosa sufficiently to reduce systemic uptake of food-borne allergens [13,14]. While no theory is exclusive, the most widely accepted belief follows a different approach: namely, that some LAB-containing fermented foods are able to promote deviation away from a pro-allergy phenotype via a mechanism of immunoregulation.

Aside from alleviating atopy, it is also possible that fermented foods containing LAB could serve to limit the establishment of an allergic phenotype during neonatal development [15]. The ‘hygiene hypothesis’ [16] proposes that, as a consequence of sterile diets and increasingly stringent hygiene practices, a lack of exposure to appropriate dietary and environ-

mental microbes early in life may be contributing to the observed rise in allergies among Western populations. In support of this theory, Alm et al. [17] recorded lower incidences of childhood allergy among Scandinavian families who consumed ‘traditional’ fermented foods containing lactobacilli than among case-matched controls who did not consume fermented foods (although the authors were unable to prove a causal relationship between diet and allergy). While the ‘hygiene hypothesis’ has yet to be tested, it is possible that signals generated by microbial exposure, including dietary LAB, could serve to prime the developing immune system ‘appropriately’, i.e. to prevent the overexpression of an allergic phenotype in early life.

3. The immunology of atopy: a potential role for fermented foods and LAB in regulating anti-allergy immune responses

Most allergic diseases reflect an imbalance in lymphocyte-governed immunity, with immune responses to allergenic molecules becoming overly biased toward a Th2 phenotype (T helper 2 lymphocyte-dominated responses). Secretion of the cytokines IL-4 (interleukin-4), IL-5 and IL-13 by allergen-sensitized Th2 cells recruits granular effector cells such as eosinophils, basophils and mast cells to the site of allergic inflammation [18–20]. These effector cells, alone or in combination with cytophilic/reaginic IgE class antibodies, promote the clinical manifestations of allergy and atopy [21,22]. In addition, IL-4 and IL-13 promote B lymphocyte immunoglobulin isotype switching to IgE [23], and serve to increase circulating levels of total and allergen-specific IgE [24–27]. However, during the early stages of T lymphocyte differentiation, it is possible that the overexpression of a Th2 phenotype can be arrested by cross-regulatory cytokines. Interferons, particularly IFN γ , can down-regulate IL-4 expression and reduce B cell immunoglobulin isotype switching [24,27]. Type I interferon (IFN α) can act as an augmentative signal for IFN γ production, can promote Th1-type immune responses and reduce IgE production [24,28,29].

Significantly, it has been well-documented that fermented foods or LAB can enhance the expression

and secretion of both Type I and Type II interferons. Enhanced levels of IFN γ have been detected in sera of healthy subjects who consumed yogurt for 3 months [30]. Peripheral blood leukocytes of both healthy and allergy-prone subjects who consumed yogurt showed enhanced capacity to produce IFN γ in in vitro cell culture [8,11,30,31], while mononuclear cells showed enhanced levels of the pro-interferon enzyme 2'-5'A synthetase [32,33]. Furthermore, adults who consumed defined immunomodulating LAB strains showed enhanced capacity for production of IFN α from their peripheral blood leukocytes [34,35]. Thus, it appears that the immunoregulatory action of LAB, present in fermented foods, is primarily responsible for enhancing interferon production at the systemic level. In theory, this mechanism could serve to regulate the overexpression of Th2-type immune responses in vivo and thus help to combat allergic responses. But what immunological evidence is there to support this hypothesis, and how might such a mechanism be effected?

4. Laboratory studies of immunoregulation by fermented foods and LAB

The ability of dietary consumption of fermented foods (or foods containing LAB) to modulate immune responses has been thoroughly studied in animal models of disease. Dietary consumption of *Lactobacillus* and *Bifidobacterium* strains has been shown to enhance protection against intracellular bacterial pathogens and to control tumor growth in mice [36–40]. These physiological responses have been shown to correspond with enhanced cell-mediated immune responses that have a Th1 cell bias, including T lymphocyte proliferative ability, mononuclear cell phagocytic capacity and natural killer (NK) cell tumoricidal activity [5,37–40]. Furthermore, oral or systemic delivery of LAB to mice has been shown to be a potent signal for activation of macrophage and NK cell cytotoxicity function, and the induction of Type I and Type II interferons [41–45]. In keeping with the concept that some strains of LAB can promote Th1-type responses preferentially, dietary consumption of several species of *Lactobacillus* has been shown to enhance the

capacity of murine splenic leukocytes to produce IFN γ following mitogenic stimulation, while IL-4 or IL-5 production is unaffected [46–49].

The ability of LAB diet-enhanced interferon production to regulate Th1/Th2-type responses has recently been studied using antigen-primed animals. Splenocytes from ovalbumin (OVA)-primed mice which had been fed *L. casei* produced enhanced levels of IFN γ when restimulated with antigen in vitro, but reduced levels of IL-4 or IL-5 [50]. Feeding OVA-primed mice with *L. casei* has also been shown to reduce antigen-specific IgE responses in vivo, as assessed by passive cutaneous anaphylaxis [51]. A murine model of food allergy has also demonstrated that systemic (intraperitoneal) administration of *L. plantarum* can down-regulate casein-specific IgE antibody levels in vivo, as well as the in vitro capacity of splenic lymphocytes to secrete IL-4 [52].

This latter result reinforces the concept that the mechanisms by which LAB regulate anti-allergy immune responses can be effected at the systemic level, regardless of the route of administration [53]. It is presumed that dietary consumption of fermented milk products facilitates the interaction of immunoregulating LAB with cells of the gut-associated lymphoid tissue (GALT) system, and there is plenty of evidence for a direct interaction of LAB with intra-intestinal lymphoid foci [54–56]. Indeed, recent studies have shown that LAB can potentiate the expression of cytokine receptors on interferon-activated human intestinal cells [57], and can regulate cellular immune phenotype and cytokine expression at intestinal sites following oral delivery in mice [58,59]. Clearly, the ability of dietary LAB to regulate immunity at a systemic as well as local level is an important factor in their potential ability to combat allergic responses.

The precise mechanisms by which LAB stimulate cells of the immune system has therefore been investigated extensively, and the ability of LAB to modulate immune responses pertinent to allergy has also been studied using in vitro immunological models. Co-culturing LAB with human or rodent leukocytes has been shown to augment the production of IFN γ by mitogen-stimulated mononuclear cells, or to induce IFN α production by isolated macrophages [34,47,60,61]. Using spleen cells derived from mice primed in vivo to OVA, the inclusion of *L. casei* in

in vitro culture has been shown to enhance antigen-stimulated production of IFN γ , and concomitantly reduce the production of IL-4, total and antigen-specific IgE [51,62]. Thus, in vitro studies have indicated that the direct interaction of LAB with leukocytes is sufficient to initiate immunoregulatory signals. Further studies have sought to identify which cells are important in effecting this response, and to define the mechanism underlying the ability of LAB to regulate immunity.

5. Lymphocyte-mediated production of IFN γ : the essential role of pro-interferon cytokines produced by accessory cells

In vitro studies have indicated that T lymphocytes are important in the production of IFN γ . Aatouri and Lemmonier [33] demonstrated that *L. acidophilus* induced IFN γ production when co-cultured with murine splenic leukocytes, but that this could be reduced by 76% and 62% following the addition to culture of neutralizing antibodies to CD4 or CD8, respectively. A further report indicated that T cell-enriched fractions (nylon wool non-adherent cells) produced IFN γ in response to co-culture with *L. casei*, but that this was absolutely dependent on the presence of adherent accessory cells; enriched T cells would not produce IFN γ in the absence of accessory cells, even if stimulated with mitogen and exposed to *L. casei* [47]. Haller et al. [63] have recently demonstrated that LAB can also induce IFN γ production by isolated human NK cells, but that co-cultured macrophages are required for this effect.

It has recently emerged that the production of IFN γ by lymphoid cells contacting LAB is dependent on the production of pro-interferon cytokines produced by accessory cells. In particular, IL-12 has been shown to be an important pro-interferon cytokine, since neutralizing antibody to IL-12 significantly suppressed the ability of LAB to stimulate IFN γ production in mixed cultures of enriched T cells and accessory cells [47]. Furthermore, while adding *L. casei* to in vitro cultures of OVA-responsive murine splenocytes suppressed antigen-stimulated production of allergen-promoting molecules (IL-4, IL-5 and IgE), the inclusion of neutralizing anti-IL-12 antibody was able to restore the allergenic

profile and completely ablated the immunoregulatory potential of *L. casei* [62]. Additional studies have indicated that some immunoregulatory LAB strains are potent inducers of IL-12, and can stimulate IL-12 production by murine splenic leukocyte cultures or isolated adherent mononuclear phagocytes in a dose-dependent manner [47,50,62]. Tejada-Simon et al. [48] have recently demonstrated that some strains of *Lactobacillus* can potentiate IL-12 production by murine peritoneal cells following oral gavage. Systemic delivery of *L. plantarum* has additionally been shown to increase plasma levels of IL-12 in mice, the first indication of the in vivo ability of immunoregulatory LAB to induce pro-interferon cytokines [52].

Interleukin-12 is known to be an effective cytokine during the early differentiation of Th0 cells, promoting development of Th1 cells and augmenting NK cell function [64]; both of these actions serve to increase IFN γ producing capacity, and can thus suppress the overexpression of a Th2 phenotype [65]. IL-12 has also been demonstrated to regulate IL-4 production by human allergen-specific T cell clones [66], and thus plays a critical role in limiting both the establishment and maintenance of Th2-type responses. Recent in vitro studies have indicated that LAB are potent stimulants for IL-12 production by human mononuclear leukocytes or isolated monocytes [67,68]. In addition, some *Lactobacillus* strains stimulate the production of IL-18 by human leukocytes [67]; IL-18 acts synergistically with IL-12 to enhance IFN γ production and to promote a Th1 phenotype [28]. Thus, the emerging scenario is that immunoregulatory LAB stimulate the production of pro-interferon monokines (IL-12 and IL-18) which, in conjunction with IFN α , induce production of IFN γ ; this biases a developing T lymphocyte-mediated immune response toward a Th1 phenotype and, more importantly, away from a pro-allergy Th2 phenotype.

6. Strain-dependent immune regulation by LAB: a caveat to the anti-allergy paradigm

It is thus clear that some strains of dietary LAB are able to regulate immunity in a way that can suppress pro-allergy immune responses, and that this

is most likely effected by their ability to bias the immune profile via the induction of appropriate (Th1-promoting) immunoregulatory cytokines. However, in keeping with the theme, it should be borne in mind that LAB have also been shown to be potent inducers of pro-inflammatory and other immunoregulatory cytokines, besides pro-interferon molecules [5,48,59,69–71]. In particular, some LAB strains are effective stimulants of IL-10 production by both murine and human mononuclear cells [67,68,72,73]. Since IL-10 is a potent deactivating signal for cytokine production and can suppress IFN γ -production and Th1 phenotype expression [74,75], it seems at odds with current immunological theory that LAB could provide the same potential stimulant for both pro- and anti-Th1 regulatory signals.

The answer seems to be in qualitative rather than absolute terms. Certainly, many strains of dietary LAB have been shown to have immunomodulatory potential, but not all promote Th1-type responses. Indeed, some strains of lactobacilli have been shown to enhance intestinal tract IgA (immunoglobulin A) or circulating IgG1 responses to foreign antigens in vivo [56,76,77], or to stimulate IL-5 secretion following co-culture with a thymoma cell line in vitro [78]. What emerges is that different strains of immunoregulatory LAB can express their regulation in different ways. Recent in vitro studies using human mononuclear cells have indicated that there are strain-dependent differences in the ability of LAB to induce immunoregulatory monokines such as IL-10 or IL-12 [67,68], with as much as a 10-fold difference in the ability of strains to induce either molecule. Hessle et al. [68] have recently shown that for two immunoregulatory strains of *Lactobacillus*, *L. paracasei* 34D induced 4- to 7-fold higher levels of IL-12 than those induced by *L. rhamnosus* 50A, whereas with IL-10 these proportions were reversed, *L. rhamnosus* 50A inducing 3- to 4-fold higher levels of IL-10 than *L. paracasei* 34D. A similar phenomenon has been reported in murine studies, where peritoneal macrophages obtained ex vivo from mice following oral gavage with LAB were shown to produce widely differing levels of IL-12 in in vitro culture, depending on the strain used [48]. What these studies imply is that not all immunoregulatory strains are likely to be effective in promoting Th1-type anti-allergy immune responses. Significantly,

perhaps, those strains which have been shown to be potent inducers of IL-12 have also been shown capable of down-regulating Th2-type effector molecules, e.g. *L. casei* (Shirota strain) and *L. plantarum* L-137 [5,47,51,52,62]. It is the tentative proposal of this review that an important criterion for the future selection of potential anti-allergy LAB strains should be their ability to induce the production of pro-Th1 molecules (IL-12, and possibly IL-18 and IFN α), and that this can be determined rapidly and simply by in vitro mononuclear cell culture.

7. The signaling mechanisms of potential anti-allergy strains of LAB

As described above, it appears that not all strains of immunoregulatory LAB are equal in their ability to regulate pro-Th1/anti-Th2 mechanisms. Interestingly, however, the recent study of Hessle et al. [68] indicated that human leukocytes exposed to *L. rhamnosus* strain 7D (a comparatively high stimulator of IL-10) could be induced to secrete higher levels of IL-12 if IL-10-neutralizing antibodies were added to culture. This opens up the possibility that each strain of immunoregulatory LAB may have the *potential* to promote Th1-type immunity, although its *de facto* ability to do so is functionally impaired. Thus, by characterizing the active molecules that induce pro-Th1 responses, it is again possible that one could select for 'promising' anti-allergy LAB strains by virtue of their expression of the active component(s). But what is this 'component' which stimulates LAB to induce pro-Th1 immunity, and what is the mode of action?

Some studies of the immunomodulatory properties of dietary LAB have drawn the general conclusion that live bacteria are more effective at modulating immunity than killed [79,80]. However, the pro-Th1 immunomodulatory strains *L. casei* Shirota and *L. plantarum* have been shown to down-regulate allergenic molecules (IL-4, IL-5 and IgE) in antigen-primed mice by either oral delivery in viable form, or systemic (intraperitoneal) delivery in heat-killed form [5,47,48,52,62]. Additionally, heat-killed preparations of LAB cells are known to be potent stimulants of IFN α , IL-12 and IL-18 in in vitro cell culture [5,47,51,52,57–63,68,69]. Solis-Pereyra et al.

[69] indicated that cell wall integrity was the critical factor in determining interferon-inducing capacity by LAB.

Lactic acid bacteria are Gram-positive bacilli, and their cell walls comprise a complex mixture of phosphorylated polysaccharides and glycolipid structures, predominant among which are peptidoglycans (PG) and lipoteichoic acids (LTA). These have been shown to be immunologically active. Phosphopolysaccharide of *Lactococcus lactis* has been shown to stimulate IFN γ production by murine leukocytes in vitro [81], while teichoic acid and muramyl dipeptide (a subfraction of peptidoglycan) have been shown to enhance IFN γ production when added to cultures of mitogen-stimulated human mononuclear blood cells [33]. Studies have indicated that Gram-positive cell wall components bind predominantly to leukocyte surface pattern recognition receptors, and are potent stimulators of monokine production via two signaling pathways: directly (via NF- κ B) and indirectly (via IFN α -induced STAT activation) [82–85]. Muramyl dipeptide and lipoteichoic acid can stimulate IL-12 production through the endotoxin receptor (CD14) on mononuclear phagocytes [86], and this is a major conduit through which Gram-positive stimulatory signals are transduced [87,88]. However, other cell surface receptors may also play an important role, including Type I macrophage scavenger receptor and Toll-like receptor 2, both of which have recently been shown to bind cell wall components of pathogenic Gram-positive bacteria [89–91]. While there have been no similar studies conducted using cell wall derivatives of non-pathogenic LAB, it does appear likely that future research in this area will be able to identify the active components of immunoregulatory strains, and to determine whether the relative expression of these components is a reliable correlate for pro-Th1 activity.

8. LAB vs. Gram-positive pathogens: different regulatory responses

As outlined above, cell wall components of pathogenic Gram-positive bacteria display similar ability to non-pathogenic LAB in the induction of immunoregulatory cytokines (e.g., IL-12, IL-18) or pro-inflammatory cytokines (e.g., IL-1, IL-6 and

TNF α), yet the in vivo consequences of exposure to these different microorganisms promotes radically different outcomes (viz. commensal colonization of the GI tract in the case of LAB, vs. pathogenesis and inflammatory responses in the case of pathogenic Gram-positive bacteria). To a large extent, these different patterns reflect the inherent virulence factors and mechanisms of pathogenicity associated with Gram-positive pathogens vs. commensals, yet the impact of cytokine induction by both sets of microorganisms will in turn greatly affect the outcome of any lymphocyte involvement, and consequently influence the pattern of T cell-mediated responses to potential allergens. Recent research has demonstrated that LAB and Group A streptococci stimulate similar patterns of immunoregulatory cytokine production and transcription factor activation in mononuclear phagocytes [67,85], but with different magnitude and kinetics: while LAB stimulate IL-12 production and a delayed, low-level secretion of IFN α , streptococci induce far higher levels of both cytokines, with rapid induction of IFN α and nuclear translocation of transcription factors in response to IL-6 and TNF α production. Miettinen et al. [85] have suggested that this very rapid and high-level expression of cytokines by streptococci could contribute to pathogenesis and tissue inflammation, whereas the more moderate and delayed production of cytokines following exposure to LAB could favor an immunoregulatory profile produced by mononuclear phagocytes.

In addition to pro-interferon cytokine induction in mononuclear phagocytes, it is also possible that LAB might induce cytokine signals in other leukocyte types that contribute to the immunoregulation of allergic responses. In particular dendritic cells (DCs), as potent stimulators of primary immune recognition in virgin (antigen-naïve) T cells, could play a pivotal role in immune dichotomization during T cell sensitization to allergens [92]. Although there are as yet no reports of DCs interacting with nonpathogenic LAB, pathogenic Gram-positive bacteria and bacterial cell wall components are known to induce IL-12 production by DCs in both human and animal model studies [93,94]. DCs express pattern-recognition receptors capable of transducing 'danger signals' from microbes [92], and their functional dichotomization into pro-Th1 or pro-Th2 type antigen-presenting cells [95] would support an active role in anti-allergy

immune activation following appropriate stimulation by LAB. Future research to characterize the ability of non-pathogenic LAB or LAB components to induce IL-12 production in DCs would seem justified, particularly in terms of elucidating the importance of LAB-exposed DCs in T cell sensitization.

9. Summary

In this review, Metchnikoff's original theory that fermented foods can benefit health has been extrapolated to the possibility that fermentative LAB could represent a dietary means of anti-allergy treatment (and possibly prophylaxis), through a mechanism of immunoregulation. We have outlined the empirical evidence that humans who consume fermented foods or products containing LAB can experience some improvements in atopic symptoms, concomitant with an increased capacity for IFN γ production, and we have used this as a basis for proposing a model by which LAB are able to effect anti-allergy immunity (Fig 1). The model proposes that certain strains of LAB induce pro-Th1 responses, primarily through a receptor-mediated cytokine pathway, and that the ability of individual cell wall components to induce pro-interferon cytokines (especially IL-12) at a sub-inflammatory level may be the key determinant of

this immunoregulatory potential. However, it should be realized that other (indirect) LAB-induced modulatory mechanisms may also be operative in combating atopy, such as their ability to liberate *de novo* immunoregulatory peptides from major food proteins via enzymatic hydrolysis; this mechanism has been demonstrated experimentally for some strains of LAB [96,97], and in one case has been shown effective at reducing *ex vivo* IL-4 production by peripheral blood lymphocytes of atopic patients [98].

Certainly, a large body of laboratory-based immunological studies, conducted over the last decade, has indicated that some strains of LAB are indeed potent enhancers of Th1-type immunity, and may therefore offer clinical benefit to allergy sufferers if incorporated into an appropriate delivery vehicle. However, a recent review [99] has highlighted the fact that in many cases definitive proof of LAB immunoregulatory activity is still required from well-designed, appropriately controlled human clinical studies. Nevertheless, the potentially important immunoregulatory role of LAB in allergy treatment and prophylaxis should not be discounted, particularly since dietary supplementation may represent a safe, non-invasive means of immunotherapy. The new decade should see an interesting time ahead for immunologists and clinicians alike who choose to undertake research in this field.

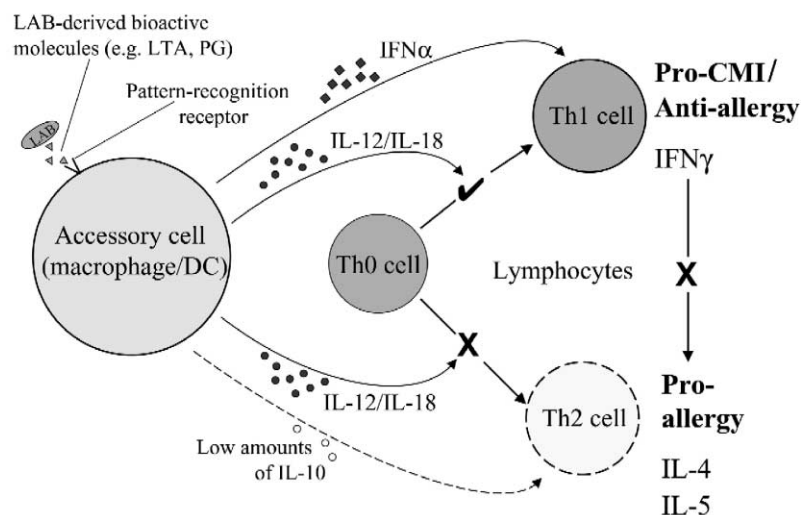


Fig. 1. Theoretical model for anti-allergy mechanisms of immunoregulating lactic acid bacteria (LAB). NB: Th cell TCR interaction with MHC II-bound allergenic peptide has been omitted for clarity.

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