

REVIEW ARTICLE

Probiotics for the airways: Potential to improve epithelial and immune homeostasis

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Abstract

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefit on the host. The therapeutic effects of probiotics have been mostly studied in the gastrointestinal tract, but recent evidence points toward the potential of these bacteria to prevent and/or treat chronic airway diseases. In this review, possible mechanisms of action of probiotics in the airways are described, with a particular focus on their capacity to modulate the epithelial barrier function and their mode of interaction with the immune system. Indeed, probiotic bacteria, mostly lactobacilli, can promote the expression and regulation of tight junctions and adherence junctions, resulting in the restoration of a defective epithelial barrier. These bacteria interact with the epithelial barrier and immune cells through pattern recognition receptors, such as Toll-like receptors, which upon activation can stimulate or suppress various immune responses. Finally, the clinical potential of probiotics to treat inflammatory diseases of the upper and lower respiratory tract, and the difference between their mode of application (eg, oral or nasal) are discussed here.

KEYWORDS

epithelial barrier, *Lactobacilli*, microbiota, probiotics, tight junctions

Abbreviations: AHR, Airway hyper-reactivity; AJ, Adherence junction; AR, Allergic rhinitis; BALF, Bronchoalveolar lavage fluid; CRS, Chronic rhinosinusitis; DCs, Dendritic cells; IEC, Intestinal epithelial cell; IL, Interleukin; LAB, Lactic acid bacteria; LPS, Lipopolysaccharides; MAMPs, Microorganism-associated molecular patterns; NGS, Next-generation sequencing; NLRs, NOD-like receptors; OVA, Ovalbumin; PRRs, Pattern recognition receptors; TGF- β , Transforming growth factor β ; TJ, Tight junction; TLRs, Toll-like receptors; TNF- α , Tumor necrosis factor- α ; Tregs, Regulatory T cells; URT, Upper respiratory tract; ZO, Zonula occludens.

1 | INTRODUCTION

The human body is colonized by approximately 100 trillion microbes, collectively known as the microbiota, which play an essential role in our well-being.¹ Thanks to the development of advanced culture-independent tools, such as next-generation sequencing, insights have been obtained in possible associations of the microbiota composition with disease.^{2,3} Together with additional experimental and clinical data on various roles of the microbiota, it is now widely accepted that the microbiota provides the host with a wide array of health effects. These health effects include (i) exclusion or inhibitions of pathogens,^{4,5} (ii) enhancement of epithelial barrier function by the modulation of signaling pathways,^{6,7} and (iii) strain-specific modulation of local and systemic host immune responses (Figure 1).⁸

The involvement of the microbiota in the development or exacerbation of chronic noninfectious diseases is also increasingly considered.⁹ An imbalance of the microbiota composition, also known as dysbiosis, has been shown in various studies to negatively impact the health status.¹⁰ Three subtypes of dysbiosis have been identified as follows: (i) loss of beneficial microbial agents, (ii) expansion of potentially harmful microorganisms, and (iii) loss of overall microbial diversity.¹⁰ Microbial dysbiosis has been identified in different chronic inflammatory diseases, including chronic rhinosinusitis (CRS),^{11,12} asthma,^{13,14} Crohn's disease,¹⁵ and ulcerative colitis.¹⁶ Interestingly, in these chronic diseases, a disturbed permeability and function of the epithelial barrier have also been demonstrated.¹⁷⁻¹⁹ It is, however, not known whether microbiota dysbiosis could be the cause or simply a consequence of the associated epithelial barrier disruption.

When looking specifically at the airways, various microorganisms such as, *Staphylococcus aureus*,²⁰ *Streptococcus pneumoniae*, and *Haemophilus influenzae*²¹ could induce pathogenic effects on the host, at

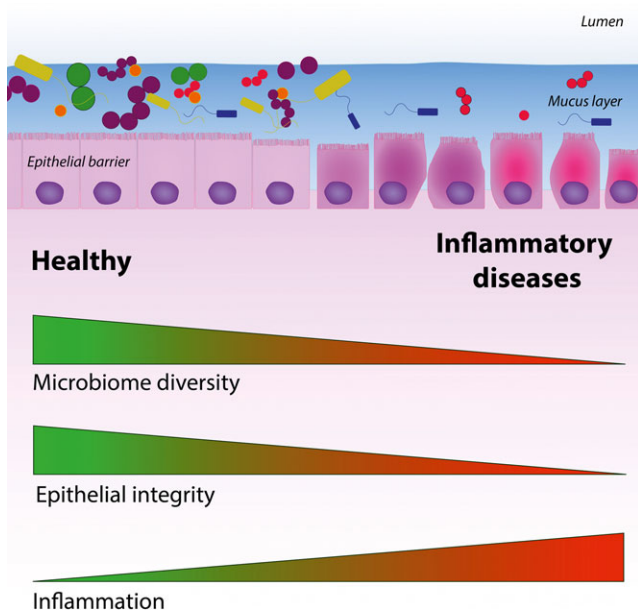
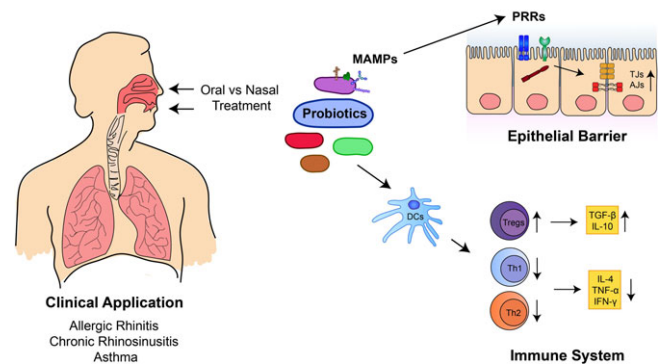


FIGURE 1 Relationship between microbiota diversity, epithelial integrity, and inflammation in disease state

Probiotics for the airways: potential to improve epithelial and immune homeostasis



GRAPHICAL ABSTRACT

The clinical potential of probiotics has gained interest as a novel oral and/or nasal treatment option for chronic airway diseases. Probiotics interact, via their microorganism-associated molecular patterns (MAMPs), with pattern recognition receptors (PRRs) on epithelial cells. This interaction can modulate tight junctions (TJs) and adherens junctions (AJs), which results in the restoration of a defective epithelial barrier. Probiotics are able to modulate host immune responses via interactions with dendritic cells (DCs), which on their turn promote regulatory T cells (Tregs) and reduce T helper 1 (Th1) and T helper 2 (Th2) cells.

least in part by increasing the epithelial barrier permeability in both the upper and lower airways. On the contrary, other microorganisms—both endogenous and exogenous—can also provide beneficial effects²² (Figure 2). These beneficial bacteria are called probiotics, defined as live microorganisms that, upon administration in adequate amounts, can provide health benefits to the host.²³ Traditionally, *Lactobacillus* and *Bifidobacterium* strains have been used as probiotics, mainly to reduce gastrointestinal tract discomfort. Other bacterial taxa have probiotic potentials, such as the *Streptococcus taxa*²⁴ or *Dolosigranulum* which is associated with respiratory health in different microbiome studies,^{25,26} but these studies are limited so that this review will mainly focus on the *Lactobacillus* strains.

Interestingly, lactobacilli were shown to be normal inhabitants of the healthy nasopharynx and upper respiratory tract (URT).²⁷⁻²⁹ For instance, Bogaert et al²⁷ showed a predominance of *Lactobacillus* species in children in summer (absolute abundance is 96%) compared to winter (absolute abundance is 10%). Additionally, lactobacilli were also detected in the healthy nasopharynx of Chinese individuals³⁰ and in the nasopharynx of healthy Belgian individuals (up to 10% relative abundance).³¹ Lactobacilli are also found in the tonsillar crypts of both healthy adults and infants.³² Nevertheless, whether lactobacilli could also function as probiotics in the nasopharyngeal niche is currently underexplored.

In this review, we summarize documented beneficial effects of probiotic microorganisms (mainly *Lactobacillus* and other species) and discuss their potential mechanisms of action in chronic diseases of the upper and lower airways. As the studies of the gastrointestinal

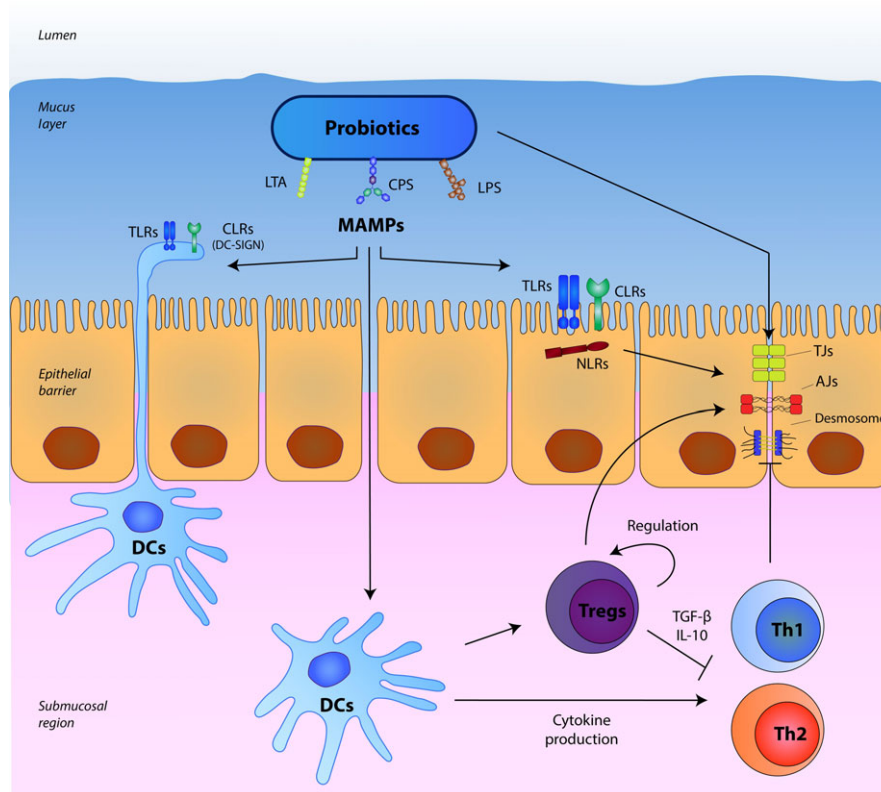


FIGURE 2 Interaction of probiotics with the epithelial barrier and the immune system. Probiotics induce their health-promoting capacities via different mechanisms. These bacteria are able to enhance epithelial barrier function via modulation of the intercellular junctions (TJs, AJs, and desmosomes) or via interaction with the different PRRs, present on the epithelial barrier. These PRRs (ie, TLRs, CLRs, NLRs) recognize the MAMPs, such as LTA, CPS, and LPS, present on the probiotic cell surface. Probiotics are also able to modulate the local and systemic immune responses. They have the ability to interact with DCs, present between the epithelial cells or in the submucosal region. This interaction can result in the activation of Tregs. Additionally, it has been suggested that Tregs play a role in maintaining epithelial barrier via the production of TGF- β and IL-10. Finally, probiotics are able to modulate the Th1 and Th2 responses, resulting in the restoration of the immune homeostasis. DCs, dendritic cells; Tregs, regulatory T cells; PRRs, pattern recognition receptors; TLRs, Toll-like receptors; CLRs, C-type lectin receptors; NLRs, NOD-like receptors; LTA, lipoteichoic acid; CPS, cell wall-associated polysaccharide; LPS, lipopolysaccharide

impact of probiotics are more advanced, some data from this field are incorporated as additional supporting evidence. We describe documented barrier-restoring capacities of probiotics, their mechanisms of interaction with the innate immune system, and the potential clinical applications of these bacteria to treat different chronic inflammatory diseases of the upper URT.

2 | PROBIOTIC REGULATION OF THE EPITHELIAL BARRIER FUNCTION

The epithelial barrier is the first physical barrier protecting the human body from the entrance of harmful substances including allergens, pathogens, and irritants.³³ Intercellular junctions, such as tight junctions (TJs), adherence junctions (AJs), and desmosomes, play a critical role in the formation and maintenance of the physical barrier. TJs are the most apically located junctions and provide the epithelia with a semipermeable size- and ion-specific barrier, restricting diffusion of macromolecular components.^{18,34,35} AJs are positioned immediately below TJs and are essential for cell-cell adhesion,

regulation of the actin cytoskeleton, intracellular signaling, and transcriptional regulation in epithelial cells.³⁶ Desmosomes form the third intercellular junction complex and are important in maintaining barrier integrity and cell adhesion.³⁷

The beneficial effects of probiotics on epithelial barrier dysfunction have been extensively studied in the gastrointestinal tract (Table 1). For instance, *Lactobacillus plantarum* MB452 has been shown to increase the expression levels of TJ-related genes in healthy intestinal epithelial cells (IECs) in vitro.³⁸ Similar positive effects on intestinal epithelial barrier integrity and TJ expression were observed with other probiotic strains such as *Lactobacillus rhamnosus* GG,³⁹ *Streptococcus thermophilus* ATCC19258, *L. plantarum* MB452,⁴⁰ and the Gram-negative probiotic strain *Escherichia coli* Nissle 1917.^{41,42} Additionally, certain *Lactobacillus* strains appear to have the capability to increase epithelial barrier integrity through the stabilization of AJs expression.⁴³ More specifically, the lactobacilli tested could increase the expression of E-cadherin and β -catenin and decrease the abundance of protein kinase C (enzyme involved in the disassembling of AJs) in T84 human colonic epithelial cell line.⁴³ Various barrier-restoring properties of probiotics have also been demonstrated in different

TABLE 1 Barrier-restoring capacities of different probiotics in the gastrointestinal tract

Strain	TJ or AJ proteins	Cells or mouse model	Barrier function	Ref.
<i>L. plantarum</i> MB452	↑ OCCL, ZO-1, ZO-2, and cingulin expression	Caco-2 cells	Increase in barrier integrity	38
<i>L. rhamnosus</i> GG	↑ ZO-1, claudin-1, and OCCL expression	Caco-2 cells	Restoration of gliadin induced epithelial barrier disruption	39
<i>S. thermophilus</i> ATCC19258 and <i>L. acidophilus</i> ATCC4356	Maintenance of actin and ZO-1, ↑ actinin and OCCL expression	HT29/cl.19A and Caco-2 cells	Increase in barrier function of untreated epithelial cells	40
<i>E. coli</i> Nissle 1917	↑ ZO-1 expression	Human small and large intestinal epithelial cells and DSS-treated mice	Upregulation of TJ expression in murine IECs and protection against DSS colitis induced increase in mucosal permeability	41
<i>E. coli</i> Nissle 1917	↑ ZO-2 expression, silencing of PKC δ	T84 cells	Restoration of a disrupted epithelial barrier	42
<i>L. acidophilus</i> , <i>L. fermentum</i> , <i>L. gasseri</i> and <i>L. rhamnosus</i>	↑ Stabilization of E-cadherin and β -catenin	T84 cell monolayers	Increase in barrier integrity	43
<i>L. rhamnosus</i> CNCM I-3690	↑ OCCLN and E-cadherin expression	Caco-2 cells and murine model of colitis induced by DNBS	Restoration of intestinal barrier function and increased levels of TJ and AJ proteins	44

in vivo models. For instance, in a murine model of colitis induced by dinitrobenzene sulfonic acid, oral administration of *L. rhamnosus* CNCM I-3690 protected epithelial barrier integrity by modulating TJ (occludin) and AJ molecules (E-cadherin).⁴⁴

In the airways, there are currently limited reports describing the regulatory properties of probiotics on the epithelial barrier. In vivo, it has been shown that oral treatment with *L. rhamnosus* CRL1505 could prevent the polycytidylic acid [poly (I:C)]-induced increased permeability of the bronchoalveolar-capillarity barrier, as determined by albumin levels in the lungs.⁴⁵ This improvement was linked to a decrease in the activation of pro-inflammatory cells and the production of pro-inflammatory cytokines in the lungs.⁴⁵ Similar findings were observed using nasally administered *Lactococcus lactis* NZ9000, which could counteract *S. pneumoniae*-induced lung tissue permeability.⁴⁶ In vitro, the stimulation of Calu-3 bronchial epithelial cells with the synthetic bacterial lipopeptide Pam3CysSK4 resulted in a concentration-dependent increase in epithelial barrier function and decrease in epithelial permeability, as a result of an increased expression of the TJ proteins claudin-1 and Zonula occludens (ZO) -1 and a decreased expression of occludin.⁴⁷

3 | PROBIOTIC INTERACTIONS WITH THE IMMUNE SYSTEM

3.1 | Interaction with pattern recognition receptors

Probiotics can modulate local and systemic host immune responses in a strain-specific manner via the expression of different microorganism-associated molecular patterns (MAMPs), such as lipopolysaccharides (LPS), flagellin, CpG-DNA, and other surface (lipo)proteins.⁵ MAMPs are recognized by pattern recognition receptors (PRRs) present on epithelial and innate immune cells (Figure 2, Table 2). The best-studied PRRs are the Toll-like receptors (TLRs), NOD-like receptors (NLRs), and C-lectin type receptors.⁴⁸ Thanks to the broad specificity of PRRs, a limited number of them can detect a wide range of MAMPs.⁴⁸ Interaction between MAMPs and PRRs leads to the activation of multiple signaling cascades that generates an appropriate molecular response against the encountered microorganism.^{49,50} In a 56-day murine ovalbumin (OVA)-induced asthma model, it has been demonstrated that oral treatment with *L. rhamnosus* NutRes1 and *Bifidobacterium breve* M-46 was able to modulate the mRNA expression levels of *Tlr9* and *Tlr3*.⁵¹ However, these results need to be analyzed with prudence, because the increase in mRNA expression levels of *Tlr3* and *Tlr9* does not necessarily prove that these 2 probiotics were recognized directly by the receptors. A similar reduction in the type 1 immune response and injury in the lungs were observed in a murine model of sepsis where treatment with *L. rhamnosus* GG and *Bifidobacterium longum* (strain not specified) via oral gavaging could attenuate neutrophil infiltration in the lungs and decreased the expression of interleukin (IL)-6 and tumor necrosis factor- α (TNF- α). The beneficial effects of these probiotics appeared to be associated with decreased gene expression levels of TLR2 and MyD88 in the lungs of the mice.⁵² Finally, oral treatment

TABLE 2 Examples of interactions of probiotics with the immune system (relevant for respiratory allergies)

Strain	Mechanism	Outcome	Ref.
<i>L. rhamnosus</i> NutRes1 and <i>Bifidobacterium breve</i> M-46	Reduction of chronic allergic inflammation by increasing the mRNA expression levels of <i>Tlr9</i> and <i>Tlr3</i>	Suppression of pulmonary airway inflammation, induction of airway remodeling and inhibition of mast cell degranulation	(48)
<i>L. rhamnosus</i> GG and <i>Bifidobacterium longum</i> ATCC	Attenuation of neutrophil infiltration in the lungs and decreased expression of IL-6 and TNF- α via decreased gene expression of TLR2 and MyD88	Reduction of immune response and lung injury	(49)
<i>L. reuteri</i> ATCC 23272	Modulation of TLR9 resulted in reduced levels of pro-inflammatory cytokines such as IL-5 and IL-13	Attenuation of inflammatory cell influx to the lungs and AHR	(50)
<i>L. reuteri</i> and <i>L. casei</i>	Modulation of DC function through dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin in vitro	Induction of IL-10 producing Tregs in vitro	(67)
<i>L. rhamnosus</i> GG and <i>B. lactis</i> Bb-12	Induction of Tregs, associated with increased expression of TGF- β in vivo	Inhibition of allergic sensitization and airway disease in an asthma mouse model	(64)
<i>L. casei</i> IBSO41 and <i>L. acidophilus</i> D031	Stimulation of DCs in vitro	Increased production of TGF- β	(69)
<i>L. plantarum</i> NCIMB8826	Inhibition of specific IgE response, high levels of specific IgG2a and increased production of IFN- γ	Inhibition of an allergic Th2 profile	(63)
<i>L. rhamnosus</i> GG	Significant decrease in eosinophils, IL-13 and IL-5 levels in the lungs, and airway hyperactivity	Prevention of birch-pollen-induced allergic asthma by LGG	(62)

with *Lactobacillus reuteri* ATCC 23272 of OVA-sensitized mice has been shown to result in attenuation of inflammatory cell influx to the lungs and airway hyper-reactivity (AHR). These beneficial effects seemed to be dependent on TLR9, because the treatment of TLR9^{-/-} mice with this *L. reuteri* strain did not attenuate inflammatory cell influx in the lungs or AHR. Additionally, the attenuation of inflammatory cell influx was associated with reduced levels of pro-inflammatory cytokines such as IL-5 and IL-13 in the bronchoalveolar lavage fluid (BALF) of the mice. However, *L. reuteri* ATCC 23272 could not alter Th1-type cytokines IL-12 and IFN- γ or the anti-inflammatory cytokine IL-10 in BALF.⁵³ Moreover, the authors showed that these effects were strain-specific, because oral treatment with *Lactobacillus salivarius* AH102 did not improve allergic airway response in the OVA-sensitized mice.⁵³ This is in agreement with the fact that the sum of all MAMPs expressed by a certain probiotic is strain-specific, resulting in their differential effects on epithelial and/or immune cells.^{5,54}

To our knowledge, there is currently no in vitro evidence showing the interaction between probiotics and PRRs using primary nasal or bronchoepithelial cells. Nonetheless, the modulating effects of TLRs by probiotics have been investigated using intestinal cell lines. For example, Ganguli et al⁵⁵ have shown the anti-inflammatory effects of *L. rhamnosus* GG and its SpaC pilus adhesin through the modulation of the TLR/IL-1R signaling pathway. The investigators demonstrated that T84 cells stimulated with *Salmonella enterica* serovar Typhimurium SL1344 or LPS can reduce IL-1 β and IL-6 secretion when treated with *L. rhamnosus* GG. These effects were due to the binding of SpaC pilus adhesin to the IECs, resulting in the downregulation of the mRNA levels of TLR3 and TLR4.⁵⁵ In addition, phenotypic comparison of wild-type and pili mutant strain of *L. rhamnosus* GG showed that the presence of pili reduced IL-8 induction in Caco-

2 cells, which was at least partially mediated by TLR2.⁵⁶ As the innate immune cells expressing PRRs provide a link between the innate and adaptive immunity, the described effects can further translate into the modulation of different T-cell subsets and the corresponding cytokines.

3.2 | Modulation of Th1/Th2 and regulatory T-cell immune response by probiotics

Probiotic applications have been shown to impact effector and regulatory T-cell responses in airway disease, resulting in the restoration of the Th1/Th2 immune balance,⁵⁷ and/or stimulation of regulatory cytokine production and the regulatory T-cell (Treg) subset.⁵⁸⁻⁶¹ This typically leads to a reduction in lung Th2 inflammatory cytokine levels (eg, IL-5 and IL-13) and the attenuation of AHR, as recently demonstrated for preventive intranasal administration of *L. rhamnosus* GG in a mouse model of birch pollen-induced allergic asthma.⁶² Additional immunomodulatory properties of probiotics in airway disease, including the stimulation of mucosal IgA levels and allergen-specific B- and T-cell responses, are well described elsewhere.⁶³

Allergic airway disease is typically a result of excessive Th2 reactions; therefore, the ability of probiotic strains to stimulate a counterbalancing Th1 immune response can be a valuable mechanism of airway disease prevention and treatment. In vitro, co-incubation with *L. plantarum* NCIMB885 induced a Th1-biased cytokine profile in murine dendritic cells (DCs), which could be linked to lipoteichoic acid composition of this probiotic strain.⁶⁴ In vivo, orally administered *L. rhamnosus* GG led to an increase in Th1 (IL-12 and IFN- γ) and a decrease in Th2 (IL-4, IL-5, and IL-13) BALF cytokine levels in a mouse model of OVA-induced airway inflammation.⁶⁵ This led to a reduction

in inflammatory cell counts in the BALF (ie, eosinophils, lymphocytes, and monocytes) and AHR.⁶⁵ The development of a Th1 over Th2 response was likewise observed as a result of intranasal co-application of *L. plantarum* NCIMB8826 and the clinically relevant house dust mite aeroallergen Der p 1 in a mouse model of Der p 1 sensitization.⁶⁶ This was reflected in an increase in specific IgG2a levels, a decrease in IFN- γ production by restimulated splenocytes, and an inhibition of specific IgE production. These effects were accompanied by the attenuation of airway inflammation after house dust mite extract challenge, as demonstrated by reduced BALF eosinophil counts.⁶⁶

Marked improvement of airway disease symptoms in murine models as a result of probiotic administration has also been linked to the induction of Tregs.^{67,68} Tregs are a subset of T lymphocytes, pivotal in maintaining immune homeostasis through the suppression of self-reactive T-cell activation and expression.⁶⁹ Their function is tightly linked to the regulatory IL-10 and transforming growth factor (TGF)- β cytokines, which can drive the activation and proliferation of Tregs along with their suppressive functions. These regulatory cytokines can be produced by DCs^{58–60} or T cells⁶¹ in response to interactions with probiotic strains. For example, *L. reuteri* ASM20016 and *Lactobacillus casei* NIZO B255 have been shown to bind DC-SIGN on DCs, which in turn drove the development of IL-10-producing Tregs.⁷⁰ Also, for *L. rhamnosus* GG, it was recently shown that glycosylated heteropolymeric pili are involved in such DC-SIGN-mediated interactions with DCs.⁷¹ In another study, *Bifidobacterium lactis* AD011 increased the production of IL-10 in a co-culture with DCs and IECs, whereas *L. casei* IBS041 and *Lactobacillus acidophilus* AD031 increased TGF- β secretion in this co-culture system.⁷² Similar results were obtained in another co-culture system containing peripheral blood mononuclear cells and *L. lactis*, that is, an increased secretion of the anti-inflammatory cytokine IL-10.⁷³ The in vitro data are further supported by in vivo studies in mouse models of allergic airway disease. Feleszko et al⁶⁷ have shown that orally administered *L. rhamnosus* GG or *B. lactis* Bb-12 can inhibit subsequent allergic sensitization and airway disease symptoms in a murine model of OVA-induced asthma. These effects are partially due to the induction of Foxp3-expressing Treg cells in the peribronchial lymph nodes, as well as increased TGF- β production by T cells in the mesenteric lymph nodes.⁶⁷ Likewise, oral treatment with *L. rhamnosus* NutRes1 and *B. breve* M-46 stimulated Foxp3 expression in blood Treg cells and decreased the levels of various pro-inflammatory cytokines such as IL-6 and IL-4 in the thoracic lymph nodes in a murine OVA-induced asthma model.⁵¹ This resulted in beneficial effects to the same extent as the corticosteroid budesonide, that is, suppression of pulmonary airway inflammation, the induction of airway remodeling, and the inhibition of mast cell degranulation.⁷⁴ Also, intranasal application of probiotic strains, such as *L. paracasei* NCC2461, has been shown to increase lung CD4⁺CD25⁺FoxP3⁺ Treg cell counts in a mouse model of allergic airway inflammation and to attenuate the allergic response.⁷⁵

Taken together, these studies emphasize the ability of certain probiotic strains to shift the immune balance away from pathological responses, suggesting their potential application for prevention and/or treatment of airway inflammatory diseases.

4 | CLINICAL APPLICATION OF PROBIOTICS FOR THE AIRWAYS

The beneficial effects of probiotics are highly influenced by different parameters such as duration and route of administration as well as the type of strain. Various studies have used murine models of allergic airway diseases to investigate the effects of probiotics. The administration route of these probiotics is typically oral, via drinking water or food,^{76–78} or through intragastric intubation.⁷⁵ More recently, intranasal administration of probiotic bacteria has been increasingly explored in different mice studies.^{62,75,79} In the study of Spacova et al, the authors demonstrated that intranasal administration of *L. rhamnosus* GG attenuated birch pollen-induced asthma in mice.⁶² However, when these mice were intragastric pretreated with *L. rhamnosus* GG, there was no significant effect on allergic sensitization.⁶² The effectiveness of intranasal application was also demonstrated in the study of Pellaton et al⁷⁵ Their findings demonstrated that intranasal application of *L. paracasei* NCC2461 can modify both the systemic and local immune response and allows for niche-specific effects compared to oral treatment. Also, clinical trials in humans to investigate the effects of probiotics on the airways are recently booming.⁸⁰ In most clinical trials, the probiotics are orally administered, for instance via capsules or supplemented in dairy products, and exert their effects mostly via modulation of the immune system. However, based on the results obtained in different murine models, we believe that local administration (eg, nasal spray) can induce more niche-specific effects both in the upper and in the lower airways, leading to a more effective treatment.

4.1 | Probiotics for the upper respiratory tract diseases

Different randomized placebo-controlled trials have been performed to investigate the potential of oral administration of probiotics in upper airway diseases.^{80,81} In the meta-analysis of Peng et al⁸², authors analyzed 11 randomized controlled trials in which probiotic efficiency for the prevention and/or treatment of allergic rhinitis (AR) was investigated. Results showed that in these 11 trials, probiotic treatment resulted in the improvement of the quality of life and nasal symptoms of the patients. However, none of the probiotic strategies tested could prevent AR nor improve the inflammatory markers in these patients. In the more recent meta-analysis of Guvenc et al,⁸¹ 22 randomized double-blind placebo-controlled studies were included, of which 17 studies demonstrated an improvement of the clinical symptoms in AR patients such as nasal blockage, rhinorrhea, and nasal itching. These beneficial effects were in 8 of the included trials linked with a decrease in inflammatory markers.⁸¹ The combination of different strains has also been studied in patients with AR. For instance, Koyama and colleagues tested a probiotic yoghurt containing a mixture of *L. rhamnosus* GR1 and *Bifidobacterium adolescentis* in 36 subjects with AR. They found no significant effects on clinical outcomes of the participants, but did observe increased serum levels of IL-10 and IL-12 during grass

pollen season, and increased TGF- β in ragweed season in the probiotic group compared to the placebo group.⁸³ Thus, although no effects on the patients' symptoms were observed, important regulatory markers were increased in these patients after probiotic administration.

Although the above-mentioned studies indicate that probiotics may improve the overall quality of life and nasal symptoms of AR patients, strong evidence is still lacking to substantiate preventive and/or therapeutic roles for probiotics in AR. Moreover, nasal application of probiotics has not yet—to the best of our knowledge—been tested in AR patients. Nasal application of probiotics can lead to the modulation of inflammation in the lower airways which was shown in a mouse model of allergic airway inflammation.⁷⁵ These results are very promising because asthma is an important comorbidity of AR.⁸⁴

In contrast to AR, the use of probiotics in patients with CRS is even less explored. In a small-scale pilot study, patients with CRS were shown to have reduced bacterial diversity with specific depletion of beneficial lactobacilli in brush samples of mucosal surfaces of the lateral, central, and medial portions of the maxillary sinuses.⁸⁵ Interestingly, these same authors could subsequently show that *Lactobacillus sakei* ATCC 15521 was sufficient to protect against *Corynebacterium tuberculostrictum*-induced sinusitis after intranasal inoculation in mice.⁸⁵ The authors focused on *L. sakei* because they found this species in higher abundances in the mucosa of healthy subjects and it was among the taxa that were significantly depleted in CRS patients.⁸⁵ This was one of the first studies to demonstrate that specific enrichment of the URT with lactobacilli can result in significant health benefits. In humans, a randomized placebo-controlled trial in CRS patients that were treated orally for 4 weeks with *L. rhamnosus* R0011 showed no improvement in sinonasal quality of life scores (SNOT-20 questionnaire).⁸⁶ Another double-blind placebo-controlled multicenter study demonstrated that oral application of *Enterococcus faecalis* Symbioflor 1 for 6 months could result in a reduction in the frequency of acute exacerbations of CRS. Furthermore, these beneficial effects were sustained for 8 months after the end of the treatment.⁸⁷ In line with these results, Kitz et al have reported a reduction in frequency and duration of recurrent acute sinusitis in children treated orally with *E. faecalis* Symbioflor-1 for 8 weeks.⁸⁸

To the best of our knowledge, only 1 placebo-controlled trial has investigated the potential of a nasal spray for the treatment of CRS without nasal polyps.⁷⁹ In this trial, 20 patients received a nasal spray containing 13 honeybee lactic acid bacteria (LAB) species (nine *Lactobacillus* and 4 *Bifidobacterium* species) for 2 weeks. After 2 weeks, the symptoms of the patients (SNOT-22 questionnaire) were evaluated, together with inflammatory markers in nasal lavage fluid and their microbiome. This study proved that the nasal LAB spray was well tolerated by the patients without any side effects.⁷⁹ However, there was no significant improvement with these honeybee isolates in symptom severity, nor changes in the immune response or microbiome of the patients.⁷⁹ Nevertheless, this study is a first important step toward the use of nasal

probiotic sprays for the treatment of CRS. Also, other studies exploring the use of a nasal spray containing probiotics for other URT infections, such as otitis media, already reported the safety of a probiotic spray in the nasal cavity.^{24,89,90} Additionally, we believe that better strain selection (especially human isolates from the URT) might be needed to observe significant effects on the clinical symptoms of patients.

4.2 | Probiotics for the lower respiratory tract diseases

When studying the treatment potential of probiotics in chronic inflammatory diseases of the URT (eg, AR and CRS), it is important to consider that these diseases are often associated with comorbidities in the lower airways, that is, mainly asthma.⁸⁴ Clinical studies investigating the potential of probiotics to treat patients with asthma are preliminary but show some promising results. For instance, Chen et al performed a randomized, double-blind, placebo-controlled trial to investigate the clinical potential of *Lactobacillus gasseri* PM-A0005 in children (6–12 years old) with asthma.⁹¹ After 2 months of daily treatment with 2×10^9 CFU/capsule of *L. gasseri* PM-A0005, they measured an improvement in asthma symptom score, together with an improvement in airway functions, that is, peak expiratory flow rates.⁹¹ Additionally, they observed a significant reduction in the blood levels of pro-inflammatory cytokines in the patients, such as TNF- α , IFN- γ , IL-12, and IL-13.⁹¹ In line with these results, Gutkowski et al showed that oral treatment with Trilac, a mixture containing *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, and *Bifidobacterium bifidum*, for 12 weeks in children with mild-to-moderate atopic asthma, resulted in an improvement in lung function and a reduction in exacerbations of asthma.⁹² In another study, van de Pol et al investigated the effect of synbiotic treatment in patients with asthma and house dust mite allergy.⁹³ They demonstrated that treatment with the combination of *B. breve* M-16V and galacto- and fructo-oligosaccharide for 4 weeks had no effect on allergen-induced changes in sputum eosinophils or lung function in these patients. However, a significant improvement in the baseline lung function and IL-5 levels in the serum was observed.⁹³ Finally, in the study of Rose et al, the authors performed a placebo-controlled clinical study where they investigated 6 months of *L. rhamnosus* GG food supplementation in infants with atopic predisposition and recurrent wheezing.⁹⁴ The results demonstrated no improvement in clinical outcomes, measured via severity scoring of atopic dermatitis and asthma symptom score (Ref Rose et al 2010). However, oral treatment with *L. rhamnosus* GG resulted in mild reduction in sensitization to aeroallergens as predictor for bronchial asthma.⁹⁴ More information regarding the involvement of airway microbial dysbiosis in asthma and the potential of probiotics to treat these patients are extensively reviewed elsewhere.⁹⁵

Although these studies are promising, more research is needed to evaluate whether targeting the URT via nasal and/or oral probiotic treatment is efficient enough to modulate the lung microbiota.

5 | CONCLUDING REMARKS

As the first culture-independent report on the presence of a healthy lung microbiota,⁹⁶ studies demonstrated the complex microbial communities residing in both the upper and the lower airways of healthy individuals.^{97,98} A dysbiosis of the microbiota has been shown in chronic airway diseases which, interestingly, seems to occur concomitantly with a defective epithelial barrier, as observed in asthma,¹⁹ AR,^{18,99} and CRS.¹⁷ Therefore, we believe that probiotics will represent a novel treatment option for chronic airway diseases. Probiotics can modulate nasal epithelial barrier via modulation of TJs and AJs and interact with the innate immune system, resulting in the restoration of mucosal barrier integrity.

Furthermore, the nature and extent of these beneficial effects might vary depending on the probiotic strain and administration route. Recent studies point toward the niche-specific effect of nasal administration of probiotics. Further research is nevertheless warranted to investigate the potential of nasal administration for the treatment of upper and lower airway diseases.

In addition, most of the results are obtained using in vitro and in vivo models of the intestine, and therefore, further research is needed to prove the beneficial effects of probiotics in the upper and lower airways.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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