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REVIEW ARTICLE

Any Benefit of Probiotics for Autoimmune Gastrointestinal Diseases?

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Abstract:
Probiotic use as capable preventive and therapeutic strategy in different diseases varying from allergic to autoimmune disease has recently been reported. Three interacting factors including an aberrant intestinal microbiota, a 'leaky' intestinal mucosal barrier, and altered intestinal immune responsiveness have been suggested to be able to create a "perfect environment" for autoimmune disease development. Regulation of intestinal microflora composition by probiotics offers the possibility to influence the development of mucosal/systemic immunity besides autoimmune diseases. Although there is a large amount of conflicting data on the preventive/therapeutic effects of probiotics in ADs; there is fairly promising evidence to recommend as well. Thus, probiotic use cannot be generally recommended for primary prevention and therapy of ADs for now.

Keywords: *Probiotics, autoimmune disease, hygiene hypothesis, yogurt, inflammatory bowel disease, ulcerative colitis*
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Introduction

Experimental and clinical trials of probiotic use as capable preventive and therapeutic strategy in different diseases varying from allergic to autoimmune disease have recently reported. Probiotics are used in allergic disease, which have shown to be beneficial in some patients with atopic dermatitis and allergic rhinitis [1]. Based on the hygiene hypothesis, it has been theorized that changes in human intestinal microflora in developed societies cause an increase in the prevalence of autoimmune disease (AD) besides allergies [2]. Regulation of intestinal microflora composition by probiotics may offer the possibility to influence the development of mucosal/systemic immunity as well as ADs. In this article we will consider the etiology of AD and its relation to gut and environmental microbiota (hygiene) before discussing the mechanisms of probiotic effect and the beneficial effects that they may confer to individuals with AD.

What Causes an AD?

The immune system normally acts to ensure tolerance to 'self', but a breakdown in the tolerogenic pathways has been hypothesized to lead to AD that may result from loss of tolerance to self antigens in general. A breakdown in the tolerogenic pathways can also lead to other so-called inflammatory diseases e.g. atopic and inflammatory bowel disease (IBD). Allergic disease may result from loss of tolerance to food and environmental antigens; IBD may result from loss of tolerance to commensal bacteria within the intestinal tract. The main characteristics of IBD and AD are tissue destruction and functional impairment as a consequence of immunologically mediated mechanisms

which are principally the same as those functioning against dangerous (pathogenic) infections. In case of ADs, a major effort was done in understanding pathogenetic mechanisms leading to the loss of tolerance to self components (autoantigens). Despite the fact that target antigens and the genetic basis of several ADs are now better understood, the initial events leading to a loss of tolerance towards self components remain unknown. One of the most attractive explanations for autoimmune phenomena has always centered on various infections as possible natural events capable of initiating the process in genetically predisposed individuals.

The most accepted conventional hypothesis explaining how infectious components cause autoimmunity is based on the concept of cross-reactivity, "molecular mimicry". This hypothesis assumes a similarity between the epitopes of an autoantigen present in the afflicted organism and the epitopes in the environmental antigen. The latter may consist of a microorganism or another external antigen that causes the autoimmune response. The other hypotheses in the AD pathogenesis such as hygiene-old friends-hypothesis, bystander immunoregulation and T regulatory cell (Treg) defects are briefly discussed below as well.

Because of our long association with environmental organisms (old friends), they are recognized by the innate immune system as harmless or, in the case of some helminths, treated as ''friends'' because a response would merely lead to immunopathology [2]. Therefore, rather than priming aggressive immune responses, these organisms prime immunoregulation. They do it by inducing an unusual pattern of maturation of dendritic cells (DC) such that these retain the ability to drive Treg. Toll-like receptor 2 (TLR2) may be involved for helminths and TLR9 for lactobacilli. It is interesting that polymorphisms of NOD2 (an intracellular receptor for bacterial peptidoglycan) are linked to increased susceptibility to both Crohn's disease and asthma [3]. Thus an extension of the ''hygiene'' mechanism suggests that in an environment that less actively primes Treg activity, immunoregulatory disorders will occur first in those individuals whose innate immune systems are least efficient at driving Treg.

The increased regulatory dendritic cells (DCreg) and Treg induced by ''old friends'' then lead to two immunoregulatory mechanisms mediated in part by release of IL-10 and TGF-β. Firstly, continuing exposure to ''old friends'' will cause continuous background activation of Treg specific for the ''old friends'' themselves, resulting in a constant background of "bystander suppression" [4]. This mechanism has been demonstrated in a model of colitis. Secondly, DCreg inevitably sample self and gut contents and so induce Treg specific for the target antigens of the groups of chronic inflammatory disorder. These mechanisms may be aborted when there are legitimate ''danger'' signals. For example, Treg function can be turned off by appropriate ''danger signals'' in vitro [5].

The unifying hypothesis explaining the simultaneous increase in T helper type 2 (Th2)-mediated allergies and Th1-mediated autoimmunity is that modern living conditions can lead to defective maturation of Treg and regulatory antigen presenting cell or DCreg [6]. Therefore, rather than Th1/Th2 balance, the crucial factor is likely to be the effector T cells/Treg balance. Thus diminished immunoregulation can lead to inappropriate immune responses to allergens, gut contents, or self. In the absence of optimal levels of immunoregulation, the individual may develop a Th1-/Th2-mediated inflammatory disorder, depending on his/her own particular Th1/Th2 bias, immunological history, and genetic background. Evidence to confirm this hypothesis has come from studies of allergic disorders, MS and autoimmune polyglandular syndromes [6].

Any Role for Hygiene (Environmental Microbiota) in AD Development?

According to the old 'hygiene (old friends) hypothesis ', the decreasing incidence of infections in developed and developing countries is at the origin of the increasing incidence of allergic diseases [7]. New practices, introduced as a result of industrialization, such as childbirth by surgical delivery, ingestion of pasteurized food, cleaner homes, and indiscriminate use of antibiotics and so on, have led in recent years to the replacement of probiotics by other microorganisms that are not as well adapted to the microenvironments of the human body. The hygiene hypothesis is based upon epidemiological data, particularly migration studies, showing that subjects migrating from a low-incidence of infections to a high-incidence country acquire the allergic and immune disorders with a high incidence at the first generation as well. Therefore, it was possible to extend the old hypothesis from the field of allergy, where it was formulated, to those of ADs such as T1D or multiple sclerosis (MS) [7,8]. However, some data and others showing a correlation between high AD incidence and high socio-economic level do not prove a causal link between infections and immune disorders. Part of the increased incidence of these diseases may be somewhat

attributed to better diagnosis or improved access to medical facilities in economically developed countries. However, this cannot explain the marked increase in immunological disorder prevalence that has occurred over such a short period of time in those countries, particularly for diseases which can be diagnosed easily, such as T1D or MS.

Proof of principle of the hygiene hypothesis is suported by animal models and to a lesser degree by intervention trials in humans. The incidence of spontaneous T1D is directly correlated with the sanitary conditions of the animal facilities, for both the non-obese diabetic (NOD) mouse and the bio-breeding diabetes-prone (BBDP) rat: the lower the infectious burden, the higher the disease incidence [8,9,10]. Diabetes has a very low incidence and may even be absent in NOD mice bred in 'conventional' facilities, whereas the incidence is close to 100% in female mice bred in specific pathogen-free conditions [11]. Furthermore, BBDP rats subject to Cesarean derivation have been noted to develop accelerated disease due to lack of contamination with microbiota in birth canal [12]. Taken together, these data open new therapeutic perspectives in the prevention of allergic and ADs.

Intestinal Microbiota and their Role in ADs?

Based on hygiene hypothesis, the sudden change in human intestinal microflora may importantly contribute to the rise in the incidence of ADs, observed in the last half a 20th century [7]. More than 17 bacterial families encompassing 500 different microbial species can be found in human adults. These commensal bacteria regulate a myriad of host processes and provide several nutrients to their host and their symbionts within the microbial community. In healthy individuals these relationships are thought to occur in equilibrium. However, disruption of this equilibrium may contribute to a variety of conditions including AD, IBD and atopic disease [13]. This connection is gaining credibility as associations between gut microbiota and either the risk for or presence of a variety of specific human diseases is demonstrated.

Accordingly, the pathogenesis of ADs has been recently thought to involve an interaction between genetically determined host susceptibility, the enteric microbiota and dysregulated immune response. Interactions between the intestinal environment, barrier function, and immune system have been shown to have a major impact in the rate of autoimmunity development. Disruption of intestinal barrier function and aberrant mucosal immune activation has been implicated in a variety of diseases within and outside of the gastrointestinal tract [2,9]. The penetration of gut bacterial antigens into lymphoid tissues is one of the suggested initial factors leading to a loss of tolerance towards self components in genetically predisposed individuals. With this model in mind, recent studies have shown a link between diet, composition of intestinal microbiota, and pathogenesis of ADs. Furthermore, this new paradigm subverts traditional theories underlying autoimmunity development, which are mainly based on molecular mimicry, and suggests that the autoimmune process can be arrested if the interplay between genes and environmental triggers is prevented by re-establishing intestinal barrier function [14].

A hypothesis previously has been proposed involving a trio of interacting factors that may create a ''perfect environment'' for ADs such as type 1 diabetes (T1D) development. These factors include (i) an aberrant intestinal microbiota, (ii) a 'leaky' intestinal mucosal barrier, and (iii) altered intestinal immune responsiveness [15]. In support of this model, modulation of T1D pathogenesis in animal models has proved successful through early intervention with a variety of dietary alterations. Indeed, the administration of a hydrolyzed casein diet or the administration of antibiotics has strengthened the hypothesis that an aberrant microbiota could accelerate disease development. More importantly, this is not a phenomenon that occurs only in rodent models of diabetes, as very recent studies have noted that humans with a propensity to develop T1D as well as other ADs possess an abnormal intestinal barrier; the so called "leaky gut" [8,14]. Together with the gut-associated lymphoid tissue and the neuroendocrine network, the intestinal epithelial barrier, with its intercellular tight junctions, controls the equilibrium between tolerance and immunity to nonself-antigens. Other modulators of tight junction proteins such as certain probiotics may also play a role in modulation of "intestinal leakiness" [8,9,13,15].

What are Probiotics?

Probiotics are usually isolated from the commensal microflora that inhabits the skin and mucosas. And they represent the species of viable microorganisms (bacteria or yeasts) that have a clear beneficial effect on the health of the host thru establishing a true symbiotic relationship with humans for the longest time. Probiotic is derived from the Greek word meaning "supporting or favoring life". The works of Metchnikoff and Tissier were the first to make scientific suggestions about the probiotic use of bacteria,

even if the word "probiotic" was not coined until 1960, to name substances produced by microorganisms which promoted the growth of other microorganisms [16].

Probiotics are first described as selective nonpathogenic living microorganisms or components of bacteria in food supplements, including some commensal bacterial flora, which have beneficial effects on host health and disease prevention and/or treatment [17]. However, experts have debated how to define probiotics. One widely used definition, developed by the World Health Organization and the Food and Agriculture Organization of the United Nations, is that probiotics are "live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host." Probiotics are also defined as ‘mono- or mixed cultures of live microorganisms which, when applied to animal or man, beneficially affect the host by improving the properties of the indigenous microflora’ [16].

A probiotic bacterium is required to fulfill certain criteria to be of benefit [1]. These include being of human origin and having generally regarded as safe status, acid and bile stability, adherence to intestinal cells, persistence for some time in the gut, ability to produce antimicrobial substances, antagonism against pathogenic bacteria, and ability to modulate the immune response. Probiotic activity has been found to be associated with Lactobacilli, Lactococci, Bifidobacteria (longum, infantis), Streptococcus (thermophilus), Enterococcus (faecium), nonpathogenic E. coli (Nissle 1917), Bacillus coagulans and Saccharomyces strains (boulardii and cerevisiae) [1,17]. The most popular lactic acid bacteria are members of the genera Lactobacilli and Lactococci, which have a long history of safe use. Lctb acidophilus is the most well-known probiotic and one of the most important for the health of the small intestine. Other examples of probiotics are Lctb rhamnosus GG (LGG), Lctb gasseri, Lctb fermentum, Lctb salivarius, Bfdbm bifidum and Streptococcus strains include cremoris, faecium and infantis.

The number of commercially available products that are supplemented with probiotics is rising. Dairy products that contain probiotics are sold in every supermarket and probiotic food supplements (for example; capsules, tablets, and powders) can be purchased in pharmacies or via the internet. For infants, infant formulas containing probiotics are also currently available. Live probiotic cultures are available in fermented dairy products and probiotic fortified foods. Examples of foods containing live probiotics are yoğurt, fermented and unfermented milk, miso, tempeh, and some juices and soy beverages. However, tablets, capsules, powders and sachets containing the bacteria in freeze dried form are also available.

A Cultural Product Related to Probiotics: Yogurt (Yoğurt)

Increasing interest has also been paid to the beneficial functions of Lactobacilli in addition to their importance in the preparation process of fermented foods such as yoğurt and cheese. Here, the reason for selecting yoğurt as a probiotic food was several-fold. It can be produced in a sustainable manner locally and therefore doesn't rely on importation, and it provides nutrition and is an excellent carrier for probiotic organisms.

Fermented foods, particularly dairy products like yoğurt, have been consumed for centuries in some cultures including Turkish. A similar health effect is also observed for lactose fermenting starter bacteria such as Lctb delbrueckii ssp. bulgaricus and Streptococcus thermophilus in fermented milk products like yoğurt. However, these traditional starters are not considered probiotics by some researchers since they lack the ability to proliferate in the intestine [16]. Therefore probiotic yoğurt including different probiotic strains (lactobacilli and/or bifidobacteria) than standard one has been produced and become popular in recent literature. Probiotic yoğurt includes a probiotic strain or multistrain probiotics that has been shown to have beneficial effects on the health of the host with HIV/AIDS and diarrhea [18]. Traditional yoğurt was compared in a study with probiotic yoğurt in non-inflammatory acute gastroenteritis. Acute non-inflammatory gastroenteritis improvement is accelerated by probiotic yoğurt consumption [19]. Probiotic yoğurt intake was associated with significant anti-inflammatory effects that paralleled the expansion of peripheral pool of putative T(reg) cells in IBD patients and with few effects in controls [20].

Yoğurt contains viable bacteria culture including Streptococcus thermophilus and Lctb delbrueckii sp. bulgaricus [1]. Although these cultures clearly fulfill the current concept of probiotics, only a small number of these bacteria have been studied. Yet some specifically have been shown to have a probiotic effect [1,21,22]. Health effects of traditional (standard) yoğurt will not be reviewed in detail here; several reviews have already been published on this topic [1,16,21]. Yoğurt has been shown to be successful for reducing the duration of symptoms in acute

non-bloody diarrhea in 6-24-month-old hospitalized infants [23]. Yoğurt feeding was associated with a clinically relevant decrease in stool frequency and duration of diarrhea in children who have reducing sugars in stools [24]. Positive changes in lipid profile were observed in both yoğurt groups [25].

Although there is well-known assumption of longer human life in the cultures consuming frequently yoğurt, the neat probiotic effect of yoğurt on the frequency of ADs is unknown.

Can Probiotics Really Prevent and/or Treat any Type of ADs?

The new version of 'hygiene hypothesis' proposes that reduced exposure to environmental and/or enteric stimuli, including microbes, underlies the rising incidence of childhood ADs [7,15,26]. This hypothesis is supported by data that highlight the importance of infant exposure to environmental microbes for appropriate development of the immune system. This might explain the observation that administration of microbes or their components inhibits AD in animals such as T1D, as mentioned above [2,13-15]. These findings raise the possibility of using live, nonpathogenic microbes (for example, probiotics) or microbial components to modulate or're-educate' the immune system.

For some time now, microbial agents have been implicated in the etiology of ADs, including insulin dependent diabetes mellitus (T1D). Recent studies, however, have revealed that exposure of genetically diabetes-susceptible animals to certain microbes or microbial agents at an early age prevent the induction and progression of disease. This suggests that microbes may act to modulate the immunological status or immune repertoire of an individual genetically programmed for T1D away from an autoimmune response [27]. Immunization with microbial agents at an early age may offer an important new direction for the immunotherapy of T1D [10,28]. The protective effect of a probiotic and a bacterial extract was reported on the onset of diabetes in NOD mice.

Similarly, there is an increasing amount of data showing that intestinal microbiota changes could contribute to the modulation of immune disorders but evidence is still slim, except in IBD. The case of probiotics in IBD is more complex because of the possible local anti-inflammatory effect, which could explain the relief of symptoms without changes in disease progression, as implicated in the hygiene hypothesis. Following a number of uncontrolled studies in a small cohort of 14 pediatric patients with newly diagnosed ulcerative colitis (UC), probiotic treatment induced a significant rate of remission compared to the control group and a lower relapse rate [29].

Supposed Mechanisms of Probiotics` Effects in the Prevention/Treatment of ADs

Some supposed mechanisms of probiotics` effects in the development of autoimmunity defined in the recent literature are discussed below (as summarized and shown in figure 1).

1- Immunoregulation by TGF-β-bearing Treg cells

CD4+/CD25+ - Tregs have shown to be pivotal players in the maintenance of immune tolerance. Their role in the prevention of autoimmunity in animal models and evidence for disturbed or dysfunction of Tregs have also been observed in patients with different ADs, including MS [6]. Recent studies provided evidence that one effect of probiotics may involve induction of differentiation of IL-10-dependent, TGF-β-bearing Tregs [6]. They also can suppress immune responses distinct from responses against the antigen in question, here antigens expressed by infectious agents (a phenomenon called bystander suppression).

2- Development of tolerogenic DCs

Lctb reuteri / casei have been also shown to prime monocyte-derived DCs through the C-type lectin DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) to drive the development of Tregs [30]. These Tregs produce increased levels of IL-10 and are capable of inhibiting the proliferation of bystander T-cells. This study suggests that the targeting of DC-SIGN by certain probiotic bacteria might explain their beneficial effect in the treatment of a number of inflammatory diseases, including AD [1].

3- Reducing proinflammatory cytokines through Th17 cells

Th17 has been also shown as pathogenic cells in some ADs such as experimental autoimmune encephalomyelitis (EAE) and arthritis [31]. Suppression of this newly discovered subset of T cells by probiotics might explain effects observed in different experimental models that all involve inflammatory responses, i.e. colitis. For instance; Lctb casei suppressed experimental arthritis by reducing proinflammatory cytokines released from Th17 cells.

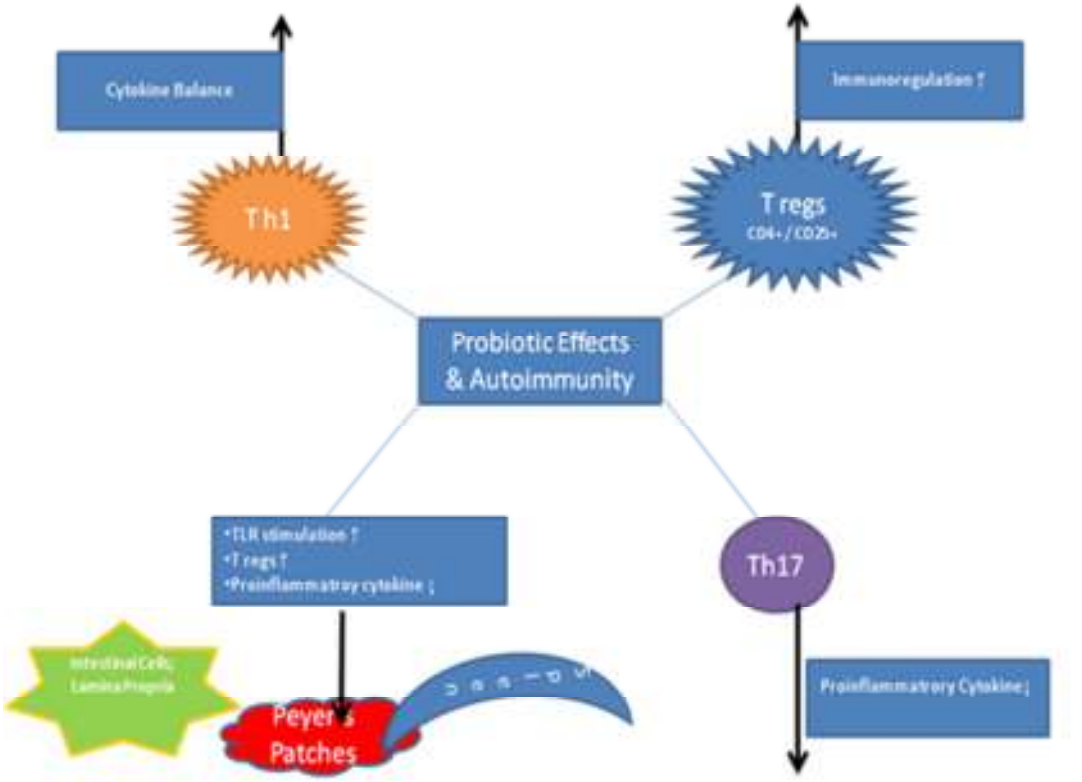

Figure 1. **This figure illustrates some supposed mechanisms of probiotic effects on the development of autoimmunity and autoimmune diseases. Probiotics seem to have a regulatory effect on Treg, Th1, Th17, intestinal cells and splenocytes.**

4- Stimulating Th1 cells

Although there are still some studies showing no significant effects of probiotics on either Th1/Th2 cell responses, certain strains of Lctb and Bfdbm modulate the production of cytokines, and may divert the immune system in a regulatory or tolerant mode. Changes in cytokine profile induced by probiotics may be probiotic strain- or site-specific and dependent on the experimental system used. For instance, Lctb reuteri induced proinflammatory and Th1 cytokines; and Bfdbm bifidum/infantis and Lctb lactis reduced Th2 cytokines and acted as potent inducers of IL-10 production [32].

5- Probiotic regulation in intestinal epithelium and upregulation of host immune responses to defend against infection

Probiotics compete with non-commensal bacteria and eliminate them by secreting antimicrobial products, increase the production of antibodies and macrophage activity and contribute to the appropriate host nutrition by producing some vitamins and by breaking down undigested molecules. These characteristics argue in favor of a symbiotic relationship between humans and probiotics [1]. Probiotic administration in humans and animals has also been shown to be beneficial in the treatment and prevention of intestinal infections and to reduce mucosal inflammation. Their ability to deviate tissue cytokine secretion from a pro-inflammatory to an anti-inflammatory profile has been specifically described. This effect probably results from the ability of probiotics to adhere to mucosal surfaces and inhibit the attachment of other pathogenic bacteria, to secrete factors that enhance barrier integrity, and to modulate cells of the immune system [33].

6- Anti-inflammatory effect of probiotics

The anti-inflammatory effect of probiotics has been attributed to increased production of IL-10 by immune cells in the lamina propria, Peyer's patches and the spleen of treated animals [34]. Moreover, a decrease in the secretion of pro-inflammatory cytokines, IFN-γ, TNF-α and IL-12 has been demonstrated [33].

7- Maturing gut barrier

Recent data indicate that commensal intestinal microbiota represents a major modulator of intestinal homeostasis.

Dysregulation of the symbiotic interaction between intestinal microbiota and the mucosa may result in a pathological condition with potential clinical repercussions. For instance, it is shown that mice reared in germ-free conditions have an underdeveloped immune system and have no oral tolerance. In contrast, pathogen-free mice are capable of reconstituting the bacterial flora with Bfdbm and tolerance development. In addition to providing maturational signals for the gut-associated lymphoid tissue, probiotics balance the generation of pro- and anti-inflammatory cytokines in the gut. After probiotic consumption, decrease in fecal α-1 antitrypsin, serum TNF-α, and changes in TGF-β and other cytokines point to down-regulation of inflammatory mediators [33]. Furthermore, probiotic bacteria may counteract the inflammatory process by stabilizing the gut microbial environment and the permeability barrier of the intestine, and by enhancing the degradation of enteral antigens and altering their immunogenicity. This gut-stabilizing effect of probiotics could be explained by the improvement by probiotics of the immunological barrier of the intestine through intestinal IgA responses, specifically [1,35].

8- Systemic TLR stimulation via non-antigenic ligands

A number of experiments indicate that infectious agents can promote protection from ADs through mechanisms independent of their constitutive antigens, leading to stimulation of non-antigen specific receptors such as TLRs. A family of pattern recognition receptors such as TLRs on gut lymphoid and epithelial cells mediates innate immune responses to bacterial molecular patterns and, thereby, orchestrates acquired immunity. An observation made for TLR-2/-3/-4/-7 and -9 that TLR stimulation could prevent the onset of T1D in NOD mice [9,10,36].

Although the beneficial effects of probiotics on wide variety of diseases have been shown, little is known about how probiotics modulate the immune system and autoimmunity development. Currently, only limited publications are available mentioning the effects of probiotics on ADs in rodent models or human. Therefore, it was important to explore the effect of human probiotics in various autoimmune experimental and clinical disease models. Here, firstly experimental and later clinical studies of probiotics in different ADs under the recent literature gathered from Medline and Pubmed are discussed.

Animal Experimental Models and Human Clinical Trials Describing Supposed Effects of Probiotics in Gastrointestinal ADs

Benefits of probiotic use in firstly animal experimental and later human clinical models of ADs including arthritis, T1D, EAE and IBD will be mentioned correspondingly in this assessment. Presumed favorable effects of probiotics in various ADs reviewed in this article are also shown in Table 1.

Ia- Probiotic Effect in Animal Experimental IBD Models

IBD is a life-long and chronic inflammatory condition of the gastrointestinal tract including the 2 major diseases, Crohn's disease (CD) and UC. A convergence of findings show that intestinal microflora play a central role in the pathogenesis of IBD and thus investigators have pursued studies to seek therapeutic effects of manipulating intestinal microflora. A reduction in microbial burden of gut by public health measures contributes to an immunological imbalance in the intestine, which has been explained by the 'hygiene hypothesis'. The question is posed to determine whether a similar explanation can be proposed for the increased incidence of IBD [9,13-15]. The extension of the hygiene hypothesis to IBD opens new therapeutic perspectives including the revisiting of probiotics and other forms of exposure to bacteria or parasite components.

A number of reports have been published that describe the influence of probiotic consumption on colitis in animal trials. In particular, the IL-10 $^{-/-}$ (knockout) mouse has been extensively studied. IL-10 knockout mice develop colitis when colonized with a conventional flora but remain disease-free when maintained under germ-free conditions. Schultz et al. colonized IL-10$^{-/-}$ mice with *Lctb plantarum 299v* two weeks before transfer from a germ free environment to a specific pathogen-free environment. This resulted in significant attenuation of disease and a significant reduction in mesenteric lymph node IL-12 and IFN-γ production [37].

Madsen et al. demonstrated a role for *Lctb reuteri* in prevention of colitis in IL-10$^{-/-}$ mice. Neonatal IL-10$^{-/-}$ mice were shown to have a decreased concentration of colonic Lctb species and an increased concentration of mucosal adherent bacteria. Oral administration of the prebiotic lactulose (shown to increase the levels of Lctb species) and rectal swabbing with Lctb reuteri restored Lctb levels to normal and reduced the number of adherent bacteria within the colon. These effects were associated with the attenuation of colitis [38].

Table 1. Probiotic effects in animal experimental autoimmune disease models and human clinical autoimmune disease trials

Disease model	Probiotics	Assessment	Outcome	Ref.
	Animal Experimental Inflammatory Bowel Disease Models			
DSS colitis	VSL#3	inflammation	↓disease activity	43
DSS colitis	E. coli Nissle 1917	inflammation	↓disease activity	43
IL-10-/- mice colitis	Lctb salivarius	↓IFN-γ	↓disease activity	39,40
IL-10-/- mice colitis	Bfdbm infantis	↓IFN-γ	↓disease activity	39,41
IL-10-/- mice colitis	Lctb reuteri	inflammation	↓disease activity	38
IL-10-/- mice colitis	Lctb plantarum	↓IL-12, ↓IFN-γ	↓disease activity	37
	Human Clinical Inflammatory Bowel Disease Trials			
Pouchitis	VSL#3	inflammation	↓ disease activity	45,47,48
Pouchitis	LGG	inflammation	↓ disease activity	46,49,50
Active UC	E. coli Nissle 1917	inflammation, induction of remission	↓ disease activity	52,53
Active UC	VSL#3	inflammation, induction of remission	↓ disease activity	45,47,48
Active UC	BbY	endoscopic and histological scores	↓ disease activity	44,45
Active UC	LGG	induction of remission	Ø disease activity	46,49,50
UC remission	VSL#3	maintenance of remission	↓ disease activity	56
UC remission	LGG	maintenance of remission	Ø disease activity	53,57
UC remission	B. breve / bifidum L.acidophilus	maintenance of remission	↓ disease activity	55
Active CD	LGG	inducing remission	Ø disease activity	58,59
Active CD	E. coli Nissle 1917	inducing remission	Ø disease activity	58
CD remission	S. boulardii	maintenance of remission	↓ disease activity	60
CD remission	LGG	maintenance of remission	Ø disease activity	59,61,62
CD remission	Lctb johnsonii	maintaining surgically induced remission	↓ disease activity	63,64
CD remission	VSL#3	maintaining surgically induced remission	↓ disease activity	65
IBS	LGG	inflammation	Ø disease activity	54
IBS	Lctb salivarius	inflammation	Ø disease activity	54
IBS	Bfdbm infantis	inflammation	↓ disease activity	53,54
IBS	Lctb plantarum	abdominal pain, bloating, and constipation	↓ disease activity	53,54
Celiac disease	Lctb sanfranciscensis	proteolytic activity	↓ disease activity	66
Celiac disease	Lctb plantarum	proteolytic activity	↓ disease activity	66
Celiac disease	VSL#3	proteolytic activity	↓ disease activity	67
Celiac disease	Bfdbm lactis	proteolytic activity	↓ disease activity	68

Abbreviations: Bfdbm**: bifidobacterium**; BbY**: Bfdbm breve strain Yakult;** CD**: Crohn`s disease;** DSS**: dextran sulfate sodium colitis;** IBS: **irritable bowel syndrome;**

UC**: ulcerative colitis;** Lctb**: lactobacillus;** LGG**: Lctb GG;** VSL#3: **a mixture of four species of lactobacilli, three species of bifidobacteria and Streptococcus thermophilus;**

↓: decreased in severity of disease; ↑: increased in severity of disease; Ø: no effect on severity of disease.

In another placebo-controlled trial the efficacy of *Lctb salivarius* UCC118 and *Bfdbm infantis* 35624 in attenuation of colitis in the IL-$10^{-/-}$ mouse model was demonstrated. Further studies examined the effect of Bfdbm infantis 35624 on early inflammation in IL-$10^{-/-}$ mice and wild-type mice of the same genetic background. Pronounced changes occurred in the Peyer's patch following probiotic consumption, with IFN-γ reduced in both wild-type and IL-$10^{-/-}$ mice [39].

The oral route of administration may not be required for certain probiotic effects. Reduced inflammatory scores and reduced production of proinflammatory cytokines have been observed in IL-$10^{-/-}$ mice that had been injected subcutaneously with Lctb salivarius UCC118 [40]. Additionally; in order to enhance the probiotic effect in these murine models, investigators have combined probiotic treatment with prebiotics, antibiotics, immunostimulatory DNA sequences or they have genetically engineered the probiotic strain to secrete antiinflammatory mediators. The prebiotic *inulin* and a combination of the probiotic organisms Lctb acidophilus La-5, Lctb delbruckii subsp. bulgaricus, Bfdbm Bb-12, and Streptococcus thermophilus significantly reduced inflammation [41]. The effect was enhanced by combination with metronidazole, suggesting a synergistic effect of the combination of anti- and probiotics in the treatment of experimental colitis [42]. Attenuation of DSS (dextran sulfate sodium) colitis was caused by *VSL#3* DNA mediated through TLR- 9 signaling. Isolated DNA of *E. coli strain Nissle 1917* showed an antiinflammatory effect in the DSS model as well. Interestingly, specific immunostimulatory DNA sequences have also been shown to attenuate the production of proinflammatory cytokines in UC patients [43]. Genetically modified probiotics have been tested for their ability to attenuate colitis in the IL-10 knockout model. *Lactococcus lactis* was engineered to secrete biologically active IL-10. A significant reduction in inflammation was observed in both murine models [44].

Ib- Probiotic Effect in Human Clinical IBD Trials

Studies on the use of probiotics in the treatment of noninfectious inflammatory bowel disorders found that 4 strains of Lctb and 1 strain of Streptococcus were effective in maintaining remission of UC and reducing the postop recurrence of CD. In the randomized controlled trials, 12 of 16 UC but only 2 of CD trials of probiotic therapy were successful. No superiority of any probiotic was clearly evident, but a multi-agent mixture, VSL3# may be better suited in UC and pouchitis [45]. And studies of probiotics e.g. LGG in CD have been disappointing, and a recent Cochrane systematic review has concluded that their use could not be recommended on the available evidence [46].

Pouchitis

The most compelling evidence for the use of probiotics in IBD comes from randomised double-blind placebo controlled trials with *VSL#3* (a mixture of four species of lactobacilli, three species of bifidobacteria and Streptococcus thermophilus) in patients with pouchitis. The efficacy of VSL#3 as a maintenance treatment in 40 patients with chronic relapsing pouchitis after antibiotic-induced remission was assessed. After 4 months fewer relapses were found to occur in the intervention group than in the control group. Moreover, all patients were subsequently found to relapse 3 months after cessation of VSL#3. Later, the same group assessed VSL#3 in the primary prevention of pouchitis in 40 patients following surgery. The incidence of pouchitis was found to be reduced and the quality of life improved in the VSL#3-treated group compared with the placebo group [45]. Finally, a further study has confirmed the effectiveness of VSL#3 as maintenance therapy in patients with recurrent or chronic pouchitis [47]. In contrast, Shen et al. have reported no significant benefit of VSL#3 in maintaining antibiotic-induced remission in 31 patients [48].

Trials of other probiotics in the management of pouchitis have yielded mixed results. One observational study of patients receiving *LGG* after pouch formation has reported a lower rate of pouchitis than in historical controls [49]. However, Kuisma et al. have found no difference in mean pouchitis scores between placebo and LGG-treated groups at the end of a 3-month study period [50]. Finally, a reduction in endoscopic and clinical disease activity associated with an increase in faecal probiotic species has been demonstrated in 51 patients with pouchitis after surgery for UC who consumed fermented milk containing lactobacilli and bifidobacteria [51].

UC

Probiotics to treat active UC

In a study comparing the effect of probiotic E. coli Nissle 1917 vs. mesalamine on induction of remission in UC, both groups had similar time to remission, demonstrating equal efficacy of treatments. Consistently, several controlled trials have demonstrated that *E. coli Nissle 1917* has similar efficacy to conventional mesalazine treatment

with fewer side effects [52]. Efficacy of direct delivery of the probiotic to the colon with E. coli Nissle 1917 enemas in left-sided UC has been demonstrated [53].

In an open-label trial, *VSL#3* was added to current regimen for patients who had failed to respond to conventional therapy for active UC. Addition of VSL#3 for 6 weeks led to either remission or response in 77% of patients as measured by the disease activity index [45].

Active UC was treated with fermented milk including *Bfdbm bifidum strain Yakult* and an *Lctb acidophillus* strain [54]. A recent clinical trial demonstrated that treatment of patients with Bfdbm fermented milk compared to placebo leads to a significant decrease in a clinical activity index score, as well as a significant decrease in endoscopic and histological scores after 12 weeks of treatment [55].

Probiotics as maintenance therapy in UC

Treatment with *VSL#3* to maintain remission in UC was found to be only 4 of 20 patients had experienced relapse at the end of the study [56]. Several studies examining the use of lactobacilli or bifidobacteria as maintenance treatment in UC have demonstrated conflicting results. Ishikawa et al. have demonstrated a reduction in the number of disease exacerbations in a group of Japanese patients receiving fermented milk containing *Bfdbm breve, Bfdbm bifidum* and *Lctb acidophilus* compared with placebo. However, this clinical benefit was not found to be associated with an increase in steroid-free remission or endoscopic improvement in disease activity [78]. Another open-label trial showed that for maintenance of remission in UC that *LGG* alone, or in combination with mesalamine, demonstrated equal efficacy to mesalamine alone [53,57].

CD

Probiotics to treat active CD

Previous two studies with *E. coli Nissle 1917* and *LGG* had evaluated probiotics in active CD patients, but neither study has demonstrated convincing efficacy, in part because of small numbers of patients [58]. A recent double-blinded placebo controlled trial randomized 11 patients with active CD to receive either LGG or placebo. There was no difference in at the rate of inducing remission for 6 months between the two groups [59].

Probiotics to maintain remission in CD

Evidence for use of probiotics as maintenance therapy in CD is not persuasive, with only a couple of studies reporting positive results. A study comparing *S. boulardii*+antibiotic+mesalazine with mesalazine alone has shown fewer relapses in the former group in patients with medically-induced remission of CD [60]. A recent double-blinded placebo controlled trial randomized 11 patients with active CD to receive either LGG or placebo. There was no difference at the rate of sustaining remission for 6 months between the two groups [59]. Another randomized, double blind study compared LGG vs. placebo in addition to standard maintenance therapy in a group of 75 children. These studies did not find any advantage for *LGG* compared with placebo in maintaining medically-induced remission [61].

Several clinical studies have been performed to analyze the effects of probiotics on maintaining surgically-induced remission. Three studies using LGG have not confirmed the effectiveness of this probiotic as a maintenance strategy after surgically-induced remission [61,62]. Another clinical trial utilizing treatment with LGG after surgical resection failed to show prevention of early endoscopic recurrence when compared to placebo. One study suggested modest but not significant improvement in recurrence rates of patients after surgical resection of diseased bowel by *Lctb johnsonii LA1* [63]. However, two randomised double-blind placebo-controlled studies have reported no effect of Lctb johnsonii LA1 in preventing recurrence in CD patients in surgically induced remission [63,64]. Lastly; Campieri et al. have shown benefit of *VSL#3* in preventing post-operative recurrence in 40 patients randomised to 3 months of rifaximin followed by 9 months of VSL#3 or to 12 months of mesalazine [65].

Irritable bowel syndrome

There is no known therapy established to alter the natural history of irritable bowel syndrome (IBS). A series of systematic reviews and meta-analyses have demonstrated that probiotics are more effective than placebo [53]. Recently, researchers demonstrated improvements in IBS symptoms by the addition of *Bfdbm infantis* 35624 in the diet with normalization of the ratio of anti-inflammatory to proinflammatory cytokines. In other clinical trials, *Lctb plantarum* 299v and DSM 9843 strains were shown to reduce abdominal pain, bloating, flatulence, and constipation. These investigators did not find any effect when *Lctb salivarius* UCC4331 was added, similar to

LGG. It was also observed that *Saccharomyces boulardii* decreased only functional diarrhea in IBS but was not effective in alleviating other symptoms of the syndrome [54].

Celiac disease

The only effective treatment for Celiac is a strict adherence to a gluten-free diet throughout the patient's lifetime. Otherwise, wheat gliadin induces severe intestinal symptoms and small-bowel mucosal damage in patients. Gluten-free products are not widely available and are usually more expensive than their gluten-containing counterparts. There is, therefore, an urgent need to develop safe and effective therapeutic alternatives, to develop high-quality gluten-free products and to investigate the potential of the bread making biotechnology following ancient protocols, which include long-time fermentation by selected sourdough LAB. There is a necessity for new biotechnologies using probiotics as starters for sourdough fermentation to investigate their potential to decrease the risk of gluten contamination in gluten-free products.

Thus, 46 strains of sourdough LAB were screened for proteolytic activity and acidification rate in gluten-free flours. The sourdough cultures consisted of *Lctb sanfranciscensis* and *plantarum* were selected and used for the manufacture of gluten-free bread [66]. Moreover, proteolytic activity by probiotic *VSL#3* was also found to have an importance during food processing to produce predigested and tolerated gliadins for increasing the palatability of gluten-free products [67]. In addition; *Bfdbm lactis* was found to inhibit the toxic effects of gliadin in intestinal cell culture conditions [68].

Conflicting Results and Reasons of Failure in Human AD Clinical Trials

Although human clinical trials for therapeutic probiotic use in AD have been somewhat disappointing, human trials are also performed unsatisfactorily. Firstly, one of the main reasons for this failure is that the topic is becoming currently popular and further studies need to be done.

Secondly; since human is a more complex organism than cell cultures and animals, performing a research in human is very difficult. As expected, most of the hopeful data firstly have come from in vitro cell culture studies and experimental animal models.

Thirdly, there are also difficulties of recognizing mechanisms implicated in ADs. As mentioned upper part of this review; there at least several hypotheses for autoimmunity development and there a lot need to be further clarified. Thus, it is very difficult to decide what kind of probiotic strain would be helpful.

Fourthly, it is very hard to measure net effect of probiotic since the effect of probiotic use is specifically dependent upon strain. Consequently, there is also a large amount of conflicting data on the probiotic use in ADs.

Fifthly, there is also fear from possible side effects of probiotics. As mentioned above, the fact that certain probiotics are known to stimulate Th1 immunity, which might be an additional safety issue. Excessive immunostimulation might aggravate or induce Th1-mediated immune responses, e.g. ADs.

Conclusion

As mentioned above, there is a large amount of conflicting data on the preventive/therapeutic effects of probiotics in ADs. Results from metaanalyses and systematic reviews that combine results of studies from different types of probiotics to examine the effects in any disease should be interpreted with caution. There are also difficulties of recognizing etiology and pathogenesis of ADs in which have many mechanisms involved. Similarly, with various strains, especially *Lctb shirota*, stimulation of Th1-mediated immune responses has been described. Additionally, if probiotics are used in patients with ADs for any reason –therapy or prevention- cautionary approach ought to be taken. Thus, probiotics cannot be recommended generally for primary prevention of ADs. Any probiotics should not be used especially in immune-compromised children; even they have at risk for ADs. Finally, there is insufficient but fairly promising evidence to recommend the addition of probiotics to foods for prevention and treatment of ADs.

Five-year view

Involvement of commensal enteric microflora and its components with strong immunoactivating properties in etiopathogenetic mechanism of multifactorial diseases, including IBD and allergy has been recently suggested. Regulation of intestinal microflora composition (e.g. by probiotics) offers the possibility to influence the development of mucosal and systemic immunity as well as it can play a role also in prevention and treatment of some

ADs. Progress has been made by the identification of receptors and pathways through which gut microbes influence development of the immune system. Such mechanistic data have moved a field that was once regarded as being on the scientific fringe to the mainstream, and support increased funding to advance this promising area of research in the hope that it might deliver the long awaited answer of how to safely prevent ADs.

Better understanding of the effects of different probiotic strains and a deeper insight into the mechanisms of the heterogeneous manifestations of AD are needed for the validation of specific strains carrying anti-autoimmune potential. Therefore, research activities are currently focusing on identification of specific probiotic strains with immunomodulatory potential and on how dietary content interacts with the most efficacious probiotic strains. Moreover, the selection of the most beneficial probiotic strain, the dose, and the timing of supplementation still need to be determined. Further studies should also clarify if any susceptible groups of ADs exist and how these groups benefit from supplementation with certain probiotic strains.

Some studies in the management of ADs suggest that therapeutic benefit requires a combination of probiotic species (as with *VSL#3 or Lacto-mix*) or that the component(s) responsible for the anti-inflammatory effect in combination preparations have specific properties that monotherapy probiotics do not. This concept also supports the use of prebiotics that increase concentrations of several commensal immunoregulatory bacteria. Prebiotic use was shown to be associated with a reduction in the faecal concentration of Bacteroides fragilis, but had no effect on lactobacilli or bifidobacteria. Genetically modified probiotics will be tested for their ability to attenuate ADs thru secreting regulatory cytokines in experimental models as well. In near future, the researchers will look for more appropriate combinations of probiotic species or modified probiotics with/without prebiotic and test them in human/rodent AD models.

Additionally, side effects are very low and they might not be nonexistent, as shown in a set of patients with different diseases. However, probiotics should not be considered as totally harmless, particularly in the immunodeficient host, and more safety studies are needed. As imagined, probiotics may have unpredictable behaviour like all microorganisms, such as unanticipated gene expression in nonnative host environment, or acquired mutations occurring spontaneously via bacterial DNA-transfer mechanisms. Certain probiotics are known to stimulate Th1 immunity, which has been suggested as one of the mechanisms by which they can suppress Th2-mediated allergic diseases. However, this presumed excessive immunostimulation might aggravate or induce Th1-mediated immune responses and diseases such as T1D, MS; and it might cause an additional safety issue.

Consequence of over-activation of the immune system by probiotics in hosts with immune dysfunctions, such as individuals genetically predisposed to autoimmunity, has raised some concerns too. With respect to the association between bacterial antigens and autoimmune responses and the adjuvant activity of LAB strains, the involvement of LAB in the pathogenesis of some models of autoimmunity in experimental animals and possibly in humans has been suggested. Thus, from a safety point of view, the potential of probiotic bacteria (especially the immunostimulatory strains), to induce destructive inflammation or autoimmunity needs to be investigated. For instance, it has been experimentally demonstrated that *Lctb casei* cell wall components (given intraperitoneally) are able to induce cardioangitis (an autoimmunity-associated heart disease) in mice [69].

Key issues

- Since conclusions on probiotics are limited to specific strains and models, they should not be generalized [32].
- Probiotics should not be considered as completely harmless, particularly in the immunodeficient host, and more safety studies are needed [69].
- Physiological use (normal route, normal dose, normal growth phase, specific strain or substrain/species) is studied in all cases, so as not to overwhelm (high dose) or circumvent natural immune processing [69].
- Do probiotics really induce/exacerbate ADs? LGG and others have specific dose- and duration-dependent immunomodulatory effects on the proliferation of B-/T-lymphocytes. Some mice orally fed lactobacilli were demonstrated to have an increased Th1 cytokine production. And this type of immunomodulatory mechanism might exacerbate Th1-dependent ADs [32,69].
- Th1-mediated immune response stimulation also seems to be dependent on type of disease model as well as probiotic strain. In SJL mice with EAE showed that different lactobacilli strains could

enhance or inhibit development of ADs [32,54]. And some immunostimulatory probiotics do not always seem to induce autoimmune responses in models that have the genetic potential to develop autoimmunity. Such as *Lctb rhamnosus* HN001 and *Bfdbm lactis* HN019 do not induce pathological inflammation in mouse model of experimental autoimmune thyroiditis [70].

- The researchers ought to look for more appropriate and safe combinations of probiotic species (as with *VSL#3 or Lacto-mix*) or modified probiotics with/without prebiotic and test them in human/rodent AD models [55].
- Research activities are currently focusing on identification of specific probiotic strains with immunomodulatory potential and on how dietary content interacts with the most efficacious probiotic strains. Further studies should be made for the identification of receptors and pathways through which gut microbes influence development of the immune system.

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