Probiotics and Lung Diseases

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Increasing awareness of the role of intestinal commensal bacteria in the development and modulation of the immune system has led to great interest in the therapeutic potential of probiotics and other bacteria-based strategies for a range of immune-related disorders. Studies in animal models have identified strong immunomodulatory effects of many nonpathogenic bacteria and provided evidence that intestinal microbes can activate a common mucosal immune response and, thus, influence sites distant to the intestine, including the respiratory tract. Respiratory effects of probiotics in animal models have included attenuating allergic airway responses and protecting against respiratory pathogens. Dendritic cells appear central to directing the beneficial immune response to probiotic bacteria and in translating microbial signals from the innate to the adaptive immune system, whereas regulatory T cells are emerging as potentially key effectors of probiotic-mediated responses, particularly in the reduction of allergic inflammation. Despite progress in basic research, clinical trials of probiotics in allergy/asthma and respiratory infection have been highly variable at best, leading to an undermining of confidence in this potential therapeutic strategy. It is clear that there is still much to learn regarding the determinants of the diverse immune responses elicited by different bacterial strains. A deeper knowledge of the interactions between administered probiotics and the existing microbiota, together with an understanding of how the dialogue between microbes and the innate immune system is translated into beneficial/protective responses, will be required before we can achieve clinically effective bacteria-based strategies that maintain and promote respiratory health.

Abbreviations

DC
dendritic cell

GALT
Nonpathogenic bacteria that promote beneficial health effects when ingested have been termed probiotics. These “beneficial microbes” are most frequently *Lactobacillus* or *Bifidobacterium* species; however, a range of lactic acid bacteria (LAB) and nonpathogenic *Escherichia coli* have also been identified as having health benefits. Furthermore, although the Food and Agriculture Organization/World Health Organization currently define probiotics as live microorganisms that when administered in appropriate dose, confer a benefit of health on the receiver, nonviable microbes have also been regarded as probiotics because in certain circumstances they exhibit beneficial effects equal to live bacteria. Prebiotic is the term given to food supplements that are generally nondigestible and stimulate the growth and/or activity of probiotic bacteria, and a preparation containing both prebiotics and probiotics is referred to as a synbiotic.

Significant attention has been focused on the role of probiotics in GI development, immune adaptation, and attenuation of GI inflammatory diseases. However, there is steadily increasing evidence that orally delivered probiotics are able to regulate immune responses outside the GI tract, including the respiratory mucosa. What follows is an outline of our current knowledge of the potential benefits and underlying mechanisms of action of probiotic bacteria in relation to the respiratory tract with a focus on modulation of allergic responses and protection against infections.

### Allergy and Asthma

The microflora hypothesis proposes that perturbations in the GI microbiota, because of antibiotic use and dietary differences in industrialized countries, have disrupted the normal microbiota-mediated mechanisms of immunologic tolerance in the mucosa leading to an increase in the incidence of allergic disease, including asthma.\[^{[1]}\] Proof of principal has been provided in murine models, wherein antibiotic administration causes altered intestinal flora, impaired barrier function, diminished T helper (Th)1 immune responses, and enhanced allergic airway responses.\[^{[2]}\] The hypothesis is further supported by epidemiologic data from various regions of the world that link variations in GI microbiota, in particular a reduction in lactobacilli and bifidobacteria, with increased incidence of allergy and asthma.\[^{[3]}\], \[^{[4]}\] Data also suggest that a balanced microbiota plays a positive role in maintaining mucosal immunologic tolerance long after postnatal development.\[^{[5]}\] The fact that this immunomodulation is mediated by harmless commensals has led to efforts to determine whether probiotic treatment could be of benefit in allergic disorders.

Resulting clinical trials have indicated that feeding mothers with LAB, such as *Lactobacillus rhamnosus* GG and *Lactobacillus fermentum*, in the prenatal and early postnatal period may be effective in the treatment and prevention of early atopic disease
However, there have also been a number of clinical trials showing no effect of the same probiotic strains on the incidence or severity of allergic disease. It should also be noted that, to date, there have been no studies of children at high risk for developing allergy that have shown significant beneficial effects of probiotics on the incidence of asthma. However, there is evidence in animal models indicating that oral administration of certain LAB can modulate allergic responses in the respiratory tract. These beneficial effects are strain specific and the observed efficacy is also likely influenced by the antigen sensitization and challenge protocols used in the animal model. The differential response to LAB in asthma models is further emphasized by at least one study demonstrating enhanced allergic airway inflammation following neonatal treatment of mice with *Lactobacillus casei*.* Although the exact mechanism(s) behind the antiallergic action of these bacteria remain obscure, several potential components of this response have been highlighted.

Asthma is a T lymphocyte-mediated inflammatory disease, and it has been suggested that the common mucosal immune system is involved, with activated T lymphocytes migrating from one mucosal site to another. In keeping with this, the beneficial effect of probiotic organisms appears to be strongly associated with changes in the balance of T-cell responses that lead to a reduction in Th2 activity. In particular, there is growing evidence from a range of model systems that the ability to induce regulatory T cell (Treg) classes that attenuate both Th1 and Th2 responses may be a critical element in the antiinflammatory action of many probiotic organisms.

**Tregs: Effectors of the Antiallergic Response?**

Diverse populations of Tregs play an important role in regulating Th2 responses to allergen and maintaining functional tolerance. Tregs can be detected at sites of inflammation, and in many situations, their ability to migrate to and remain in inflamed tissue is important for their function in vivo. In rodent asthma models, CD4+CD25+Foxp3+ Tregs are recruited into the lungs and draining lymph nodes and can suppress allergen-induced airway eosinophilia, mucous hypersecretion, and hyperresponsiveness. Indeed, CD4+CD25+ transferred from *L reuteri*-fed nonsensitized mice can attenuate the allergic airway response in ovalbumin (OVA)-sensitized animals. *L rhamnosus* GG has also been shown to reduce the murine allergic airway response, with associated increases in Foxp3+ T cells, but only when the bacteria are administered in the neonatal period. This led to the suggestion that probiotic intervention might be successful primarily in the initial stage of intestinal colonization, a time point that is believed to be crucial for the maturation and balance of the immune system. However, as described previously, it is clear that certain strains of LAB can have profound immunoregulatory and antiallergic effects when administered to adult mice. Interestingly, Lyons et al demonstrated that one particular strain of *Bifidobacterium* induced Tregs only when fed to mice in the perinatal period, whereas another strain was able to induce Tregs in both adult and neonatal mice. Only this second strain attenuated the allergic airway response in adult OVA-sensitized mice. This suggests that a combination of bacterial strain-specific characteristics and host-specific processes, such as immunologic maturity, mucosal lymphoid antigen sampling, and gut barrier integrity, may be important for the induction of host regulatory responses.

LAB can induce a regulatory response that does not require prior exposure of Tregs to a specific allergen. Once activated, Tregs can suppress effector T cells in an antigen-nonspecific way called “bystander suppression,” and in vivo transfer studies demonstrate that Tregs can create a regulatory milieu that promotes the outgrowth of new populations of Tregs with antigen specificities distinct from those of the original population. In this way, certain LAB may induce Tregs in the gut-associated lymphoid tissue (GALT) that can spread to the airways in response to immune challenge and inflammation (Fig 1). This is supported by the finding that oral treatment with *L reuteri* results in an increase in Tregs in the draining lymph nodes of the lung, relative to vehicle-treated control subjects, only following airway challenge in sensitized mice.
Figure 1  Proposed gut-lung axis of probiotic action. Microbes in the intestine are sampled by DCs either directly from the lumen or following translocation through M cells to the GALT. A combination of signals from the microbes results in phenotypic changes in the DCs and the production of Th1 type and/or regulatory mediators. IL-12 promotes Th1 cells and activation and IFN-γ production by NK cells. Regulatory cytokines such as IL-10, TGF-β, and the activation of IDO and subsequent production of immunoactive KYNs promotes Tregs and depletes Th2 cells. Following immune challenge in the airway, cells activated in the GALT and MLN traffic to the respiratory mucosa where they promote protective and antiinflammatory responses. AHR = airway hyperresponsiveness; BALT = bronchus-associated lymphoid tissue; DC = dendritic cell; GALT = gut-associated lymphoid tissue; IDO = indolamine 2,3 dioxygenase; IFN = interferon; Kyn = kynurenine; MLN = mesenteric lymph node; NK = natural killer; TGF = transforming growth factor; Th = T helper; Treg = regulatory T cell.

However, despite growing evidence supporting an association between the antiinflammatory effects of LAB and an ability to induce Treg, a causal relationship has yet to be clearly established. Tregs are believed to be involved principally in the resolution of established inflammation,[14] and whereas adoptive transfer of Tregs from LAB-fed and helminth-infected mice can suppress airway inflammation, [15] [16] more research is required to determine the extent to which LAB-induced Tregs contribute to protection against an allergic airway response.

Dendritic Cells: Key Translators of Microbial Signals

It is an attractive concept that by controlling the maturation and function of dendritic cells (DCs), mucosal immune responses can be modulated. Given that DCs are pivotal in early bacterial recognition and can induce a range of Treg subtypes, there has, understandably, been great interest in interactions between commensal organisms and DCs.

Consequently it is becoming apparent that although Tregs may be major effectors of immune regulation mediated by probiotics, the functional changes in DCs following interaction with the bacteria is critical in orchestrating these responses. Specifically, the ability to induce IL-10 production by DCs, suggesting a regulatory phenotype, seems to be key to the immunoregulatory action of many probiotics.[19] Recently, Kwon et al.[20] confirmed that regulatory DCs expressing high levels of IL-10, transforming growth factor-β, COX-2, and indoleamine 2,3-dioxygenase (IDO) drive the generation of CD4+Foxp3+
Tregs following administration of a mixed-strain probiotic preparation in mice. The enzyme IDO is the rate-limiting step in the conversion of tryptophan to immunoactive kynurenines. DC expressing IDO contribute to the generation and maintenance of peripheral tolerance by depleting autoreactive T cells and by inducing Treg responses. Hayashi et al observed that the ability of bacterial DNA-derived CpG motifs to attenuate the allergic airway response was dependent on increased IDO activity in the lung, whereas the antiinflammatory effects of L reuteri in the airway of OVA-sensitized and -challenged mice is associated with increased systemic, but not localized lung, IDO activity. Significantly, the maintenance of a clinically unresponsive state following aeroallergen exposure in atopic individuals has been associated with increased IDO activity and IL-10 production. Overall it appears that the ability of certain microbes to promote IDO activity, in addition to IL-10 expression by DCs, may be important in the generation of a regulatory immune response and the establishment of tolerance.

**Probiotics and Lung Infection**

The increase in antibiotic resistance and need for new and improved strategies to tackle infectious disease have led to an examination of the therapeutic potential of commensal induced modulation of the mucosal immune response. Consequently, it has been discovered that certain LAB do have protective effects against bacterial and viral infections in the GI and respiratory systems. Administration of probiotics has been associated with lower incidence of ventilator-associated pneumonia, reduced respiratory infections in healthy and hospitalized children, and reduced duration of common cold infection. It should be noted that in addition to causing morbidity and mortality directly there is good evidence that respiratory infections, particularly viral infections, are a contributing factor not only to the exacerbation of asthma, but also to development of the disease. Indeed, it has been suggested that the focus of potential beneficial effects of probiotics in asthma should be directed at identifying organisms capable of reducing viral infections in early life.

In mouse studies, intranasal administration of LAB protects against respiratory pathogens. However, direct exposure of the probiotic organism to the airway mucosa is not required, and LAB can protect host animals from airway infection through an interaction with GALT, such as Peyer patch cells, and indirect enhancement of respiratory immunity (Fig 1). The protective effects of both intranasal and oral probiotics are generally associated with upregulation of natural killer (NK) cell and/or macrophage activity in the airway mucosa.

NK cells are the main components of host-nonspecific cell-mediated immunity, recognizing and helping to control a wide range of pathogens, including viruses, bacteria, and intercellular parasites. NK cells are activated by IL-12 that is produced by antigen-presenting cells, such as macrophages, DCs, and Langerhans cells. Here again, data suggest that the dialog between DCs and bacteria is key to controlling the immune effects of LAB beyond the GI tract. Koizumi et al demonstrated that feeding mice with Lactobacillus pentosus significantly enhances NK activity of spleen cells and induced NK1.1-positive NK and NK T cells to produce interferon (IFN)-γ. The increase in IFN-γ production did not occur through direct action of L pentosus on NK cells but was dependent on IL-12 produced by CD11c+ DCs following a toll-like receptor (TLR) 2- and/or TLR4-dependent interaction between the DC and LAB. Strains of LAB differ greatly in their ability to induce high levels of IL-12 in human DCs and consequently DC-dependent IFN-γ production by NK cells.

Alveolar macrophages provide the first line of defense against organisms that reach the lower airways. In addition to their phagocytic function, alveolar macrophages can synthesize and release various protein and lipid mediators on contact with pathogens or pathogenic substances. LAB have been well characterized in terms of an ability to induce cytokine production following contact with mononuclear phagocytes. The ability of orally administered L casei to dose-dependently enhance the phagocytic activity of alveolar macrophages likely contributes to the accelerated recovery of the innate immune response and improved outcomes following Streptococcus pneumoniae respiratory infection in malnourished mice and in young mice infected with Pseudomonas aeruginosa. Here again, the suggestion is that stimulation of the GALT leads to a general enhancement or priming of the innate immune response in the airway. However, recently Lactobacillus salivarius and L fermentum strains were shown to enhance both natural and acquired immune responses, as evidenced by activation of NK cells and the expansion of Tregs, whereas Bifidobacterium infantis can induce Foxp3+ T cells that protect mice against Salmonella typhimurium infection. Therefore, it is likely both branches of the immune system contribute to LAB-induced protection against respiratory pathogens. In addition, a number of LAB can produce antibacterial compounds, including Lactobacillus plantarum, which can inhibit the induction of virulence factors and thus the pathogenicity of P aerogenosa, suggesting that intrinsic antibacterial effects of LAB could contribute to protection against respiratory pathogens if administered directly to the site of infection.

**Challenges to Probiotic-Based Therapies**

It is likely that the antiinflammatory efficacy of a probiotic results from a combination of signaling pathways activated as a result of a specific pattern of microbe-derived ligands interacting with the corresponding receptors on host cells (Fig 2). Little is known, however, concerning the nature of the probiotic-host cell interactions, or how these interactions could be manipulated to obtain stronger regulatory responses. Factors to be considered include localization of particular bacteria in the GI tract and strain-specific cell wall components and metabolic products.
The probiotic-DC dialogue. Probiotic bacteria can interact with DCs via various surface molecules, which act to signal through microbe-associated molecular pattern (MAMP) receptors such as TLRs that can be extracellular or associated with endosomes such as TLR-9, and lectins, including the C-type lectin DC-SIGN. Important MAMPs include LTAs, PGNs, LPSs, and a range of CPSs including PSA. In addition to cell surface components, secreted products of bacteria may also influence DCs, including AHLs, part of the quorum sensing system of gram-negative bacteria. It is likely that specific combinations of signals instigated by these interactions determine the response of DCs and thus the immunoregulatory capacity of individual bacterial strains. +ve = gram-positive bacteria; -ve = gram-negative bacteria; AHL = acyl-homoserine lactone; CPS = cell wall-associated polysaccharide; DC-SIGN = DC-specific intercellular adhesion molecule 3-grabbing non-integrin; LPS = lipopolysaccharide; LTA = lipoteichoic acid; PGN = peptidoglycan; PSA = polysaccharide A; TLR = toll-like receptor. See Figure 1 legend for expansion of the other abbreviation.

Although animal studies have provided clear evidence that certain LAB can have profound immunoregulatory effects and regulate immune responses beyond the GI tract, the fact remains that the results of clinical trials have been highly variable. With particular regard to asthma there have been no beneficial effects reported. It is clear that candidate probiotic strains display a range of immune effects and therapeutic efficacies in specific disease states or model systems. However, strain differences are unlikely to explain all of the observed variability, as in clinical tests the same strains have produced conflicting results. Kuitunen et al. reported that probiotic supplementation of pregnant mothers and their offspring conferred protection from allergic disease only to cesarean-delivered children, suggesting that probiotic treatment may be beneficial only in subpopulations of patients (ie, those with abnormal or disrupted gut microbiota). It is also clear that the immunoregulatory actions of certain LAB can be inhibited in the presence of other strains. L reuteri, a poor inducer of IL-12 from murine DCs, inhibits IL-12, IL-6, and TNF-α induction by the otherwise strong cytokine inducer L casei, whereas both Bifidobacterium bifidum and L reuteri can inhibit Lactobacillus acidophilus-induced IL-12 production by DCs and accordingly abrogate IFN-γ production by NK cells. This suggests that the benefits of mixed strain probiotic preparations may actually be less than the sum of their parts. How the existing commensal LAB and other bacteria composing the host microbiota might influence immunomodulatory action of a single orally administered strain is also unknown. Other factors that have not been fully explored and may influence therapeutic efficacy include the fact that the immune response to candidate probiotics may depend on the growth phase of the bacteria. Furthermore, there has also been little examination of the immunomodulatory effects of long-term exposure to probiotics, and one study in mice suggests that some of the extraintestinal immune effects may be lost with sustained treatment.

Conclusions

For the reasons outlined previously, the therapeutic efficacy of live probiotic strains may be limited. However, alternative approaches may be developed. To date a number of microbial cell wall components, including polysaccharides and lipoteichoic acids, as well as potential secreted products, have been identified as being critical to the immunoregulatory effects of certain bacteria and/or to mimic the effect of whole organisms, including the ability to attenuate the allergic airway response in mice. Such research may lead to the development of therapies that effectively deliver critical triggers to the innate immune system in such a way as to mimic the immunoregulatory effects of whole probiotic organisms.
while bypassing some of the strain- and host-specific factors that might hinder the efficacy of live bacteria. Regardless of the approach taken, it is clear that without the identification of the critical characteristics of effective probiotic strains or a clear understanding of their mechanism of action, testing of probiotic-based treatment will remain highly empirical, and as such the outcome of clinical trials will continue to be variable and may serve to obfuscate the true potential of microbial-based therapies for respiratory disorders.

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