

ALTERNATIVE/COMPLEMENTARY MEDICINE

The immunomodulatory effect of probiotics beyond atopy: an update

Giovanna Vitaliti, MD, Piero Pavone, MD, Francesco Guglielmo, MD, Giuseppina Spataro, MD, and Raffaele Falsaperla, MD

*Pediatric Complex Operative Unit and Pediatric Acute and Emergency Unit, Policlinico-Vittorio-Emanuele Hospital, University of Catania, Catania, Italy***Abstract**

Background: In the past decades, the theory of “allergen avoidance” was considered the standard treatment for preventing the onset of allergic diseases. Recently, the concept of “immune tolerance” has replaced this old theory, and induction of tolerance by exposure is actually considered the appropriate method for preventing atopic diseases and other immunomediated pathologies. On the other hand, it is obvious that for public health reasons, abandoning current medical and hygienic practices is not desirable; therefore, safe alternatives, such as probiotics, have been suggested for providing necessary microbial stimulation. **Objective:** The purpose of our review is to describe the immunomodulatory and anti-inflammatory properties of probiotics, reporting literature data on their effect when used for the treatment of immunomediated diseases. **Materials and methods:** Articles reporting the evidence on the use of probiotics in immunomediated diseases, such as atopy, cow’s milk allergy and rheumatoid arthritis (RA), and in inflammatory diseases, such as inflammatory bowel diseases (IBDs), with or without statistical meta-analysis, were selected in three different search engines: (1) MEDLINE via PubMed interface, (2) Scopus and (3) Google Scholar for all articles published from inception to July 2013. Titles and abstracts of identified papers were screened by two independent reviewers to determine whether they met the eligibility criteria of interest to develop our review. Subsequently, full texts of the remaining articles were independently retrieved for eligibility by the two reviewers. **Results and Discussion:** The recent literature is focusing its interest towards the immunologic properties of relatively harmless organisms, including lactobacilli and bifidobacteria, helminths and saprophytic mycobacteria that may skew immune responses towards immunoregulation by inducing Treg cells, rather than eliciting a pro-inflammatory immune response. For this reason, recent researches have been addressed on the use of probiotics to promote immunoregulation in atopic diseases, such as atopic/eczema dermatitis syndrome and food allergy, as well as in inflammatory-based diseases such as IBDs, RA and bronchial asthma.

Introduction

The prevalence of allergic diseases is increasing in western countries, and it is estimated that 20% of the population is affected by any kind of allergy. Moreover, there are no indications that this trend will reverse. As a multi-factorial pathology, a hereditary predisposition for allergy is thought to be involved, even if environmental factors appear to be required to trigger the disease [1]. The “hygiene hypothesis”, according to which an insufficient or aberrant exposure to environmental microbes is one of the causes that favours the onset of allergy, has been suggested to be the first cause of this increasing prevalence. The habits of people in western countries, such as improved hygiene, widespread vaccination, use of antibiotics to reduce the prevalence of infectious diseases, reduction of family size and consumption of almost sterile food, have reduced their exposure to microbes [2] and modified their innate immune system, which has been adapted to deal with an

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almost sterile environment. The absence of such an appropriate microbial exposure may pose a problem for the development of the innate immune system in growing children, so that in this age group there is an opportunity to direct the immune phenotype away from the allergic one [3].

In the past decades, the theory of “allergen avoidance” was considered the standard treatment for preventing the onset of allergy [4], meeting a limited success, because allergen avoidance relieves the symptoms but does not treat the disease. Recently the concept of “immune tolerance” has replaced the old theory of “avoidance” and induction of tolerance by “exposure” is actually considered the appropriate method to prevent allergy. On the other hand, it is not possible to put apart current medical and hygienic practices; therefore, recent research has directed its efforts towards finding safe alternatives, such as probiotics, that have been suggested for providing necessary microbial stimulation [3].

Immunologic features of the gut

The largest part of the human lymphoid tissue is composed of the gut-associated lymphoid tissue (GALT) that is involved

Correspondence: Giovanna Vitaliti, AOU Policlinico-Vittorio-Emanuele, Via Plebiscito 628, 95124 Catania, Italy. Tel: +39-0957435451. E-mail: giovitaliti@yahoo.it

in the major defence against foreign antigens, as the primary element of the total immunologic capacity of the host.

In the gastrointestinal tract (GT), there are about 1014 commensal bacteria encompassing several hundred different species that live in a symbiotic relationship with mammalian hosts [5] by encoding physiological components (such as enzymes) that complement host capacities or deficiencies [6]. The immune system developing in the gut (GALT) should be tightly regulated to avoid any collateral damage to self and food antigens and to innocuous bacteria such as those composing the commensal flora [7].

In the gut mucosa, non-pathogenic bacteria are in a constant contact with GALT, epithelial cells and dendritic cells (DCs), these latter extend their dendrites across the epithelial barrier and express molecules fundamental for the development of tight junctions [8]. Moreover, bacteria can get in contact with other types of cells including M cells and immune cells, interacting also with epithelial cells and DCs from the rest of the intestinal mucosa. Intestinal bacteria of the commensal flora are recognized by pattern-recognition receptors (PPRs), such as toll-like receptors (TLRs) and NOD-like receptors (NLRs), sited on intestinal epithelial cells [9]. Rakoff-Nahoum et al. [10] showed that the loss of gut epithelium integrity in mice deficient of PPRs and TLRs after chemical exposure resulted in a higher rate of intestinal and systemic infections, underlying the importance of this type of interaction.

Such equilibrium among the host commensal microbiota, the gut and its immune system, established by permanent contact, enforces the gut tolerance that is somehow disturbed by the introduction of pathogens [7]. The DC recognition of viral and microbial products, known as microbe-associated molecular patterns (MAMPs), by PPRs, including TLRs and NLRs, together with inflammatory cytokine release, drives and sustains effector T helper (Th) cell responses [11,12]. Therefore, DCs are at the interface between innate and adaptive immunity, directing and orchestrating different classes of immune responses [13,14], and for this reason they are designated as tolerogenic or immunogenic molecules, as they can elicit tolerance by enhancing T-cell immunity [7]. Interleukins (ILs), such as IL-12 and IL-10 produced by DCs, drive the differentiation of CD4⁺ T cells into pro-inflammatory (Th1) and regulatory (Treg) T cells, respectively. Transforming growth factor (TGF)- β , among the cytokines secreted by DCs, plays a crucial role in the differentiation of adaptive forkhead box P3 (FoxP3) Treg but, together with IL-6, promotes the generation of IL-17-producing cells in mice [15]. In contrast, IL-1b and IL-21, which promote human Th17 development, are responsible for the amplification of allergic diseases [16,17]. It has also been supposed that a direct interaction between commensal bacteria and DCs could also occur *in vivo* [18], even if the precise nature of the molecular signals developing from this interaction has to be clarified yet. As for Treg cells, this kind of anti-inflammatory cells is responsible for the production of IL-10 and IL-12, with anti-inflammatory properties, that drive an immunomediated shift from a pro-inflammatory T-cell pattern (such as Th1 pattern) to an anti-inflammatory one (such as Th2). In regard to probiotics, it seems that while lactobacilli are supposed to signal through TLR1, TLR2, TLR3, TLR4,

TLR6, TLR7 or TLR9, bifidobacteria prime human DCs via TLR2 [19,20]. Baba et al. [21] and Smits et al. [22] reported that Gram-positive and Gram-negative commensal bacteria induced phenotypic and functional DC maturation. Therefore, DCs that have been in contact with these commensal bacteria convert naïve CD4⁺ T cells to suppressor T cells [21]. Nevertheless, how DCs sense harmless commensals and dangerous pathogens, considering that MAMPs are shared by commensals and pathogens, remains an important question to answer. The intestinal microbiota thus interacts with the local immune system, promoting intestinal homeostasis, and it is possible to hypothesize that the interaction between intestinal bacteria and DC is partially mediated by the secretion of soluble bacterial compounds, such as proteins, with a homeostatic effect [21].

Recently it has been demonstrated that the microbiota does not directly interact with immune cells because it is separated by the gut epithelial barrier, composed of physical, chemical and electrical components [23]. The glycocalyx, produced by epithelial cells, is negatively charged and resists most of the microorganisms that are also negatively charged [23]. Paneth cells and epithelial cells release a number of antimicrobial peptides that control different classes of microorganisms. Finally, epithelial cells are connected with one another by tight junctions that prevent microorganisms from entering via the intercellular space. However, a subset of antigen-presenting cells (APCs) characterized by the expression of CX3CR1 can creep between epithelial cells and can contact directly the intestinal lumen [9,24].

After the epithelial barrier, the first cells to contact microorganisms are APCs and intraepithelial lymphocytes. In the gut, these cells can be found scattered in the lamina propria of both small and large intestines, in the GALT and in the mesenteric lymph nodes (MLNs) [25–29]. In the mouse, APCs can be divided into subgroups, depending on the expression of CX3CR1 (the receptor of fractalkine) and CD103 (a-E integrin) [9,24]. The counterpart of CX3CR1⁺ APCs has not yet been described in the human system, while CD103⁺ DCs have been found in human MLNs [30,31]. CD103⁺ DCs are endowed with the capacity to drive Treg cells via a mechanism that is dependent on retinoic acid and TGF- β in both the mouse [30,31] and human [32] systems. In addition, they constitutively express a molecule called IDO, which is fundamental for their tolerogenic potential [33]. CD103⁺ DCs can also imprint T cells with gut-homing properties in the mouse [34] and human [35] systems. Hence, under steady-state conditions, CD103⁺ DCs are specialized in driving mucosal tolerogenic responses. Regarding CX3CR1⁺ APCs, it is not yet clear what their function *in vivo* is as they are incapable of migrating out of the gut [33] and they have poor APCs. However, a recent report has shown that these cells acquire migratory properties in the absence of the microbiota, indicating that they may exert a DC-like function under certain conditions [30]. Nevertheless, it has not yet been cleared up whether these cells are the equivalent of human macrophages, described as inflammatory anergic cells, because of their ability to phagocyte and kill bacteria, without inducing an overt inflammatory reaction [35].

In the mouse Peyer's patches, there are also several subsets of DCs that can be distinguished based on cell surface

markers, particularly chemokine receptors, that are responsible for their localization (CX3CR1, CCR6 and CCR7). CCR6 are found in the subepithelial dome and contribute to antigen-specific Th cell activation in response to bacteria [36], while the function of CX3CR1+ DCs, which are in close contact with the FAE, has not yet been elucidated. As expected, DCs expressing CCR7 are found in T-cell areas [37]. A population of CD103+ DCs has been described, but its localization and function are unknown; however, the population is expanded in response to probiotics [38]. Plasmacytoid DCs are also found in the PP. These cells are impaired in their ability to release type I interferons and are non-inflammatory in nature [39].

Hence, DCs at steady state are set to a non-inflammatory mode that allows to induce Treg-cell development, restimulate Treg cells and induce IgA class switching. All of these activities allow to maintain the homeostasis of the gut and to tolerate food antigens and the microbiota. However, it is not clear whether the microbiota is tolerated or ignored because systemic injection of intestinal microbial strains induces a ready immune response to the bacteria, suggesting that there is no systemic tolerance to the microbiota [40], while orally administered food antigens do not induce systemic immunity [41].

Probiotics: a unique definition

There has been a long debate on how to define “probiotics”. The World Health Organization and the Food and Agriculture Organization of the United Nations have defined “probiotics” as “live microorganisms, which, when administered in adequate amounts, confer health benefit on a host” [42]. Thus, according to this definition, it is important to state what kind of benefits probiotics are able to promote.

Probiotics differ from “prebiotics”, which are non-digestible food ingredients that selectively stimulate the growth and activity of beneficial microorganisms that are already present in the host’s gut. When probiotics and prebiotics are mixed together, they form “symbiosis” [43].

Actually, many kinds of foods (e.g. yogurt, fermented and unfermented milk, miso, tempeh, some juices and soy beverages) and dietary supplements (e.g. capsules, tablets and powders) contain probiotics. In probiotic foods and supplements, the bacteria may have been present originally or added during preparation [43].

Some probiotics, such as bifidobacteria, can manifest a similar composition to those found naturally in people’s gut, especially in breast-fed infants (who present a natural protection against different kinds of diseases). Generally the bacteria belong to two main groups: *Lactobacillus* or *Bifidobacterium*. Within each group, there are different species (e.g. *Lactobacillus acidophilus* and *Bifidobacterium bifidus*), and within each species, there are different strains (or varieties) [43].

Objective: The aim of our review is to highlight the mechanism of immune tolerance induced by probiotics and the importance of their immunomodulatory and anti-inflammatory effect in paediatric immunomediated diseases, when used as a therapy of support to the standard treatment of these types of diseases.

Materials and methods

Articles reporting the evidence on the use of probiotics in immunomediated diseases, such as atopy, cow’s milk allergy (CMA) and rheumatoid arthritis (RA), and in inflammatory diseases, such as inflammatory bowel diseases (IBDs), with or without statistical meta-analysis, were selected. Those reporting the evidence on the use of probiotics in immunomediated comorbidities were also considered. Studies that assessed the use of probiotics in other kinds of diseases not involving the immunologic system were excluded.

Given the lack of an electronic database that contains the publications of all medical journals and the fact that the restriction of only one database could be associated with a systematic bias, it is necessary to combine multiple databases for a comprehensive literature search. For this reason, an electronic literature search was carried out in (1) MEDLINE via PubMed interface, (2) Scopus, (3) Google Scholar for all articles published from inception to July 2013. Database-specific search strings were developed, and search terms describing “probiotics”, “immunotherapy”, “atopic diseases”, “immunomediated diseases”, “asthma”, “inflammatory diseases” and “paediatric diseases” were included. A combination of medical subject headings and keywords was used.

Titles and abstracts of identified papers were screened by two independent reviewers to determine whether they met the eligibility criteria of interest to develop our review. Subsequently, full texts of the remaining articles were independently retrieved for eligibility by the two reviewers.

A limit of this review is the use of only three electronic databases, but they were the only databases to which our university had full access.

Results and discussion

Potential immunologic mechanisms of probiotics

The initial immunologic explanation of the hygiene hypothesis has been based on the assumption that a lack of microbial antigen-induced immune stimulation causes the deviation from a Th1- to a Th2-type cytokine profile, resulting in the development of enhanced Th2 responses to allergens [44–46]. However, the prevalence of Th1-associated diseases, such as type 1 diabetes mellitus, Crohn’s disease and multiple sclerosis, was also increasing, and chronic parasitic worm infections, such as those provoked by helminths, inducing strong Th2 responses and high IgE levels, are not always associated with an increased risk of allergy [44].

An alternative interpretation conceives anti-inflammatory immune responses to be of fundamental importance in the development of mucosal and systemic tolerance [45], as those directed by Treg cells that control, by the production of IL-10 and TGF- β , both Th1 and Th2 responses in immunomediated disorders [45,46].

The recent literature is focusing its interest on the immunologic properties of relatively harmless organisms, such as lactobacilli, bifidobacteria, helminths and saprophytic mycobacteria, having the ability to drive the human immune response towards immunoregulation by inducing Treg cells to the detriment of any pro-inflammatory immune

response (Table 1). For example, *L. paracasei* seems to inhibit in vitro the secretion of both Th1 and Th2 cytokines, while inducing the development of a population of CD4+ T cells secreting TGF- β and IL-10, recognized in the pattern of Treg cells and implicated in oral tolerance and gut homeostasis [47].

It has also been showed that *L. reuteri*, *L. casei* and *L. lactis* have the property in vitro to prime human monocyte-derived DCs, which subsequently stimulate IL-10-producing Treg cells, through the binding of the C-type lectin DC-specific intracellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) [48].

Also bifidobacteria seem to be implicated in the process of immune homeostasis, and the *Bifidobacterium* genomic DNA has been reported to induce the secretion of IL-10 by peripheral blood mononuclear cells (PBMCs) from healthy donors in vitro [49].

According to these assumptions, we could speculate that the hygiene hypothesis has been replaced by the microbiota hypothesis proposing that the loss of exposure to these harmless microorganisms in the westernized environment has caused an increasing prevalence of immune disregulatory disorders [50,51]. The epidemiological findings and the literature data available so far suggest that an involvement of both the decrease of the immune suppression operated by Treg cells and a lack of immune deviation from a Th2 to Th1 profile [52], as well as the impact of the IgA antibody responses on the gut microbiota, which contribute to pathogen and allergen exclusion in the gut lumen, may also be involved [53]. Moreover, the gut microbiota seems to be related not only to food antigens but also to aeroallergens and the subsequent manifestation of airway symptoms. In mouse models it has been demonstrated that antibiotic therapies, leading to bacterial and fungal microbiota changes, could predispose a host to develop allergic airway diseases [54]. Furthermore, Forsythe et al. [55] have recently shown that oral treatment with *L. reuteri* inhibits the allergic airway response in mice. These results confirm that in allergic sensitization, other events that occur outside the lung, involving the host-microbiota communication, are responsible for the pathogenesis of the disease. This occurrence could be considered as part of an active communication between the gut microbiota and the respiratory immune system. As a matter of fact, inhaled particles, fluids and microbes are also swallowed, and their antigens are exposed to both the respiratory mucosa and the GT. Ingestion of these antigens can induce oral tolerance to them, and the GT may act as a sensor for the development of the tolerance of inhaled antigens [56], by the induction of Treg cells. The latter may migrate to other tissues, such as mucosal tissues, throughout the body and thus populate the respiratory tract as well [57,58].

Recently, it has been accepted that the immunomodulatory effects of lactobacilli are heterogeneous and strain dependent. This assumption has allowed recognizing immunogenic effects in new lactobacilli strains and phenotypes as well. As mentioned above, monocytes and macrophages recognize conserved molecular patterns of bacterial components through TLR and therefore are responsible for the activation of different transcription factors responsible for the

Table 1. Resume of the main immunologic features of probiotics.

Probiotics	Type of study	Immunologic properties	Reference in the text
<i>L. paracasei</i>	<i>In vitro</i>	- Inhibition of the secretion of Th1 and Th2 cytokines	[47]
<i>L. reuteri</i>	<i>In vitro</i>	- Induction of the development of a population of CD4(+) T cells secreting TGF- β and IL-10	[48]
<i>L. casei</i>		- Priming of the human monocyte-derived DCs, stimulating the secretion of IL-10-producing Treg cell	
<i>L. lactis</i>			
<i>L. reuteri</i>	<i>In vivo</i> (animal model)	- Inhibition of the allergic airway response in mice	[55]
<i>L. salivarius</i> CECT5713	<i>In vitro</i>	- Promotion of a Th1 response	[59]
<i>L. fermentatum</i> CECT5716	<i>In vitro</i>	- Induction of IL-10-secreting T cells	[59]
<i>B. breve</i> UCC2003	<i>In vitro</i>	- Production of an EPS sited on its surface that provides resistance to both bile and acid	[64]
<i>B. adolescentis</i>	<i>In vitro</i>	- Induction of DC maturation and polarized naïve CD4+ T cells towards IFN- γ and IL-10 production	[67]
<i>B. bifidum</i>			
<i>B. longum</i>	<i>In vitro</i>	- Promotion of an increase of CD4+CD25+ and FoxP3 T cells and induction of IFN- γ in Th2-skewed peripheral blood mononuclear cells and DC T-cell co-cultures	[67]
<i>B. bifidum</i>		- Ability to reorient established Th2 responses towards Th1 and Treg cell profiles	
		- Polarization of naïve T cells into functional Treg cells	
<i>B. bifidum</i> LMG13195	<i>In vitro</i>	- Promotion of the differentiation of functional CD25(high)FoxP3(high)CD127(low) Treg cells	[68]
		- Induction of the highest IL-10 levels with respect to the pro-inflammatory cytokines IFN- γ , TNF- α and IL-17	

production of cytokines involved in the development of T-cell differentiation into Th1, Th2 or Treg cells [50,51]. Therefore, the change in the macrophage-derived cytokines profile can develop a specific immune response. Lara-Villoslada et al. [59] found out that two different strains of *Lactobacillus*, in particular *L. salivarius* CECT5713 and *L. fermentum* CECT5716, when co-incubated with murine bone-marrow-derived macrophages, had stimulated the production of cytokines, but the effect of each strain was different. The cytokine pattern induced by *L. fermentum* CECT5716 encouraged the Th1 response, meanwhile the higher production of IL-10 induced by *L. salivarius* CECT5713 had the opposite effect [59]. Therefore, authors concluded that *L. fermentum* CECT5716 commonly acts as an immunostimulant agent, while *L. salivarius* CECT5713 performs an anti-inflammatory/regulatory effect. These results confirm the strain-specific effect of each probiotic, and the evidence that the selection of a strain for any specific application should be based on the particular properties of each strain and the basic health condition of the host [59].

As for the bifidobacteria, it has been proved that these probiotic species have also some immunomodulatory properties. One of the proposed mechanisms, by which bifidobacteria mediate this immunomodulation, is the production of exopolysaccharide (EPS) capsule [60]. Bacterial EPS consists of a repeating mono- or oligosaccharide subunit connected by varying glycosidic linkages, thereby generating structurally diverse homo- or heteropolymers [60]. EPSs are particularly thought to be important for the host–microbe interactions, where they aid in adherence and colonization within the human host [61] and function in immunomodulation [62]. Although very little is known about the function of bifidobacterial EPSs, they have been indicated to improve the tolerance of the bacterium to the gut bile and acid [63], and they are also an important growth substrate for elements of the gut microbiota [60]. Fanning et al. [64] have found out that the commensal *Bifidobacterium*, *B. breve* UCC2003, contains a bidirectional EPS-encoding genetic locus responsible for the production of an EPS sited on its surface that provides resistance to both bile and acid in vitro. In mice, this surface EPS aids in long-term persistence and also mediates immune evasion, especially avoiding B-cell responses. Colonization of mice with EPS+, but not EPS–, *B. breve* also favours protection of the host against enteric pathogen challenge. This finding suggests that commensal bacterial surface EPSs can facilitate colonization of their host through evasion of potentially damaging immune responses, providing beneficial effects on the host, reducing external pathogen colonization. From a stricter immunologic point of view, it seems that polysaccharide immunogens are typically T-cell-independent B-cell antigens. Previous studies have shown that capsular polysaccharides induce only weak antibody responses, without involving the T-cell immune system [65]. Indeed, IL-12 has been shown to enhance humoral immunity in mouse models, through mechanisms that are both dependent and independent of interferon- γ (IFN- γ), specifically T-cell-independent antigens [66]. Therefore, the reduction of these cytokines mediated by bifidobacterial EPS also correlates with the reduction in overall antibody levels and affinity towards polysaccharides on the surface of commensal

Bifidobacterium such as *B. breve* [64]. These findings are consistent with an EPS-mediated strategy that prevents a commensal bacterium from evoking a strong adaptive immune response within the local environment.

Recently, in 2012, Moussu et al. [67] found that other *Bifidobacterium* species are also able to implement the immune system *in vitro*, and, in particular, they demonstrated that *B. adolescentis*, *B. bifidum* and *B. longum* induced DC maturation and polarized naïve CD4+ T cells towards IFN- γ and IL-10 production. *B. bifidum* favoured the increase of CD4+CD25+ and FoxP3 T cells, and it was the most potent inducer of IFN- γ in Th2-skewed PBMCs and DC T-cell co-cultures. It also induced a significant decrease in airway hyperresponsiveness in mouse models sensitized to ovalbumin. Sublingual administration of *B. bifidum*, together with recombinant Bet v 1, enhanced tolerance induction in mice sensitized to birch pollen, with a downregulation of airway hyperresponsiveness, lung inflammation and Bet v 1-specific Th2 responses [67]. The authors concluded that *B. bifidum* has got the ability to reorient established Th2 responses towards Th1 and Treg cell profiles, representing a valid candidate adjuvant for specific immunotherapy of type I allergies. *B. bifidum* has also been shown to have the ability to induce antigen-specific Treg cells and peripheral tolerance to restore human homeostasis. In particular, *B. bifidum* LMG13195 strain has been demonstrated to induce the *in vitro* polarization of naïve T cells into functional Treg cells [68]. López et al. [68] found that DCs exposed to *B. bifidum* LMG13195 membrane vesicles most strongly promoted the differentiation of functional CD25(high)FoxP3(high)CD127(low) Treg cells as well as induced the highest IL-10 levels with respect to the pro-inflammatory cytokines IFN- γ , TNF- α and IL-17, suggesting the potential use of *B. bifidum* LMG13195 membrane vesicles as a safe and clinically effective adjuvant for immunotherapy. All these evidences taken together indicate how important the use of lactobacilli and bifidobacteria is in driving the polarization of the host immune system towards immune tolerance, even if more *in vivo* studies are needed to confirm their real immunomodulatory efficiency.

In literature other probiotics of non-conventional lactic acid bacteria have been demonstrated to have immunomodulatory properties. Foligné et al [69], at the Pasteur Institute in France, demonstrated that probiotic species with allochthonous or extremophile origin, with similar structure and function, may also have specific health benefits to a host. They explored the *in vitro* immunomodulatory or immune-stimulatory activities of 25 wine lactic acid bacteria belonging to *Oenococcus oeni* and *Pediococcus parvulus*, and they found that while cytokines released by PBMCs following the stimulation by *P. parvulus* strains showed little variation, *O. oeni* strains induced a pattern of cytokines that were strain specific. The latter were then further analysed under various conditions for growth, dose and culture medium and subsequently evaluated for the induction of oral tolerance and safety, especially of two strains of *O. oeni*, in mice fed with a high dose of bacteria for a week. They gathered evidence on the *in vivo* anti-inflammatory potential of the selected *O. oeni* strain IOEB 9115, by inducing colitis in a mouse model using an experimental 2,4,6-trinitrobenzene sulfonic acid

The authors found that even if the results did not match the anti-inflammatory levels obtained with certain conventional probiotics, strain IOEB 9115 significantly lowered colonic injury and alleviated colitis symptoms. The authors concluded *O. oeni*'s measureable immunomodulatory potential, and the "natural" tolerance of its certain strains towards acid, ethanol and phenolic compounds suggest a possible use of selected strains isolated from wine as live probiotics [69].

Probiotics and atopy

In the case of allergy, it has been observed that children with allergy have a different microbiota composition from healthy infants, and this assumption supports the rationale for modulating the gut microbiota. Literature data have demonstrated that children with allergy have an aberrant microbiota even before the onset of allergy, with higher levels of clostridia and lower levels of bifidobacteria [70,71]. Moreover, a qualitative difference has been observed in the microbiota of children with allergy, in the sense that infants with atopic dermatitis have been found to have a more adult-type *Bifidobacterium* microbiota, with high prevalence of *B. adolescentis*, while healthy infants were colonized mainly by *B. bifidum*, mostly present in breast-fed infants [72,73]. However, children affected by respiratory allergic symptoms did not exhibit an aberrant microbiota composition [73].

He et al., in 2002 [74], found that the bifidobacteria from infants with atopic dermatitis induced a higher secretion of pro-inflammatory cytokines in vitro, whereas the bifidobacteria from healthy infants induced a higher secretion of cytokines with anti-inflammatory properties [74]. Moreover, bifidobacteria from patients with allergy differed from healthy infants in the in vitro adhesion to intestinal mucosa by the interaction with adhesion molecules [75,76]. This difference may result in reduced stimulation of the immune system through the GALT.

Not only does the composition of the intestinal microbiota but also its metabolic activity in children with allergy differ from the healthy patient, and a relationship with the child's ethnic origin has been demonstrated. In fact, Swedish children, who have been demonstrated to have a higher risk to develop allergic diseases, were found to have significantly increased levels of faecal butyrate, isovalerate and caproate compared to Estonian children, who presented a lower risk for developing allergies [77].

Until now, a limited number of probiotic strains have been tested for their efficacy in the treatment and prevention of allergy in infants. The most common allergic disease in early infancy is the CMA, which sometimes may manifest even during exclusive breast-feeding. In these cases, standard treatment involves the feeding of extensively hydrolysed formula [78]. Recently, it has been demonstrated in infants that supplementation of hydrolysed formula with *B. lactis* Bb-12 or *L. rhamnosus* GG leads to an earlier recovery than standard treatment alone [79]. In children with atopic dermatitis, a combination of two *Lactobacillus* strains, in particular *L. rhamnosus* 19070-2 and *L. reuteri* DSM 122460, was found to significantly reduce the clinical scoring of atopic dermatitis (SCORAD) in 1- to 13-year-old children with a

positive skin prick test (SPT) for cow's milk, but the SCORAD of children with no positive SPT remained unchanged. Interestingly, in this study more than half of the subjects reported an improvement in their eczema, whereas in the placebo group only 15% of children reported an improvement of their symptoms [80]. The two studies just discussed used different probiotic preparations, and, according to this assumption, the observed differences in outcome may be explained. But these differences may also relate to the diverse age of the studied patients. In the peculiar age group of young infants, in fact, the immune system is still developing with a possibility to direct it towards tolerance. In older children, the allergic phenotype is already established, and the eventual immunomodulatory therapy should be able to relieve only the symptoms [81].

Studies on the use of probiotics in children affected by cow's milk allergy

CMA is the most common food allergy in early childhood [82]. Its estimated incidence, emerging from perspective studies, is 2% to 5% [83]. The majority of infants become tolerant before the age of 3 years [84]. On the other hand, children with CMA at early infancy have an increased risk of atopic diseases in later life [85]. It has been hypothesized that an immaturity of local and systemic immune responses leads to the development of CMA in genetically predisposed children [86] and is associated with an altered composition of the intestinal microbiota in the western society [87]. Based on these findings, it has been proposed that specific probiotics could potentially restore intestinal homeostasis both in mouse model [88] and in children affected by atopic dermatitis [89], thus favouring a prevention of the progression of allergy through interaction with the intestinal immune cells [89]. Probiotics have been extensively studied in vivo for the treatment of atopic diseases such as atopic eczema/dermatitis syndrome (AEDS) [80,90–94] and allergic rhinitis [95].

The first studies of probiotic treatment on AEDS have reported a positive effect on the severity of AEDS [92,96]. Advantageous effects of probiotics on AEDS and faecal α 1-antitrypsin/TNF- α were also described by Majamaa and Isolauri [96], whereas Viljanen et al. [97,98] found no beneficial effects of probiotics on AEDS or faecal inflammatory markers in the infants with CMA by using the same probiotic strain (*Lactobacillus* GG).

Regarding food allergy as mere expression of CMA, previous studies on probiotic supplementation of infants with a certain or suspected diagnosis of CMA have been reported [90,96–98].

Recently, Canani et al. [99] demonstrated how *Lactobacillus* GG favour a higher improvement of cow's milk tolerance in children affected by CMA, when this probiotic is added to the standard diet with hydrolysed formula, compared to those children fed with only this formula. This effect could be attributed to the ability of *Lactobacillus* GG to balance the generation of cytokines, such as IL-4, IL-5, IL-10, IFN- γ , TGF- β , and TNF- α , possibly involved in IgE- or non-IgE-mediated CMA, which can contribute to the downregulation of inflammatory processes [100–102]. These effects were strain specific because studies

conducted with other *Lactobacillus* species did not show comparable results [103].

Anti-inflammatory effects of probiotics

Anti-inflammatory and regulatory mucosal immune responses are essential for withholding detrimental inflammatory reactions to dietary antigens and non-pathogenic microbes, a phenomenon referred to as mucosal tolerance. As mentioned earlier, there are several active mechanisms through which mucosal tolerance is established and maintained, which involve some processes as clonal deletion, anergy and antigen-specific suppression by T cells. Distinct regulatory mechanisms modulating both Th1 and Th2 responses have recently been discovered, and they have been attributed to the effects of novel T-cell subclasses with suppressive and regulatory functions. Gut-derived Th3 and TR1 cells exert their effects through the production of cytokines, mainly TGF- β and IL-10, respectively. The thymus-originated CD4+CD25+ regulatory T cells act through a contact-dependent mechanism involving membrane-bound TGF- β [104]. In this context, the indigenous microbiota contributes to the anti-inflammatory tone of the mucosal immune system [105]. The importance of host–microbe interaction is most vital in the neonatal period, when the establishment of a normal microbiota provides the host with the most substantial antigen challenge, with a strong stimulatory effect for the maturation of the GALT. Indeed, in a mouse experimental model, it has been demonstrated that mice reared in germ-free conditions displayed defective oral tolerance and regain the ability to establish oral tolerance if the intestinal microbiota is reconstituted with bifidobacteria at the neonatal age but not later [47,106].

According to the evidence that probiotics show anti-inflammatory properties, they have also been used in in vivo models for reducing inflammation in all the diseases in which inflammatory processes are the main pathogenic event for the development of the disease.

Probiotics and inflammatory bowel diseases

A large body of evidence from both experimental animal work and clinical observations suggests that the enteric microbiota has not only immunomodulatory effects but also anti-inflammatory properties. It has been hypothesized that the enteric microbiota may play an important role in driving the abnormal inflammatory response in the genetically susceptible host, leading to chronic inflammation in IBD, using probiotics as a means to reconstitute microbial and immunologic homeostasis [107].

Combinations of different probiotic strains have demonstrated efficacy in the control of pouchitis, an inflammation of the ileal–anal anastomosis, surgically produced after colectomy for ulcerative colitis (UC). A randomised, placebo-controlled study, using high doses of once-daily probiotic therapy, showed that probiotics were effective in the maintenance of antibiotic-induced remission in chronic pouchitis [108]. By contrast, the use of single probiotic strains, such as *L. rhamnosus* GG, or a combination of *L. acidophilus* and *B. lactis* did not show any clinical improvement in patients affected by acute active pouchitis [109]. In the treatment of

acute Crohn's disease, in children with mild-to-moderate disease activity, *L. rhamnosus* GG in combination with prednisone and immunomodulatory drugs showed a better clinical improvement [110]. However, in adult patients with active Crohn's disease, the same probiotic was ineffective, although patients were on steroid diet regimen and received antibiotics before starting the probiotic supplementation [111]. In the maintenance of surgically induced remission, a few studies with different outcomes have been published. In a randomised placebo-controlled prospective study, oral administration of *L. rhamnosus* GG for 1 year could not prevent Crohn's recurrent lesions after surgery and did not reduce their severity [112]. By contrast, administration of VSL#3 (a mixture of different probiotic strains) demonstrated efficacy in preventing post-operative recurrence when compared to mesalamine [113] up to 1-year post-surgery.

A randomised, open-label, multi-centre trial showed that VSL#3 added to low doses of balsalazide (a mesalazine prodrug) was more effective than monotherapy with standard doses of balsalazide or mesalazine in the treatment of acute mild-to-moderate UC [114]. Recently it has also demonstrated the efficacy of *Lactobacillus* GG in combination with mesalazine to sustain remission in patients with quiescent UC [115]. The role of bifidobacteria in medically induced remission of UC was evaluated in a small human trial showing less relapses in patients supplemented with bifidobacteria than the control group, during a 2-month follow-up, as well as inflammatory activity in the colon, evaluated by the analysis of pro-inflammatory cytokine (IL-1b, TNF- α) and the quantification of NF- κ B, was significantly decreased in the probiotic treatment group [116]. This positive effect on UC patients was similarly found in patients fed with a fermented milk product containing probiotic bifidobacteria [117]. Finally, a randomised controlled pilot trial with *B. longum* in combination with a inulin-type prebiotics showed resolution of inflammation in patients with UC and reduced inflammatory activity in the mucosal tissue (with a decreased production of TNF- α , IL-1 α and defensins) [118].

To date only a few randomised, double-blind, placebo-controlled trials are published, and it seems that high doses of probiotics, most likely a combination of different lactobacilli and bifidobacteria, have a promising efficiency in decreasing clinical inflammatory score and maintaining patients in medically induced remission, when associated with the standard therapy. Future research might identify clinical phenotypes of patients with IBD (based on the insights in genetic predisposition, microbiota asset and immune response) that are likely to respond to probiotic therapy.

Probiotics and rheumatoid arthritis

Literature data have developed interest in the properties of some probiotics to improve the standard treatment of RA. Nowak et al. [119], in 2012, demonstrated how oral administration of *L. rhamnosus* attenuates various types of experimental arthritis, showing how the EPS properties of this *Lactobacillus* can suppress active collagen-induced activity, by the inhibition of arthritogenic antibodies production, and

Table 2. Efficacy of probiotics as therapy of support in immunomediated diseases.

Type of study	Population	Probiotic strain	Disease in which the probiotic was used	Beneficial effect	Immunogenic mechanism	Reference in the text
Randomised controlled clinical trial	Infants	<i>B. lactis</i> Bb-12	CMA	Better prognosis and earlier recovery than standard treatment alone	NP	[79]
Randomised controlled clinical trial	Children	<i>L. rhamnosus</i> GG <i>L. rhamnosus</i> 19070-2 <i>L. reuteri</i> DSM 122460	AEDS	Reduction of SCORAD in children with SPT positive for CMA Improvement of eczema Improvement of SCORAD Decrease of faecal alpha 1-antitrypsin	NP	[80]
Randomised controlled clinical trial	Children	<i>Lactobacillus</i> GG (ATCC 53103)	CMA		Promotion of endogenous barrier mechanisms/decrease of intestinal inflammation	[96]
Randomised controlled clinical trial	Infants	<i>Lactobacillus</i> GG (ATCC 53103)	AEDS/CMA	Increase of faecal IgA	NP	[97]
Randomised double-blind placebo-controlled clinical trial	Infants	<i>Lactobacillus</i> GG	AEDS/CMA	Reduction of SCORAD in IgE-sensitized infants, but not in non-IgE-sensitized children	NP	[98]
Randomised placebo-controlled clinical trial	Infants	<i>Lactobacillus</i> GG	AEDS/CMA	No beneficial effect was reported	NP	[90]
Randomised double-blind clinical trial	Infants	<i>Lactobacillus</i> GG	AEDS/CMA	Acquisition of tolerance towards CM SPT turned negative	Balance in the generation of pro-inflammatory and anti-inflammatory cytokines	[99]
Randomised placebo-controlled clinical trial	Adults	VSL#3	Refractory pouchitis	Probiotics were demonstrated effective in maintaining antibiotic-introduced remission for at least a year	Reduction of TNF- α , IFN- γ , inducible NOS, and matrix metalloproteinases 2 and 9	[108]
Randomised double-blind placebo-controlled clinical trial	Adults	<i>L. rhamnosus</i> GG as single strain vs <i>L. acidophilus</i> and <i>B. lactis</i> <i>L. rhamnosus</i> GG <i>Lactobacillus</i> GG	Refractory pouchitis	No clinically beneficial effect	Increased tissue IL-10	[109]
Open-label pilot study	Children		Crohn's disease (active phase)	Clinical improvement	Improvement of the gut barrier function	[110]
Randomised placebo-controlled clinical trial	Adults		Crohn's disease (active phase)	No clinically beneficial effect	NP	[111]
Randomised placebo-controlled clinical trial	Adults	<i>L. rhamnosus</i> GG	Crohn's disease (maintenance of surgical-induced remission)	No clinically beneficial effect	NP	[112]
Randomised placebo-controlled clinical trial	Adults	VSL#3	Crohn's disease (maintenance of surgical-induced remission)	Prevention of post-operative recurrence	NP	[112]
Randomised placebo-controlled clinical trial	Adults	VSL#3	Chronic pouchitis	Prevention of post-operative recurrence	Increase of tissue levels of IL-10 in patients with chronic pouchitis	[113]
Randomised controlled clinical trial	Adults	VSL#3	Chronic pouchitis	Reduction of remission in balsalazide/VLS ³ group than in balsalazide or mesalazine group	NP	[114]
Randomised controlled clinical trial	Adults	<i>Lactobacillus</i> GG	Quiescent ulcerative colitis	Efficiency in maintaining remission	NP	[115]
Randomised placebo-controlled clinical trial	Adults	BIFICO	Ulcerative colitis	Prevention of flare-ups in chronic colitis	Inhibition of the activation of NF- κ B; decrease of the expressions of TNF- α and IL-1b	[116]
Randomised placebo-controlled clinical trial	Adults	BFM	Ulcerative colitis	Maintenance of remission and preventive effects on the relapse of the disease	Increase of the expression of IL-10	[117]
Randomised double-blind controlled trial	Adults	<i>B. longum</i> + synergy one (prebiotic)	Active ulcerative colitis	Improvement of the full clinical appearance of chronic inflammation	Reduction of mRNA levels for human β defensins 2, 3, and 4	[118]
Experimental animal model	Rats	<i>L. casei</i>	Rheumatoid arthritis	Reduction of pain, cartilage destruction, and lymphocyte infiltration	Reduction of TNF- α and IL-1a Decreased expression of pro-inflammatory cytokines, TNF- α , IFN- γ , and matrix metalloproteinases	[120]
Experimental animal model	Newborn Balb/c mice	<i>L. rhamnosus</i> GG + <i>B. lactis</i> (Bb-12)	Asthma	Improvement of symptoms and airway hyperreactivity	Upregulating anti-inflammatory cytokines Antigen-specific recall proliferation by spleen cells and Th2-type cytokine Increase in numbers of TGF- β -secreting	[122]

(continued)

Table 2. Continued

Type of study	Population	Probiotic strain	Disease in which the probiotic was used	Beneficial effect	Immunogenic mechanism	Reference in the text
Experimental animal model	Balb/c mice	<i>L. casei rhamnosus Lcr35</i>	Asthma	Oral treatment with <i>Lcr35</i> prior to sensitization can attenuate airway inflammation and hyperactivity in a mouse	CD4+CD3+ T cells in mesenteric lymph nodes	[124]
Randomised double-blind placebo-controlled clinical trial	Mothers	<i>Lactobacillus GG</i>	AEDS	Prevention of early atopic disease in children	Decrease of total cell counts and the proportion of eosinophils in BAL fluid	[125]
Double-blind placebo-controlled clinical trial	Adults	<i>L. rhamnosus</i> (ATCC 53103)	Birch-pollen allergy	No beneficial effect	Decrease of total serum IgE levels NP	[126]

AEDS: atopic/eczema dermatitis syndrome; BIFICO: bifid triple viable capsule; BFM: bifidobacteria fermented milk; CMA: cow's milk allergy; NP: not reported; SPT: skin prick test.

in particular *L. rhamnosus* KL37 has been demonstrated to depress the production of anticollagen IgG and affect the development of collagen-induced arthritis in DBA/1 mice.

Previously, in 2011, So et al. [120] demonstrated that *L. casei* could act as a potent nutraceutical modulator for osteoarthritis treatment by reducing the inflammatory response, pain and articular cartilage degradation. They found that oral administration of *L. casei*, together with type II collagen (CII) and glucosamine (Gln), more effectively reduced pain, cartilage destruction and lymphocyte infiltration than the treatment with Gln or *L. casei* alone. This co-administration also decreased the expression of various pro-inflammatory cytokines, such as IL-1b, IL-2, IL-6, IL-12, IL-17, TNF- α and IFN- γ , mostly responsible for the cartilage inflammation and destruction, and matrix metalloproteinases (MMP1, MMP3 and MMP13), while favouring an increased production of anti-inflammatory cytokines (IL-4 and IL-10). These results are concomitant with reduced translocation of NF- κ B into the nucleus and increased expression of the tissue inhibitor of MMP1 (TIMP1) and CII in chondrocytes. In the same year, *L. casei* has been also demonstrated to reduce the inflammatory joint damage associated with collagen-induced arthritis in mouse models, by the inhibition of COX-2, thus inhibiting the pro-inflammatory cytokines involved in these pathways [121].

In this field literature data are still limited; nevertheless, it seems that further studies are being directed in order to better understand the anti-inflammatory properties of probiotics in the improvement of the efficiency of RA treatment.

Probiotics and asthma

Recently, successful probiotic treatments in animal models of asthma have been described [122–124], showing in a mouse model of allergic airway inflammation as some probiotic species, such as oral *L. rhamnosus* (*Lcr35*), treatment prior to allergic sensitization can attenuate airway inflammation and hyperreactivity [124]. However, the immunomodulatory and anti-inflammatory effects of probiotics on the development of allergic asthma and allergic rhinitis in human clinical trials and reported cases remain controversial [125–128]. Despite conflicting results, it is generally thought that the use of probiotics on allergic diseases is a promising area of research [129,130] in the treatment of both bronchial asthma and recurrent respiratory infections. A number of studies suggest that the immunosuppressive cytokines, such as IL-10 and TGF- β along with Treg cells, are the main agents of probiotics to suppress allergic inflammation [44–54]. Nevertheless, there have been few mechanistic studies of probiotic activity in allergic disease and even in asthma. Jang et al. [131] confirmed the involvement of Treg cells in the protective effect of *Lcr35* on allergic asthma in a mouse model, suggesting that Treg cells may be involved in the protection conferred by oral *Lcr35*. Thus, it has been demonstrated that probiotics have beneficial effects on atopic diseases; moreover, they seem to be efficient in the treatment of asthma, and, actually, more recent research fields are addressing their interest on the use of probiotics in preventing recurrent respiratory diseases.

Conclusions

Recent literature is focusing its efforts on providing evidence that probiotics have an immunomodulatory and anti-inflammatory effect. The main features of the studies reported in this article are resumed in Table 2. Probiotics have strain-specific and species-dependent properties, thus, knowing every single property of each probiotic strain will help to treat efficiently immunomediated diseases, especially those involving Treg cells, such as atopic diseases.

Probiotics have demonstrated to be safe in long-term therapies, and the earlier the treatment is performed the most efficient it is, especially in atopic diseases. It has been demonstrated that younger children with atopic diseases benefit more from the probiotics use for immunomodulation than older children. Nevertheless, further studies should clarify if probiotics could be considered the only therapy or the support treatment to the standard therapy of allergic and immunomediated diseases.

Declaration of interest

All authors declare not to have any competing interest in the publication of this paper.

All authors have equally contributed to the conception and design, acquisition of data, drafting the manuscript and critical revision of the manuscript.

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