# Cross-Talk between Probiotic Bacteria and the Host Immune System[1,2]

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## Abstract

Among the numerous purported health benefits attributed to probiotic bacteria, their capacity to interact with the immune system of the host is now supported by an increasing number of in vitro and in vivo experiments. In addition to these, a few well-controlled human intervention trials aimed at preventing chronic immune dysregulation have been reported. Even though the precise molecular mechanisms governing the cross-talk between these beneficial bacteria and the intestinal ecosystem remain to be discovered, a new and fascinating phase of research has been initiated in this area as demonstrated by a series of recent articles. This article summarizes the status and latest progress of the field in selected areas and aims at identifying key questions that remain to be addressed, especially concerning the translocation of ingested bacteria, the identification of major immunomodulatory compounds of probiotics, and specific aspects of the host-microbe cross-talk. The interaction with immunocompetent cells and the role of secretory IgA in gut homeostasis are also evoked. Finally, a brief overview is provided on the potential use of recombinant DNA technology to enhance the health benefits of probiotic strains and to unravel specific mechanisms of the host-microbe interaction. J. Nutr. 137: 781S–790S, 2007.

Modulation of host immunity is one of the most commonly purported benefits of the consumption of probiotics. Increasingly growing, but still limited, clinical evidence exists to support this concept. Nevertheless, general claims regarding probiotic modulation of host immunity overstate our current knowledge of both the fate of ingested probiotic products and their specific effects on molecular and cellular components of the immune system, even though progress has recently been made in analyzing possible mechanisms involved in host-microbe interactions. The direct antagonism toward infectious organisms by probiotics, although a clearly important application, is generally not featured in this article and has been reviewed recently (1–5). This article summarizes the status of the field in selected areas referring to specific examples. It aims at identifying the major gaps that remain in our knowledge and outlines possible avenues to fill those gaps rather than reviewing the abundant literature in this research area. Complementary information can be found in a number of recent reviews (6–9).

## Fate of ingested bacteria in the gastrointestinal tract

It is commonly suggested that probiotics must "persist and multiply" in the target ecosystem to be effective. However, the interaction of orally ingested probiotics with the intestinal epithelium or other immunologically active intestinal cells has just begun to be rigorously studied. A number of studies with a variety of probiotic strains have been conducted to determine the extent to which probiotics "colonize" or, more correctly, transiently persist in the intestine. The combined results demonstrate conclusively that ingested strains do not become established members of the normal microbiota but persist only during periods of dosing or for relative short periods thereafter (10–14). There is also evidence that common probiotic strains differ in their degree of persistence (10,15). This may reflect in part their capacity to resist the harsh conditions encountered in the upper digestive tract.

Presumably, to modulate immunity, probiotic organisms must "talk" to immune cells that are endowed with recognition receptors or that are otherwise sensitive to probiotic-derived products (e.g., metabolites, cell wall components, DNA) (**Fig. 1**). There is no a priori reason that introduced strains would need persist and multiply to encounter intestinal immune cells. In fact, general acceptance that "colonization or persistence" is required

[1] Published as a supplement to *The Journal of Nutrition*. The articles included in this supplement are derived from presentations and discussions at the World Dairy Summit 2003 of the International Dairy Federation (IDF) in a joint IDF/FAO symposium entitled ''Effects of Probiotics and Prebiotics on Health Maintenance—Critical Evaluation of the Evidence,'' held in Bruges, Belgium. The articles in this publication were revised in April 2006 to include additional relevant and timely information, including citations to recent research on the topics discussed. The guest editors for the supplement publication are Michael de Vrese and J. Schrezenmeir. *Guest Editor disclosure*: M. de Vrese and J. Schrezenmeir have no conflict of interest in terms of finances or current grants received from the IDF. J. Schrezenmeir is the IDF observer for Codex Alimentarius without financial interest. The editors have received grants or compensation for services, such as lectures, from the following companies that market pro- and prebiotics: Bauer, Danone, Danisco, Ch. Hansen, Merck, Müller Milch, Morinaga, Nestec, Nutricia, Orafti, Valio, and Yakult.

[2] Author disclosure: no relationships to disclose.

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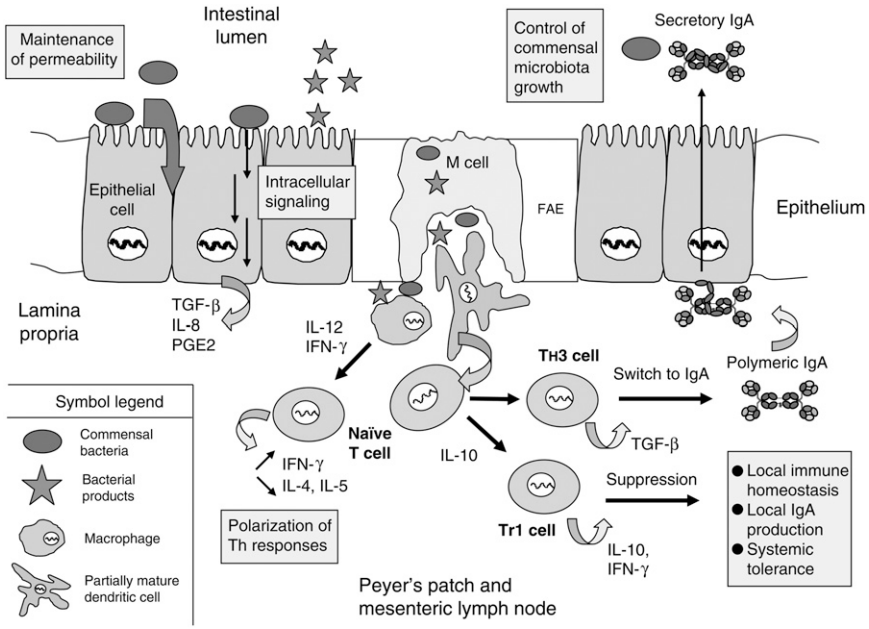


**Figure 1** Schematic representation of the multiple consequences of the cross-talk between the probiotic bacteria and the intestinal mucosa. At the intestinal epithelial level, probiotic bacteria may allow beneficial effects through transient colonization and/or release of bioactive compounds. This translates into reinforcement of the intestinal barrier as well as direct modulation of epithelial cell functions including cytokine and chemokine release. Although a limited event, translocation of bacteria to the lamina propria may affect innate and adaptive immunity by activating production of cytokines by monocytes/macrophages. Sampling by M cells in Peyer's patches (*PP*) and subsequent engulfment by dendritic cells (*DC*) of the innate immune system may contribute to present microbial antigens to naïve T cells in the PP and mesenteric lymph nodes (*MLN*). This allows IgA antibody-mediated mucosal response to take place against the bacterium to prevent overgrowth and spreading beyond MLN but also, for example, to the antigen coded by a recombinant probiotic strain used as a vaccine. Remarkably, the same processing pathway plays a critical role in the shaping of the mucosal immune system toward a noninflammatory, tolerogenic pattern that takes place through the induction of regulatory T cells. *Author's caution:* The scheme is a simplified synthesis obtained from data collected in vivo and in vitro in various experimental models; the specific effects of a particular probiotic on the development of local and systemic responses must be considered on a case-by-case basis.

for probiotics to be efficacious typically illustrates that perceived benefits of probiotics are often ill-defined. The field instead needs to consider specific immunological applications, whether prophylactic or therapeutic, and then proceed to address mechanisms by which ingested probiotic organisms might be used to prevent or treat enteric disorders. Such studies will require the formulation of detailed hypotheses regarding the way orally ingested probiotics interact with specific types of host immune cells.

Given the diversity of inflammatory or immune responses that can be mounted by the intestinal epithelium, association of probiotics with epithelial cells might be sufficient to trigger signaling cascades that ultimately activate underlying immune cells in the lamina propria. Alternatively, probiotics may also release soluble factors that themselves trigger signaling cascades at the level of the epithelium or associated immune system (16–18) (Fig. 1).

Certainly much attention has been given to the adhesive properties of probiotic organisms, and ability to adhere to host cells or mucus is commonly considered to be a requirement for probiotics. However our perceptions about probiotic adhesion, especially to epithelial cells, have been derived almost entirely from in vitro studies, which very partially mimic the complexity of the intestinal ecosystem. Our knowledge of the mucus gel and its importance as a defensive entity is significantly limited because conventional fixation of intestinal tissues (with aldehyde fixative) results in detachment and loss of surface mucus. The significance of this experimental limitation was demonstrated by Matsuo and coworkers (19), who used Carnoy's solution (ethanol- and acetic acid-based) to guarantee the preservation of surface mucus in paraffin sections of human colon samples. Bacteria were observed within laminated arrays of sialo- and sulfomucins in an outer layer, indicating the importance of the mucus gel in preventing direct adherence of gut bacteria to the epithelial surface. This raises the question of the importance of epithelial adhesion and the physiological significance of in vitro systems often based on epithelial cell lines that do not produce mucus. However, these simplified systems are useful and important tools to identify possible signaling pathways and molecular markers, which could be studied further in animal models or human intervention trials. Certainly much additional work is needed to determine whether certain strains are able to reach and adhere to the epithelium in vivo, eventually forming a biofilm, as well as to identify the physiological consequences of such action. These questions might best be addressed with recombinant or mutated probiotic strains overexpressing or lacking genes that encode putative adhesion factors or with specific transgenic knockout mouse strains.

Nonetheless, a large number of in vitro studies have been reported that examined epithelial cell responses to adherent probiotic strains. Initially, these studies demonstrated the ability of probiotic strains to regulate the secretion of a variety of cytokines and pro- or antiinflammatory molecules, especially in cocultures of intestinal cell lines and immune cells (20–22). Refinement of analytical methods has led to the identification of cell signal transduction proteins specific for gram-positive probiotic strains as compared with pathogenic bacteria (23,24). Recent in-depth mechanistic studies revealed complex steps involving transcription factor shuttling between the cytosol and nucleus of epithelial cells (25–27). To better account for the physiological context of the observed responses, it appears critical to design more sophisticated in vitro models involving

multiple cellular partners (21) or ex vivo culture models (e.g., Ussing chambers) (28) using samples derived from healthy or diseased intestinal tissues. This would enable the particular role of probiotic strains in the context of specific immunological or inflammatory conditions to be examined. Nevertheless, observations derived from such systems will have to be confirmed in vivo, as they may not account for factors such as the peristaltic movement of the bowel or the interaction with the enteric nervous system.

### Bacterial translocation in the gastrointestinal tract

The impact of bacterial adhesion on translocation across the epithelium represents another recurrent question. Translocation of commensal bacteria to mesenteric lymph nodes (MLN)[6] has been clearly demonstrated (29–32) and presumably is central to the development and activation of the intestinal immune system. This work should be expanded to screen a wide range of well-characterized and labeled probiotic strains, considering that some strains may be capable of modulating tight junctions and thereby crossing the epithelium. Also needed are noninvasive methods for measuring bacterial translocation because this will make it possible to evaluate the importance of this process in terms of immunological responsiveness to probiotic or commensal bacteria.

Sampling of luminal bacteria by dendritic cells (DC), which have been shown to anchor between epithelial cells (33,34) through receptors for the tight junction protein they express, may also occur. It was demonstrated that dendrite protrusions can cross the epithelial junctions to "capture" bacteria from the lumen. It has also been suggested that DC may sample translocated bacteria that enter the lamina propria because of a low degree of physiological leakiness in the epithelial barrier.

An alternative pathway for crossing the epithelium relies on bacterial adhesion to M cells covering the Peyer's patches. Following capture by DC in the subepithelial dome region, the activation of IgA responses is triggered locally and at distant mucosal sites, which might be a desired outcome. Using *Enterobacter cloacae* as a model commensal bacterium, a recent study suggested that sampling is indeed likely to occur through the specialized M cells (35). This resulted in the detection of a few live commensal bacteria in the subepithelial dome region underlying M cells. These bacteria appeared to be phagocytosed by $CD11c^+$ DC that become activated as reflected by their capacity to express CD86. Because the commensal-loaded DC are restricted to draining MLN, this may guarantee local induction of immune responses while limiting the level of penetration of commensals and avoiding systemic inflammatory reactions that may be deleterious to cohabitation with the host. Recombinant strains genetically labeled for in situ detection and identification would greatly facilitate investigation of M-cell binding and subsequent translocation, as this was reported with *E. coli* Nissle 1917 (36). Unfortunately, M cells cannot be propagated in primary culture, and physiologically relevant M-cell lines do not exist.

Genetically tagged bacterial strains will also be crucial for determining the regions of the gastrointestinal tract that are most immunologically responsive to ingested probiotic strains, another key consideration that is undefined at present. Given the central role of Peyer's patches for the development of secretory IgA (SIgA), it might be hypothesized that probiotic strains targeting M cells should be identified for applications that seek to bolster intestinal immunity. On the other hand, probiotic strains adapted to the colonic environment and possessing antiinflammatory properties may correspond to good candidates to fight inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC). So far, specific probiotic strains or mixtures have shown significant efficacy solely in treatment of pouchitis (37) and UC (38) in humans. These clinical effects may be based on direct immunomodulating effects of these bacteria as well as on their capacity to act on the resident microbiota or to improve the intestinal barrier integrity, thus limiting bacterial translocation.

### Immunomodulatory compounds of probiotic bacteria

The immunostimulatory properties of commensal bacteria are best exemplified by studies with gnotobiotic animal models, which demonstrate that essentially all aspects of the intestinal immune system are underdeveloped in germ-free animals but rapidly restored on the introduction of even single bacterial species (39–42). The development of the localized mucosa-associated immune system is only in part genetically determined: it is also functionally dependent of the bacterial microbiota (43). Not clear, however, is the extent to which antigenic components of bacterial cell walls mediate the state of physiological inflammation that characterizes the stable association between a mammal host and its resident microbiota.

More reports of systematic investigation of host cell responses to distinct microbe-associated molecular patterns (44) of probiotic strains, mainly *Lactobacillus* or *Bifidobacterium* species, should shed light on molecular and cellular processes underlying the cross-talk between these nonpathogenic bacteria and the host (45,46). Recognition of microbe-associated molecular patterns is known to be mediated by pattern recognition receptors, including the Toll-like receptor family (TLRs), that signal the presence of specific microorganisms to the host (47). For example the lipoteichoic acids of gram-positive bacteria, pathogenic or nonpathogenic, are able to activate cellular responses via TLR2 (48–50). On another hand, Travassos et al. (51) reported that the peptidoglycan of both gram-positive and gram-negative bacteria is not sensed through TLR2, TLR2/1, or TLR2/6 but most likely through an intracellular receptor (Nod1/Nod2); however, no probiotic bacteria were included in this study. More recently, Mazmanian et al. showed that the ubiquitous gut microorganism *Bacteroides fragilis* could activate maturation of the developing immune system through the zwitterionic surface polysaccharide PSA (52).

As mentioned above, it has been established that the effect of probiotic bacteria may also result from soluble factors that alter epithelial permeability (16), inhibit the inflammatory cascade (17), or mediate activation/maturation/survival of dentritic cells (53). Native DNA carrying specific unmethylated CpG motifs could similarly provide some basis for the discrimination among different bacterial species in the gastrointestinal tract. DNA isolated from the probiotic mixture VSL#3 containing 8 lyophilized lactic acid bacterial strains (*Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium breve*, *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus plantarum, and Streptococcus salivarius* subsp. *thermophilus*) elicited noninflammatory responses from epithelial and immune cells (54). In addition to inhibition of IL-8 secretion from epithelial cells and attenuation of *Bacteroides vulgatus*-induced interferon-$\gamma$ from mouse

[6] Abbreviations used: CD, Crohn's disease; DC, dendritic cells; GALT, gut-associated lymphoid tissue; IBD, inflammatory bowel disease; MLN, mesenteric lymph nodes; SIgA, secretory immunoglobulin A; TFF, trefoil factors; TLR, Toll-like receptor; TTFC, C subunit of the tetanus toxin; UC, ulcerative colitis.

splenocytes, it was found that VSL#3 DNA inhibited systemic TNF-$\alpha$ production and improved the histological score of inflammation in IL-10 knockout mice (54). In a similar approach and also using the VSL#3 mixture, Rachmilewitz et al. (55) reported that the chromosomal DNA of this probiotic preparation was responsible, via TLR9 signaling, for the antiinflammatory effect observed in a mouse Dextran sodium sulfate-induced colitis model. In their experimental setting, nonviable $\gamma$-irradiated probiotics were equally effective as live ones. Signaling through TLR-5 has also been reported to occur; for example, the commensal *E. coli* strain MG1655 and its associated flagellin were shown to trigger proinflammatory responses in enterocytes both in vitro and ex vivo (56).

Altogether, these results shed light on the potency of probiotic bacteria to regulate immune responses and highlight a complex interaction between the host immune system and different bacterial compounds, including chromosomal DNA and cell wall components as well as soluble metabolites (Fig. 1). Studies of this type may ultimately lead to a better understanding of the molecular basis of the variation in immunomodulation capacity that clearly exists among various lactic acid bacterial strains (57–60). The use of transgenic knockout mice invalidated for specific receptors or transcriptional regulators will also help to unravel key host factors mediating the response to microbial stimuli (see below).

### Interaction of probiotics with immunocompetent cells

Tolerance and homeostasis in the intestine are maintained by specialized subsets of lymphocytes. Subsets of $CD4^+$ T cells have drawn most of the attention so far, and several phenotypes have been described, depending on the type of cytokines or surface molecules they express. At least 3 subsets of regulatory $CD4^+$ T cells have been characterized that may play a role in gut homeostasis: Th3, Tr1, and $CD25^+$ (61,62). There also seem to be roles for other T-cell types, including NK T cells (63) and $\gamma\delta$-intraepithelial lymphocytes (IEL) (64,65), but the proof of their direct involvement is in need of additional study. Recently, the presence in the gut of evolutionarily conserved mucosal-associated invariant T cells that are MR1 (monomorphic major histocompatibility complex class I-related molecule)-restricted and require the presence of the commensal microbiota for their expansion in the lamina propria has been documented (66), but their function remains to be elucidated.

The microbiota has a positive impact on immune regulatory functions of the gut, and disruption of these immune regulatory functions by an imbalanced microbiota may lead to exacerbated effector responses and chronic inflammatory diseases (67). Typically, both UC and CD are characterized by a loss of tolerance to gut commensals (68,69), as illustrated for example by the observations that treatment with broad-spectrum antibiotics abolishes clinical symptoms (70). Some interest has therefore recently emerged to address the potential role of probiotics in the induction (or restoration) of regulatory-type immune responses in the gut. Experiments in rats and mice using several strains of *Lactobacillus* have shown an increase in proportion of $CD25^+$ T cells in the lamina propria (71) and a decrease in T-cell reactivity (72–74). In a clinical study, the ingestion of *L. rhamnosus* GG was associated with a rise in mitogen-induced IL-10 from peripheral blood mononuclear cells that translated into high serum concentrations of IL-10 (75). However, this strain proved unprotective against IBD in humans (76). In vitro studies have suggested that undefined components of lactobacilli may have antiproliferative effects on T cells and suppressive effects on cytokine secretion by T cells (77). Such effects appear to implicate the emergence of a Tr1-like cell population capable of releasing TGF-$\beta$ and IL-10 in the culture medium (78). However, the change in immune balance might also result from an indirect mechanism based on luminal modification of the antigen after treatment with probiotic strains (79). For example, a decrease in CD3-mediated secretion of the Th2 prototype cytokine IL-4 and suppression of T-cell proliferation were observed in mice exposed to milk caseins previously treated with *Lactobacillus rhamnosus* GG enzymes (80,81).

It is clear that the nature of the intestinal T-cell response is regulated by local DC populations that interact with these cells (82) and that, conversely, regulatory T cells interacting with DC restrain their maturation, thus amplifying tolerance (83,84). DC initiate immune responses in vivo by presenting antigens to T cells, whereas secretion of immunoregulatory cytokines influences polarization of T-cell responses (Th1, Th2, Th3, or regulatory T cells). The relative roles of DC and T cells in regulating immune tolerance to intestinal bacteria and in inflammatory sites where tolerance has been abrogated are ill-defined. It has been shown that *Lactobacillu*s and *Bifidobacterium* strains differentially influence cytokine production by in vitro–matured DC, which suggests that the in vivo activity of regulatory T cells might be influenced by DC that have been exposed to specific commensal microorganisms including probiotics (85,86). Exposure of DC to a selection of probiotic bacteria in vitro was shown to instruct the DC to drive regulatory T cells to produce IL-10 (87). This capacity is not restricted to lactic acid or even gram-positive bacteria, as it was observed that *Bordetella pertussis* and *Vibrio cholerae* compounds can selectively commit DC to induce polarizing signals via different mechanisms (88). The demonstration that mucosal DC differ from systemic and spleen DC in their capacity either to suppress or prime immune responses (89–91) argues in favor of a mucosa-specific cross-talk between the intestinal microbiota and the host (92). In addition, human monocytes and monocyte-derived DC were shown to exhibit different patterns of cytokine release and receptor expression in response to exposure to gram-positive or gram-negative bacteria (93). These observations underline the necessity to isolate cell populations from the most appropriate tissue or fluid when pursuing ex vivo analysis of the immunomodulation capacity of probiotics.

Apart from a few studies (47,54,55), the wide range of existing animal models, particularly transgenic knockout mice with specific cellular or molecular deficiencies (e.g., B cells, T cells, TLRs), have merely been used to investigate immunological responses to either commensal or probiotic bacteria. In this respect, IL-12p40 promoter/luciferase transgenic mice (94) represent a valid tool to address the effects of particular bacteria on the modulation of the host immune response. Specifically, this model allowed identification of a subset of DC in the terminal ileum constitutively producing IL-23 under the influence of intestinal bacteria, which could explain clinical manifestation of CD in this part of the gut (95,96).

Most of the studies conducted so far have used a variety of "simplified" in vitro systems in which many potential players of the mucosal regulatory response were lacking. For example, the key role of the epithelial cell in the whole process would be better integrated using appropriate in vitro coculture systems (21,33). Special attention should be paid to the fact that the methods used for DC isolation and maturation may influence how they respond to microbial stimulus. In parallel, ex vivo studies that target individual cellular components of the mucosal immune system are now feasible, because of laser microdissection techniques associated with microarray technology (97,98).

Although in vitro assays correspond to relatively flexible tools to initiate mechanistic studies, much remains to be done to establish their predictive value as to the targeted health benefit.

### Role of the secretory IgA in the gut homeostasis

SIgA is the most abundantly produced immunoglobulin at the surface of mucous membranes in mammals. SIgA contributes to specific immunity against invading pathogenic microorganisms (99). In the gut, SIgA production depends on intricate mechanisms involving antigen sampling by M cells (100), processing by underlying antigen-presenting cells (101), T-cell activation (102), and B-cell switch in the Peyer's patch and neighboring lamina propria (103) (Fig. 1). Multiple cytokines including IL-4, TGF-$\beta$, IL-5, IL-6, and IL-10 are instrumental to intestinal SIgA production, yet discrepancies between in vitro and in vivo data remain, leading to controversy as to their physiological function. The same set of cytokines are required for maintaining tolerance and IgA switch and production, thus establishing a link that can partly explain why mucosal SIgA are considered noninflammatory in the mucosal environment (104).

Commensal bacteria act as an important antigenic stimulus for the maturation of gut-associated lymphoid tissue (GALT) implicated in the induction of local immune responses (105,106). In what can appear as a paradox, probiotics and nonpathogenic commensals boost overall SIgA antibody responses and thereby trigger intestinal immune exclusion and subsequent elimination (107–110). The mechanisms whereby probiotics modulate immune responses leading to tolerance or SIgA activation appear to be highly dependent on the strains. Changes in the intestinal microbiota result in induction of specific mucosal SIgA responses through a pathway independent of T-cell help and subsequent antibody maturation (111). This ensures control of the endogenous microbiota through a broad spectrum of reduced-affinity SIgA, in contrast to the mechanisms involved in the recognition of pathogen antigens. The adaptive SIgA responses to the intestinal microbiota could allow the host to respond to fluctuations in commensal bacteria without eliciting a deleterious response and thus contribute to mucosal homeostasis (35,112). Additionally, the sampling of low amounts of antigen associated with SIgA may be important in inducing and maintaining tolerance to intestinal bacteria. SIgA capable of entering Peyer's patches across M cells and target DC (113) may direct bacteria in the form of immune complexes into the GALT to permit continuous immune stimulation under noninflammatory conditions (114).

The crucial role of SIgA in maintaining bacterial homeostasis is further reflected by its contribution to microbial biofilm formation in vitro (115). The potential role of biofilms in the complex bacteria-bacteria or bacteria-host interactions that take place in the gut remains largely unexplored. Biofilms have been proposed to ensure a mode of steady-state growth of the endogenous microbiota (116). SIgA-mediated biofilm formation might also explain why bacteria that bind SIgA have a selective advantage in the gut (117). The association of SIgA with biofilm formation in the gut has been demonstrated recently in a more physiological context in sections from rat, baboon, and human tissues (118).

SIgA were reported to be involved in multiple functions including bacterial binding (119,120), antibody anchoring at mucosal surfaces (121), and interaction with mucus (122). An intriguing recent study provided evidence that a 30-mer peptide comprising amino acids 38–67 from human secretory component found in mucosal and gland secretions (123) exhibits prebiotic properties when incubated with various bifidobacterial strains (124). This suggests a relevant function for free secretory component that may benefit gut bacteria. The bifidobacterial growth was stimulated 100 times more effectively than with equimolar amounts of the carbohydrate *N*-acetylglucosamine. The bifidogenic effect of milk might thus not be caused solely by its "free" sugar content as generally thought but can be contributed by SIgA known to be heavily complexed with carbohydrates (125).

### Recombinant lactic acid bacteria with enhanced health effects

The potential of lactic acid bacteria to act as a live mucosal delivery system has been investigated during the last 2 decades (126–130). Although strain-specific immunoadjuvant properties have been demonstrated for a number of *Lactobacillus* species (131), the intrinsic antigenicity of lactic acid bacteria seems to be rather low by mucosal routes. This has not prevented the use of these microorganisms as effective carriers for protective antigens. The most complete studies have been carried out with the C subunit of the tetanus toxin (TTFC). Both persisting (i.e., *Streptococcus gordonii, Lactobacillus plantarum*, and *Lactobacillus casei*) and nonpersisting (i.e., *Lactococcus lactis*) species have been investigated as live vaccine vehicles. The strains producing sufficient antigen concentrations induced high serum IgG concentrations after nasal or intragastric administration, which turned out to be protective in many instances. Also, local TTFC-specific SIgA were induced (132). This approach has now been extended to additional antigens (128–130).

In parallel to this work, Steidler et al. (133) demonstrated that host immune responses could be enhanced by codelivery of IL-2 or IL-6 and TTFC. The approach of delivering cytokines with known modulatory properties was further extended by the construction of recombinant *L. lactis* strains secreting murine IL-10 (134). The authors successfully demonstrated that these strains were able to prevent or treat inflammation in 2 murine colitis models. Notably, this effect was obtained with much lower doses of IL-10 than those required when the cytokine was used as a free polypeptide. Steidler et al. further constructed a safe (no antibioresistance marker and chromosomally integrated transgene) biologically contained strain secreting human IL-10 (135). Authorization to conduct a small human intervention trial (targeting IBD) with this strain has been obtained in the Netherlands, and the trial has recently been completed (136). The search for novel therapeutic approaches for acute and chronic colitis based on live recombinant lactic acid bacteria was also extended by the construction and in vivo evaluation of *L. lactis* strains secreting bioactive murine trefoil factors (TFF). TFF are excellent candidates to restore disrupted intestinal epithelial barrier, but they are mostly ineffective when administered orally. Vandenbroucke et al. (137) demonstrated that intragastric administration of TFF-secreting *L. lactis*, in contrast to purified TFF, led to effective prevention and healing of acute DSS-induced murine colitis and was successful in reducing established chronic colitis in IL-$10^{-/-}$ mice.

Additionally, production and mucosal delivery of different types of bioactive molecules such as single-chain Fv antibodies, allergens, or digestive enzymes have been achieved in lactic acid bacteria (130). Targeted diseases included microbial infections such as vaginal candidiosis (138) and dental caries (139), allergies (140–143), autoimmune diseases (144,145), HPV-induced tumors (146), and metabolic defects such as pancreatic insufficiency (147). Moreover, efforts have been devoted to improve the efficacy of lactococci or lactobacilli as delivery systems. For example, mutants were generated that release intracellular

compounds more efficiently (148), but their in vivo immunogenicity has not been reported as yet. More recently, cell wall mutants of *L. plantarum* and *L. lactis*, defective in alanine racemase (*alr* gene), were constructed and characterized (149,150). Using TTFC as a model antigen, Grangette et al. (151) demonstrated that each of these mutants behaves as a substantially improved antigen delivery system compared with its wild-type counterpart. The potency of the *L. plantarum* Alr- mutant was further confirmed using a weak immunogen, i.e., the *Helicobacter pylori* urease B, as protective antigen (152). Notably, in this study, a significant reduction in the pathogen load in the mouse stomach was achieved after immunization with the recombinant mutant strain, in contrast to results obtained with its wild-type counterpart.

Although recombinant strains would not be accepted today in functional foods, their future use in therapeutic approaches can be foreseen provided that the benefit/risk balance is positive for consumers. It might be expected that such strains would be formulated as pharmaceutical preparations and prescribed by medical doctors. By no means are they intended to be included in retail products.

In addition to these "designed strains," mutants in specific genes encoding for potential probiotic functions (adhesion factors to mucus, resistance to acid, specific cell wall components, etc.) could be engineered to compare their biological effect with that of their wild-type counterpart. This strategy should help unravel mechanisms underlying the cross-talk between probiotic bacteria and their host or identify key probiotic compounds. Currently available and rapidly growing genomic information should greatly facilitate this approach (153,154). Grangette et al. (50) recently provided an illustration of this approach. Based on in vitro tests (cytokine secretion profile from stimulated human peripheral blood mononuclear cells), these authors selected a mutant of *L. plantarum* impaired in its capacity to incorporate D-alanine in teichoic acids (Dlt- mutant), for its antiinflammatory potential. Notably, in correlation with in vitro tests, the Dlt- mutant proved to be more protective in a mouse model of colitis than the wild-type strain. Finally, as mentioned above, fluorescently labeled (155) or genetically tagged bacteria could be used to better explain the fate of bacteria after ingestion, and this may help identify the principal immune cells that recognize and process them.

The fact that probiotic bacteria interact with the host immune system is now well accepted and illustrated by in vitro and in vivo experiments and is becoming progressively supported by human intervention trials. However, our current understanding of the molecular mediators involved in the cross-talk between beneficial or commensal bacteria and the host remains fragmentary as compared with the knowledge developed for specific pathogens. Although mechanistic studies have become more sophisticated in recent years, the information remains limited. Different active compounds have been identified in a few probiotic strains, but their respective contribution to specific immune effects remains to be analyzed in more detail, as most of these compounds also exist in the endogenous intestinal bacteria. The mucosal immune system has therefore to process a significant number of similar signals and yet guarantee immune homeostasis. Use of the rapidly evolving "omics" technology will undoubtely help progress in this area, as it will provide a more holistic view of the cross-talk between partners.

### Conclusion

In conclusion, it is evident that the analysis of the impact of probiotics on the host immune system has entered a new and fascinating phase of research and that this effort is likely to offer novel and useful means to modulate host immunity for protection from, or treatment of, a wide variety of human and animal disorders.


### Acknowledgments

We thank Guénolée Prioult for critical reading of the manuscript and Thierry von der Weid for helpful discussions.


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