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Are vaccination models suitable to determine whether probiotics have beneficial health effects in the general population?

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Abbreviations: CFU, Colony forming units; DC, Dendritic cells; EFSA, European Food Safety Authority; iT-regs, Inducible T-regs; nT-regs, Natural T-regs; PBMC, Peripheral blood mononuclear cells; T-regs, T regulatory cells

The European Food Safety Authority (EFSA) has indicated that stimulation of protective antibody titers from vaccination could be used to substantiate a supplement or food health claim on the function of the immune system related to defense against pathogens in healthy individuals. Vaccination allows exposure of the immune system to controlled quantities of antigen and also for assessment of median antibody responses and percentage of responders/non-responders, which provides indication of an integrated immune response to challenge. Probiotic vaccination studies have shown enhanced antibody titers, lower percentages of non-seroconverters and greater percentages reaching minimum cut-off titer values in healthy adults, elderly and children. These results indicate that probiotics are a good candidate to stimulate responses to vaccines and thus, according to EFSA, enhance the function of the immune system related to defense against infection. However, animal research has recently indicated that Foxp3+ T-regulatory cells, recognized suppressors of immune activity, were paradoxically associated with reduced respiratory viral morbidity without compromising viral clearance. These effects conflict with vaccine research findings, which suggest a depletion of Foxp3+ T-regs enhances the immune response. Many probiotics exert anti-inflammatory influence on the immune system and induce T-regs. Given this, caution regarding the applicability of the vaccination model as indicated by EFSA must be exercised. Induction of T-cell immune modulatory pathways may also explain the reduced duration of respiratory illness observed in probiotic clinical studies.

Background

Probiotic supplements are purported to prevent infectious illness and enhance health. Initially recognized to reduce symptoms of gastrointestinal illness, in particular antibiotic associated diarrhea and acute infectious diarrhea, the list of illnesses for which probiotics may be beneficial has grown to include oral health, respiratory illness, metabolic dysfunction and some cancers.[1-5]

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Probiotics are likely to exert their health effects via a combination of mechanisms, such as competitive displacement of pathogens in the mucosa, the production of antimicrobial proteins that are toxic to pathogens, the production of metabolic substrates for mucosal integrity and the modulation of immune function. While there has been an extensive body of research undertaken, the evidence base for probiotics has been described as uncertain, confusing and conflicting.[6,7] This has, however, not prevented claims of efficacy being promulgated within the media and for probiotic supplements to enjoy broad popularity. In order to protect consumers from unsubstantiated claims regarding enhanced immune function, regulatory agencies have indicated the need for immune challenge tests related to improved protection from pathogens. In this context, vaccination-induced antibody response has been proposed as the ideal model for determining the effect of probiotic supplementation on immune function. This conflicts with growing evidence that probiotics exert anti-inflammatory influence on the immune system.[8,9] We propose in this mini-review that probiotic upregulation of T-cell dominant immune-regulatory pathways via the induction of FoxP3+ T-regulatory cells (T-regs) optimizes the dynamic balance between suppression and effector mechanisms without compromising respiratory viral host defense. This conflicts with a vaccine model of immune enhancement given that T-regs compromise vaccine efficacy. Caution regarding the applicability of the vaccine model is warranted in relation to viral respiratory infection.

Probiotics for Health

Initial evidence for the benefits of probiotic supplementation was for symptoms associated with gastrointestinal illness. While differential findings are reported between population cohorts and probiotic strains, meta-analyses indicate that probiotics reduce the risk of developing antibiotic associated diarrhea by ~50% and infectious diarrhea by 15–50% along with moderate reductions in severity and duration of illness.[10-13] Understanding of the role of authochnous bacteria also led to interest in the effects of probiotic supplements for other illnesses, particularly common infectious illness (common cold) in the healthy population. In the

generally healthy population consumption of a combination probiotic containing *Lactobacillus plantarum* HEAL 9 (DSM 15312) and *Lactobacillus paracasei* 8700:2 (DSM 13434) reduced the incidence of the common cold by ~20% ($p < 0.05$) and led to a 27% reduction in days of illness ($p < 0.05$).[14] In two independent studies of 142 elderly individuals (n = 57 and 85 respectively), supplementation with *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1 was associated with a 2.6-fold lower risk of catching a cold.[15] Other studies have shown benefits for respiratory symptoms in athletic cohorts,[16] children[17] and shift workers.[18] However, these effects are not uniform. Gender differences have been noted in one study[19] and in a number of other studies, probiotic supplements have had no significant effect on respiratory symptoms.[20,21] A recent meta-analysis found that while evidence is weak, overall, some probiotics have beneficial effects on the incidence, duration and severity of acute respiratory illness.[5]

While various mechanisms are likely to underpin the beneficial effects reported for probiotic supplementation on respiratory illness, it is direct modulation of the immune system that has been the primary focus of research. Human intervention studies have found contradictory effects depending on strain and dosage of bacteria, duration of supplementation, outcome markers and cohort under investigation. Overall, the effect of probiotics in vivo can only be described as modest at best. Berggren et al.[14] reported that consumption of *L. plantarum* and *L. paracasei* at 10^9 CFU/day for 12 weeks in 272 healthy individuals counteracted the proliferation of B lymphocytes compared with those on a placebo while no significant cellular immune response to probiotics was found for NK cells, T-lymphocytes or on T-helper, T-suppressor and T-cytotoxic cells. In contrast, de Vrese et al.[22] reported a significant increase in T-cytotoxic cells and T-suppressor cells but not in T-cell activation, natural killer cells, B-cells or phagocytic activity after 14 d of daily supplementation of a probiotic mixture with vitamins and minerals over two winter/spring periods in 479 healthy adults. Finally, a study examining dosages of 0, 10^8, 10^9, 10^{10} and 10^{11} colony forming units (CFU)/day^{-1} of a mixture of *Bifidobacterium animalis ssp. lactis* (BB-12) and *Lactobacillus paracasei ssp. paracasei* (CRL-431) in 71 healthy adults, reported no effects on phagocytic activity, fecal IgA, or whole blood cytokine production.[23] However, when correlated with the recovery of BB-12 in feces the production of whole blood interferon-γ was significantly reduced in the probiotic group. Overall, these data indicate that probiotic supplementation has little effect on the immune system of healthy individuals.

Health Claims Related to Immune Function

With an increasingly health conscious, informed and connected population, there is strong commercial interest in the use of claims for health and immune function to promote probiotic supplements within the general population. Thus far however, the equivocal results from studies of probiotic supplements have led to an almost outright rejection of commercial applications proposing health and immune claims, particularly by EFSA.[24] In considering future research design EFSA published a Scientific Opinion – Guidance on the scientific requirements for health claims related to gut and immune function.[25] A key issue noted in the paper relates to claims on immune function related to defense against pathogens. The Guideline notes that immunological parameters measured need to be relevant (to host protection from infection by the pathogen/s of interest) preferably shown in the same intervention study that shows clinical benefit (page 10).[25] Such advice arguably makes it difficult to measure a small number of discrete parameters of the immune system and argue that observed changes are practically meaningful. However, EFSA note "that stimulation of protective antibody titers (in response to vaccination) could be used to substantiate a health claim on the function of the immune system related to defense against pathogens".[25] Given that vaccine antibody responses are correlated with protection from pathogen infection, a heightened antibody response observed in conjunction with probiotic supplementation could be considered a beneficial response of supplementation.

Immune Suppression and Viral Infection

A recent animal study leads to questions over the utility of the vaccine model for evidence of probiotic-enhanced immune activity for defense against viral infection. In mice, Liu et al.[26] report that respiratory syncytial virus specific CD4+ T cells expressing the forkhead transcription factor FoxP3+ regulated effector CD8+ T cell responses in vivo to diminish RSV-induced illness without affecting viral clearance. T-reg cells regulate immune homeostasis and control the extent and degree of inflammation.[27] Natural T-regs (nT-regs) are generated in the thymus while inducible T-regs (iT-regs) are induced from CD4+CD25- T cells. Given the role of T-regs in suppression of inflammatory activity, T-regs can diminish the ability of the immune system to control and clear infection, thus impairing host defense if the balance between suppression and effector cells is skewed. This observation holds true for vaccination studies in which ablating or expanding T-regs has been associated with an inverse relationship to antibody response and clinical benefit.[28,29]

Further complicating the relevance of a vaccination model as a surrogate end-point for probiotic-related enhancement of immunity, is that many probiotic supplements exert an anti-inflammatory influence on the immune system, particularly the adaptive immune system, and promote iT-regs differentiation from CD4+CD25- T cells ex vivo and in animal models.[30] Some probiotic strains also increase the activity of nT-regs cells. In a human peripheral blood mononuclear cells (PBMC) co-culture, *B. lactis* W51, *L. acidophilus* W55 or *L. plantarum* W62 or an *Escherichia coli* control strain differentially increase T-reg number.[31] In this study, *L. acidophilus* W55 elicited the greatest increase in iT-regs while *L. plantarum* W62 had no significant effect above PBMC cultured in medium alone. No proliferation of nT-regs was observed. In a murine model of CD8+ T-cell mediated skin inflammation, daily intra-gastric feeding of mice with 200 μl of live *L. casei* DN-114 001 (10^8 CFU/ml) was found to enhance the frequency of nT-regs in the skin and increase the production of the anti-inflammatory cytokine interleukin-10 in draining lymph nodes.[32] Finally, Karimi[33] et al. reported that nine days of oral treatment of BALB/c mice with *L. reuteri* (10^9

CFU/day) led to a 1.6-fold increase in T-regs, which was also associated with an attenuation of inflammatory mediators to the lung in an experimental asthma model. These studies provide initial evidence that probiotic supplementation induces suppressive mechanisms of the immune system.

Evidence that probiotic bacteria modulate T-cell phenotype is consistent with understanding of the role of commensal microbiota in immune development and homeostasis. Probiotics may promote iT-regs via bacterial exposure to CD103+ dendritic cells (DC) in Peyer's patches followed by subsequent interaction of the DC with CD4+CD25- T-cells in gut associated lymphoid tissue and mesenteric lymph nodes.[34] Homing of the iT-reg cells via the common mucosal immune system, an interconnected system linking inductive sites to effector sites throughout the mucosa, may explain the extra-intestinal immune effects of probiotics. Whether the animal and ex vivo findings regarding the effects of probiotics on T-regs translate to human clinical research is yet to be determined. Initial studies should examine whether probiotic supplementation alters non-specific iT-reg numbers in peripheral blood. Furthermore, expression of chemokine receptors may provide indication of the target tissue for these iT-regs. Future work also needs to determine whether probiotics enhance the proliferation of respiratory virus antigen-specific T-regs during an active infection. These studies may shed light on whether a T-cell dominant immunoregulatory model rather than a vaccination model of heightened immune activation more closely resembles the beneficial effects of probiotic supplementation for defense against respiratory infection.

Does the Peripheral Induction of T-regs by Probiotics Shorten the Duration of Respiratory Illness Observed in Clinical Intervention Studies?

Probiotic-induced induction of T-regs also provides a potential model to explain the reduced duration and severity of respiratory illness evident in clinical human studies. The beneficial effects of probiotics in shortening the duration of symptoms has been proposed to be due to their ability to accelerate protective immunity and to subsequently clear pathogens more quickly.[6] Viral infection-induced symptoms are the result of inflammatory mediators, in particular cytokine secretion.[35] Further evidence of this comes from animal studies in which ablation of T-regs results in an increase in effector CD8 T-cell frequency and heightened illness symptoms.[36] This suggests that induction of a pro-inflammatory response by probiotics would instead worsen the severity of symptoms and lengthen the duration of illness. In contrast, the findings by Liu et al.[26] that T-regs reduce viral illness, suggests that the mechanisms for such an effect with daily probiotic supplementation may be related to immune suppression rather than enhanced immune activity.

The need for evidence that changes in immune function are relevant to defense against infection has led to recommendations by regulatory agencies that vaccine models are a surrogate for evidence of an integrated immune response to challenge. Animal evidence suggests that antigen specific T-cell dominant immune regulatory pathways are associated with reduced respiratory viral symptomatology that does not affect viral clearance. Probiotics supplements have been shown to exert anti-inflammatory influence on the immune system and induce T-regs. This raises some doubt over the utility of vaccine models for probiotic-induced immune modulation related to viral respiratory infection. Furthermore, it provides a model to explain the beneficial effects of probiotic supplementation on respiratory illness duration and severity.

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