REVIEW

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How can probiotics and prebiotics impact mucosal immunity?

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The study of probiotics and prebiotics is an expanding field of interest and scientific research that has resulted in insights related to the host immune response. Recent advances have naturally led to key questions. What are the specific probiotic components that mediate immunomodulation? Can we extrapolate the results of in vitro studies in animal and human trials? Which biomarkers and immune parameters should be measured in probiotic and prebiotic intervention studies? These questions were part of a discussion entitled "How Can Probiotics and Prebiotics Impact Mucosal Immunity" at the 2009 Annual Meeting of the International Scientific Association for Probiotics and Prebiotics (ISAPP). This review highlights recent knowledge about the modulation of mucosal immunity by probiotics and prebiotics, as well as considerations for measuring their effects on mucosal immunity. A list of biomarkers and immune parameters to be measured in human clinical trials is included.

Introduction

The immune system provides the first line of defense against pathogens and ingested toxins. A growing body of evidence suggests that host-microbial interactions may result in dysregulated mucosal immune responses, causing chronic inflammation such as Crohn disease or ulcerative colitis. A modest stimulation of the immune system by commensal bacteria may prevent infections. Immunomodulation is interpreted more broadly and includes antibodies, complement and cytokines, effects on gut barrier function and induction of antimicrobial compounds by the host. Microbes in the gastrointestinal tract (GIT) can exert numerous effects on different cells of the mucosal immune system and, in turn, induce the production of cytokines, which prime additional immune cells (**Fig. 1**). Depending on the immune stimulus, Toll-like receptors (TLRs) on the surfaces of immune cells are differentially stimulated and allow the immune system to discriminate between pathogens and the gut microbiota.[1] The soluble cytoplasmic NOD-like receptors, NLRs, also mediate communication between the GIT and gut microbiota. The NLRs and TLRs act synergistically, resulting in the induction of immune cascades such as the NFκB pathway, which ultimately leads to the induction of chemokines and cytokines.[2]

Probiotics, "live microorganisms which when administered in adequate amounts confer a health benefit on the host"[3] and prebiotics have been used more or less successfully to improve the host immune response in different conditions. The most recent definition for prebiotics was defined at the 2008 ISAPP meeting, which states, "A dietary prebiotic is a selectively fermented ingredient that results in specific changes, in the consumption and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health." Without a doubt, our understanding of the mechanisms of action of probiotics and prebiotics has been facilitated by recent advances in genomics, transcriptomics, metabolomics and advances in studies of immune function. Studies of interest include investigations of interactions of probiotics and prebiotics with the host at the mucosal interface, including the GIT. In addition, bacterial components may be responsible for immunomodulation and by using transcriptomics transcriptional effects on both the host and probiotics may be determined.

Researchers are capable of studying immunological effects on the host after exposure to intact bacteria, cell surface-associated factors, metabolites and secreted proteins. These effects are dependent on the bacterial strain and ultimately the effector molecules produced by probiotics or beneficial microbes in general. Challenges include the determination of biomarkers and evaluation of the bases for human trials. What are the biomarkers and what do we consider positive results? What is the relevance of results obtained from in vitro studies, and how do the laboratory data compare to in vivo studies? What is the relevance of immunomodulation for clinical trials, and how can laboratory studies be linked to a measurable health benefit? The first part of this review discusses the effects of probiotics, their effector molecules and prebiotic compounds on mucosal immunity, while the second part addresses the extrapolation of the immunomodulatory effects of probiotics and prebiotics in vitro to in vivo models such as interventional studies in humans. Additional questions that

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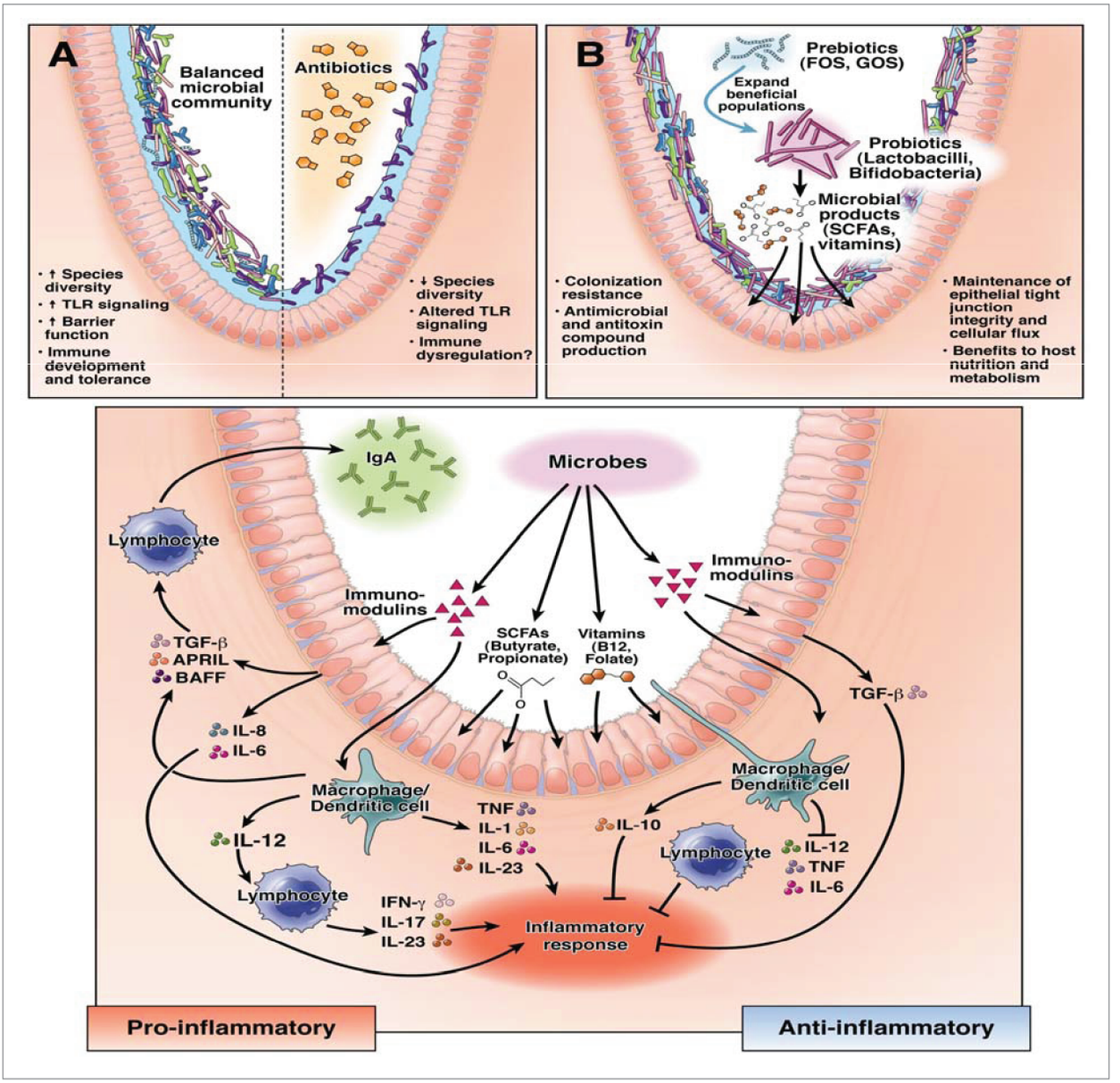

Figure 1. Microbial manipulation strategies and effects on intestinal biology. Reprinted from reference 72 with permission from Elsevier.

require discussion and further research are outlined in **Table 1**. This review highlights the discussions and research reviewed by the discussion group: "How Can Probiotics and Prebiotics Impact Mucosal Immunity" from the 2009 Annual Meeting of the International Scientific Association for Probiotics and Prebiotics (ISAPP).

Current Knowledge About Probiotics and Prebiotics and Their Effects on Mucosal Immunity

Probiotics and probiotics-derived effector molecules; gut barrier function and immune defense. Gut barrier function is vital for maintenance of gut health, with barrier dysfunction contributing to intestinal diseases including Crohn disease and irritable bowel syndrome (IBS). Probiotics and their effector molecules can influence the gut barrier by numerous methods including modulation of mucus production, reduction of bacterial adhesion, enhancement of tight junctions, enhancement of cell survival and induction of defensins or IgA. These effects can be accomplished by indirect influences on the permeability of tight junctions[4] and direct alterations of the tight junction by modulation of tight junction proteins and protein distribution in the membrane.[5-8] Moreover, activation of TLR2 [which responds to the presence of gram-positive cell wall components such as lipoteichoic acid (LTA) and peptidoglycan] by the gut microbiota is necessary for maintenance of gut homeostasis and protection from injury.[9]

A study by Mennigen et al.,[8] demonstrated that the probiotic VSL#3 mixture protected the epithelial barrier in a murine

model of colitis by preventing apoptosis and maintaining tight junction protein expression. Previous work demonstrated that conditioned media from *Bifidobacterium infantis* prevented a reduction in transepithelial resistance of intestinal epithelial cells and reduced ileal and colonic permeability in IL-10-deficient mice.[10] Another study demonstrated that prior exposure of an intestinal epithelial cell line to viable *Lactobacillus acidophilus*, but not heat-inactivated *L. acidophilus*, limited the adverse effects induced by an *Escherichia coli* strain, such as a decline in transepithelial resistance, increased epithelial permeability and physiological dysfunction. Additionally, viable *L. acidophilus* reduced phosphorylation of tight junction proteins induced by *E. coli*.[6] Two proteins, p40 and p75, were purified from the culture supernatant of *Lactobacillus rhamnosus* LGG, and both proteins prevented TNF-induced apoptosis and intestinal barrier disruption in colonic epithelial cells.[11]

The effects of probiotics and their effector molecules on barrier function in clinical trials are sparse. In one study, the efficacy of a placebo, VSL#3 and probiotic-derived sonicates, was studied for modulation of intestinal permeability in patients in the intensive care unit with multiple organ dysfunction syndrome.[12] Patients that received VSL#3 exhibited a larger increase in serum IgA and IgG concentrations than the patients who received placebo or sonicates. However, no significant difference in intestinal permeability was observed between the patients who received VSL#3, probiotic sonicates and placebo.[12] In another study, a short-term improvement in mucosal barrier function in patients with IBS was observed after administration of a probiotic fermented milk (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *L. acidophilus* and *Bifidobacterium longum*) compared to a milk beverage containing no bacteria.[13] These studies emphasize the importance of strain selection and viability. In addition, as noted in other studies with prebiotics and probiotics, effects on gut barrier function varied between strains and effector molecules.[6,10,14] It is clear that probiotic immunomodulatory components consist of a varied array of effector molecules (**Table 2**), including surface layer proteins, cell wall polysaccharides, adhesins, teichoic acids and heat shock proteins. Other immunomodulatory components have been understudied or remain to be characterized. While in vitro work to identify important candidate effector molecules (**Table 2**) continues, the effects on mucosal immunity in human intervention studies still needs to be determined.

Live bacteria, dead bacteria or bacterial supernatants. *Effects on host gene expression and mucosal immune responses.* Studies have been performed using live, dead and bacterial supernatants to assess the mucosal immune response. In particular, probiotic *Lactobacillus plantarum* WCFS1 has been studied using in situ-based -omic models in the GIT. Gene expression of beneficial bacteria[15] and the mammalian host[16,17] has been studied in parallel. Gene expression profiles in ceca of germ-free mice fed a standard low fat or western diet were investigated to determine the effects of host diet on bacterial (probiotic) gene expression in the GIT.[15] The western diet was high in simple sugars and fat, and the standard diet (low fat diet) was high in complex plant polysaccharides.[15] In addition to transcriptomics studies, colonization levels of *L. plantarum* were measured in mice fed both diet types, and was determined to be ten times higher in mice receiving the standard feed compared to the western diet. Gene expression was compared to a reference group of differentially expressed genes, namely mid-logarithmic *L. plantarum* grown in vitro in MRS, chemically defined medium and chow medium. The main results from this study indicated that bacterial genes involved in carbohydrate metabolism and cell surface functions were upregulated in mice fed the standard diet compared to the culture media. In addition, it was determined that the western diet resulted in a nutritionally restricted, growth-limiting environment for *L. plantarum* in the distal gut.[15] In mice fed both diets, gene sets encoding cell surface-related functions were differentially expressed, which included genes involved in D-alanylation of LTA, a probiotic effector molecule (**Table 2**). The authors suggested that the probiotic bacteria modified its gene expression to reduce levels of LTA on the cell surface, as a means to restrict exposure to components of the host immune system in the GIT of mice fed both diet types.

The growth phase and viability of the probiotic cultures is also a factor that varies between reported experiments. For example, in a study by van Baarlen and co-workers,[17] differences in gene expression in the duodenum of healthy humans was observed depending on the growth phase and viability of the probiotic *L. plantarum* WCFS1. Individuals were administered logarithmic, stationary or dead cells and samples were taken from the duodenum for analysis after 6 hours. Results from this small randomized, double-blind, placebo-controlled crossover study identified gene expression patterns at the mucosal surface and within cellular pathways that were related to immune tolerance. While all three conditions resulted in the induction of genes involved in the immune response, differences in the type of induction and number of genes induced were observed between logarithmic and stationary phase cells. Logarithmic phase cells induced a response targeted towards metabolic functions such as nucleic acid metabolism and cytoplasmic organization. However, stationary phase bacteria resulted in induction of genes such as the NFκB and JUN transcription factors involved in the establishment of immune tolerance.[17] Interestingly, the

Table 1. Key questions require further study and discussion

• What are the specific probiotic components that mediate immunomodulation?
• What other microbes, perhaps as yet undiscovered or understudied exhibit immunomodulation properties?
• What is considered healthy and how do we measure health benefits in human trials and animal studies?
• What are the factors influencing responses in clinical trials?
• Which biomarkers and immune parameters should we use in probiotic and prebiotic intervention studies in humans?
• What are the systemic effects of prebiotics on the gut microbiota and direct effects on the gut mucosa?
• What are the best target groups to use for human trials?
• How comparable are in vitro, versus in vivo and ex vivo studies?
• How comparable are results from animal models to human clinical trials?

Table 2. Potential effector molecules derived from probiotics

Bacteria	Molecule	Effect	References
Lactobacillus reuteri	Secreted factors by *L. reuteri* biofilms	Suppression of human TNF production by LPS-activated monocytoid cells	54
L. reuteri	Secretion of reuterin (antimicrobial glycerol derivative) *by L. reuteri* biofilms	Secretion of reuterin by biofilms demonstrated	54
Intestinal bacteria	Butyrate (a short chain fatty acid)	Increased intestinal barrier function in Caco2 cell lines	55
Lactobacillus acidophilus NCFM	S layer protein	DC-SIGN ligand involved in the modulation of DCs and T cells functions	56
Faecalibacterium prausnitzii	*F. prausnitzii* supernatant	Reduced the severity of TNBS colitis and tended to correct the dysbiosis associated with TNBS colitis	57
Lactobacillus casei Shirota	Cell wall polysaccharide moiety	Inhibit macrophage and splenocyte activity	58
Bifidobacteria infantis	*B. infantis* conditioned medium	Reduced colonic permeability in mice and attenuated inflammation in IL-10-deficient mice	10
Lactobacillus helveticus	S layer protein	Inhibition of *Escherichia coli* 0157:H7 adherence to intestinal cell lines	59
Lactobacillus rhamnosus GG	Modified teichoic acids	Increased sensitivity to gastric juice and human beta-defensin-2 but no difference in immunomodulation	60
L. rhamnosus GG	P40 and P75 secreted proteins	Prevention of cytokine-induced apoptosis in human and mouse intestinal epithelial cells	11
Lactobacillus salivarius UCC118	Secretion of bacteriocin Abp118	Protected mice against infection with the pathogen *Listeria monocytogenes*	61
Escherichia coli Nissle 1917	Flagellin	Induction of beta-defensin-2 in intestinal cell lines	62
Lactobacillus johnsonii La1	GroEL	Bind to components of the gastrointestinal mucosa and stimulates interleukin-8 secretion in macrophages and HT29 cells in a CD14-dependent mechanism	63
Lactobacillus delbrueckii ssp. *bulgaricus* OLL1073R	Extracellular polysaccharides (EPS)	Stimulation of mouse splenocytes and significant increase in interferon-gamma production	64
Lactobacillus plantarum NCIMB8826	Modified teichoic acids	Protective against colitis	23
L. johnsonii NCC533	Adhesin; elongation factor Tu (EF-Tu)	Mucin binding, proinflammatory response in the presence of soluble CD14	65

dead cells (heat-killed stationary phase) induced the highest fold changes, and the mucosal responses to dead and stationary phase bacteria were comparable to each other but different than the responses to logarithmic phase bacteria. These studies demonstrated the importance of reference sets of gene expression data used for comparisons in vivo, in addition to diet, growth media and growth phase of probiotics. Factors such as the stability of each probiotic strain, expression and stability of effector molecules, growth phase of the probiotic strain and site of action of probiotic-derived effector molecules should be considered for in vivo studies. Finally, can immunomodulation in the laboratory be connected with health benefits in humans and animals?

Prebiotics and mucosal immunity. Prebiotic benefits include modulation of the gut microbiota, increased mineral adsorption, modulation of lipid metabolism and inhibition of pathogens.[18] While studies of prebiotics have documented their effects on mucosal immunity, the majority of these reports included animal models, with relatively few human studies published to date. Some published studies have included other factors such as antioxidants and vitamins, which may also contribute to mucosal immunity. In studies with synbiotics, it is difficult to distinguish the effects of prebiotics from that of probiotics.

A few studies examined immunomodulation by dietary prebiotics (reviewed in ref. 19). Prebiotic supplementation increased fecal secretory IgA and postnatal immune development in infants.[20] In disease states, moderate effects were seen in patients with Crohn disease,[21] but results were encouraging enough to warrant further exploration. Because prebiotic compounds per definition modulate the composition of the gut microbiota, it is difficult to know whether these effects are direct or are the consequences of a shift of certain bacterial groups. Clinical trials have included an analysis of microbial composition and associated effects on short chain fatty acids (SCFA). Products of bacteria that have been reported to show anti-inflammatory properties include SCFAs (**Table 2**).[22] Other bacterial metabolites that could be responsible for the modulation of the immune system, remain to be characterized.

Additional animal and human studies are needed, as there is no clear consensus on the effects of prebiotics on mucosal immunity. Points to consider include the nature of prebiotics and

consequent indirect interactions such as system-wide changes of gut microbiota and the subsequent effects on mucosal immunity and/or health benefits and direct interactions of prebiotics with the mucosa. The challenge is determining these effects on health and designing better trials including the use of biomarkers (discussed below).

Can We Extrapolate Immunomodulatory Effects of Probiotics and Prebiotics In Vitro to Humans?

Factors influencing the immune response in vivo and in human clinical trials. Extrapolation of immunomodulatory effects found in the laboratory and in animal studies with outcomes in human trials presents a difficult challenge. Immunomodulatory effects conferred by *L. plantarum* WCFS1 in vitro,[23] in animal models,[16,23] but also in humans[24,17] highlights the difficulties of comparing similar effects by a single strain in different contexts. A few examples show correlations between in vitro and in vivo immunomodulatory properties of lactic acid bacteria.[23,25,26] The study by Foligne et al.[25] for instance, demonstrated the possibility of translating results from an in vitro assay with peripheral blood mononuclear cells (PBMCs) obtained from different donors, to a murine model of colitis using the IL-10/IL-12 ratio. Generally, the discrepancies between in vitro and in vivo results observed in published trials can be partly explained by the host contribution (genetic factors, different baseline immune functions between individuals, microbiome diversity, differences in the body sites targeted, intra-person variation) as well as environmental factors (diet, stress, etc.) partially controlled by each individual. Different factors can affect outcomes in studies reporting immunomodulatory effects of probiotic or prebiotic interventions in vivo.

Variation between individuals and effects on host response. Genetic factors. Genetic variability strongly influences the immune response of the host. Specific polymorphisms in genes encoding antigens (human leukocytes[27]) or proteins involved in the immune response can influence quantities of cytokines[28] in a healthy population or disease state. A growing body of evidence indicates that individuals respond to bacterial signals (TLR ligands) differently due to single nucleotide polymorphisms (SNPs) within TLR genes.[29] A frameshift mutation in NOD2 is a strong risk factor in patients with inflammatory bowel disease (Crohn disease), and this evidence supports the importance of SNPs and inter-individual differences in TLR signaling.[30,31]

Microbiome. Preliminary observations that all vertebrates have a microbiome that co-evolved with the host, resulted in the hypothesis that the adaptive immune system (memory-based) evolved in the vertebrate lineage because of the intimate co-existence with complex communities of beneficial microbes.[32] Whether this is true or not still needs to be demonstrated, but it certainly highlights the complex links between our microbiome and our immune system. Because we are in the early stages of characterizing the phylogenetic or functional core microbiomes in human and animal models, it is difficult to fully understand relationships between the host microbiota and immune system. However, the relative paucity of microbes such as *Faecalibacterium prausnitzii* in inflammatory bowel disease (Crohn disease) has been demonstrated by different studies.[33] Baseline immune functions and microbiomes may differ and depend on each other so that aggregate responses and disease susceptibilities in individuals may represent a combination of microbial composition, microbial functions and host genotypes.

Influence of environmental factors controlled by the host such as diet. The gut microbiota is a dynamic environment, and changes induced by the diet, the uptake of antibiotics or even physiologic stresses may induce rapid changes in microbial composition and functions,[34] with a concomitant impact on the host immune response. Rapid changes in microbial composition have been well documented with a high fat diet.[35] These results should encourage investigators to control diets of subjects and support the use of standardized diets when measuring the host immune response. Dietary components may have a direct effect on the response of the immune system and vitamins, for example, may have an effect on cytokine production.[36] To make the story more complex, effects of dietary components may be partly determined by genetic polymorphisms of cytokines such as TNF.[37]

Consideration of body site contribution. Small intestine versus colon. Immune parameters measured in different body sites will certainly be different and may have an impact on results and outcomes of studies with probiotics and prebiotics. In the gut, the colon has been more extensively investigated than the small intestine or other mucosal sites of the GIT such as mouth or stomach,[38] and certainly merits further investigation. As the composition of the mucus layer and mucus barrier is different at these sites, the contribution of the mucus layer and its interaction with probiotics and prebiotics or other members of the gut microbiota should be considered and investigated further.

Which biomarkers and immune parameters shall we use in probiotic/prebiotic interventions in humans? No universal biomarkers have been identified to assess how probiotics and prebiotics impact mucosal immunity. However, specific biomarkers can be defined for certain populations and disease states, and are summarized in **Table 3**. Measuring these parameters is still challenging in healthy people, and limited by the ability to analyze markers only in blood, saliva, fecal samples or urine. In an experimental setting with disease patients, it is usually possible to get biopsies from the gut. New techniques and potential biomarkers are still emerging and may offer promising alternatives in the future. For instance, recent discoveries on the role of C-reactive protein (CRP) isoforms and their role in inflammation could facilitate an improved understanding of the importance of different isoforms.[39] Metabolites that distinguish patients with IBD, IBS or other gut diseases have emerged.[39,40] Choices of immune markers will certainly depend on the population studies and diseases studied in probiotic and prebiotic interventions.

Consideration of interventional trials with probiotics and prebiotics. *Choices of animal models.* Different animal models have been used to study the effects of probiotics and prebiotics on mucosal immunity. Effects in mouse models of IBD have included IL-10 knockout mice, trinitrobenzene sulfonic acid (TNBS)- or dextran sodium sulphate (DSS)-induced colitis. Based on the characterization of the microbiota in different knockout mice

Table 3. Possible immune biomarkers and parameters for assessment of mucosal and systemic immunity in vivo in response to probiotic or prebiotic interventions

Marker	Advantages	Drawbacks	References
Cytokines (TNF, IL6, IL10, IL12) FoxP3	Easy to measure	Level in blood may not reflect levels in other body sites	66
CRP and acute phase reactants	Easy to measure Different conformational change	Measure other outcome	39
Antibody (IgA)	Easy to measure	Not helpful without immune challenge	67
Gene expression profile in tissues and peripheral blood (targeted vs. global)	Extensive survey of gene activated	Difficulties for obtaining tissues in healthy humans	17, 24
Proteomics and metabolomics in urine and blood and fecal water	Extensive survey of metabolites produced	Difficult to analyze Lack of references	44, 68, 69
Calprotectin	Easy to measure in stool	Standardization necessary	70, 71, 67

and wild-type mice using genotyping and 16S rRNA gene analyses, large differences in the composition of the microbiota can be observed in different animal models,[41] as well as between animal models obtained from different vendors.[42] Differences in microbial composition between C57BL/6 mice obtained from the Jackson laboratory and Taconic laboratory were investigated recently using a high density microarray (PhyloChip). These mice differed with respect to the relative proportions of Th17 cells that contribute to inflammation and may be mediators in immune protection and immunopathology. Comparative analyses of 766 bacterial taxa detected in both groups of mice demonstrated large differences in the relative abundance of more than half of the taxa, with two taxa [*Lactobacillus murinus* and segmented filamentous bacterium (SFB)] being more than 25-fold more abundant in Taconic mice. The Taconic mice in this study had elevated proportions of Th17 cells.[43] Most importantly, it was demonstrated that colonization of mice housed in the Jackson laboratory with one SFB species (Arthromitus spp.), was sufficient to induce the appearance of Th17 cells derived from a $CD4^+$ T helper cell lineage.[43] Furthermore, this colonization was correlated with enhanced expression of genes associated with inflammation and antimicrobial defenses, resulting in enhanced resistance to the murine intestinal pathogen *Citrobacter rodentium*. Thus, characterizing the microbiomes in animals at sites where immune parameters will be measured, before intervention, may provide further insights into the possible roles of certain microbes and outcomes with probiotics or prebiotics. Murine models with "humanized" microbiomes offer promising systems for evaluation of microbial effects on host immune responses. Thus, humanized microbiome murine models (for example with a baby microbiota)[44] have similarities with that of formula-fed neonates.[45] However, the relevance of these humanized rodent models to human diseases remains to be seen.

• *Response to vaccine challenges.* Vaccine challenges are particularly useful to assess the efficacy of probiotics that may enhance the immune status in a healthy population. Probiotics enhance the immunogenicity of several vaccines including rotavirus, influenza, poliovirus, hepatitis B and pneumococcus.[46-48] Measurement of antibody responses to vaccines is straightforward, and such human studies may provide excellent opportunities to evaluate the immunologic efficacy of probiotics.

• *Non-responders.* Separating responders from non-responders may facilitate interpretations of effects of the probiotics or prebiotics on the immune system. Only a few in vivo trials have been performed.[49]

• *Influence of age, gender, diet.* Because immune parameters differ according to age, gender, health status, activity and dietary habits, human studies should use crossover placebo-controlled design while trying to match and compare different groups.

• *Core or functional microbiomes.* In the future, integrated approaches using microbiology, genomics, transcriptomics and proteomics tools would certainly highlight the mechanisms and the microbes that may influence the immune system.[50] It is still debatable whether a core microbiome exists at the phylogenetic level[51] or functional level.[52] However, recent studies such as those by Gordon and colleagues suggest the existence of a functional core at the level of shared gene families, which suggests that different species could fulfill similar functional roles in the GIT. As pointed out by Shop et al.[53] however, an interesting follow-up question regarding the outcome of this study is whether the lack of shared phylotypes is still as pronounced if rare populations are taken into account.

Summary and Conclusions

Research scientists face numerous challenges including the demonstration and articulation of specific beneficial effects and associated mechanisms by probiotics and prebiotics. A clear definition of human health, if one exists, is a central consideration. What is considered "healthy" as a baseline condition may vary depending on age, gender, ethnicity, diet and numerous environmental factors. Although we have some answers with certain parameters (leukocytes, cytokines), baseline parameters including quantitative "normal" ranges of immune biomarkers remain to be defined. Non-invasive techniques that evaluate blood, fecal water or urine metabolites need to be further developed. Many studies are needed to understand various metabolites and how they may provide measurable standards for interventional studies. Additionally, the involvement of neuro-immune interactions in the GIT is an understudied area of research with respect to probiotics and prebiotics.

The difficulty to establish health claims such as "strengthen the immune response" for probiotics has been illustrated recently in Europe by the absence of health claims related to the stimulation of the immune system authorized for probiotic foods by the European Food Safety Authority (http://www.isapp.net/docs/ISAPP_responds_to_EFSA_oct09.pdf). A growing body of evidence has documented the beneficial effects of probiotics and prebiotics in disease treatment and management studies. However, long-term studies are still needed to identify their prophylactic effects towards inflammatory disorders in different populations.

Before using probiotics in interventional studies, many factors remain to be considered. For example, viable cells are generally more effective at stimulating adaptive immunity, and the method of cell killing should be considered if nonviable cells are used. If cell supernatants are used, the active component(s) should be purified and the stability and physiologic effects of these compounds must be considered. Doses of bacteria and growth phase at time of harvest are additional considerations in tandem with traditional methods of determining strain robustness or functional effects. On the host side, genetic influences, site of action, the mucus barrier, route of administration, diet and microbial composition of the host contribute to results of interventional trials published to date. In future trials, individual factors that potentially affect the efficacy of probiotics and prebiotics must be addressed. Continuing advancement in technologies, knowledge of the immune system, gut microbiota and improved biomarkers are essential to making human interventional studies with probiotics and prebiotics successful.

Conflicts of Interest

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