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MiniReview

Microbes versus microbes: immune signals generated by probiotic lactobacilli and their role in protection against microbial pathogens

Martin L. Cross *

Human Nutrition and Health Research Group, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

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Abstract

Probiotic lactic acid bacteria can signal the immune system through innate cell surface pattern recognition receptors or via direct lymphoid cell activation. In some cases, this action has been shown to be sufficient to modulate local- and systemic-level in vivo immune responses. Practical applications of probiotics include their use in anti-tumour and anti-allergy immunotherapy, but there is also increasing evidence that some probiotics can stimulate a protective immune response sufficiently to enhance resistance to microbial pathogens. This review outlines the experimental and clinical evidence for enhanced anti-microbial immune protection by probiotic lactic acid bacteria, focussing on those studies where a correlative or suggestive link has been shown between immune modulation and enhanced protection.

Keywords: Probiotics; Lactic acid bacteria; Immune modulation; Bacterial pathogens; Protection; *Lactobacillus*

1. Introduction

For nearly a century, Professor Elie Metchnikoff's belief that the 'friendly' microbes present in fermented foods could contribute to human health and well-being has been upheld by microbiologists [1]. However, it is only in the last two decades that the role of these gut-colonising 'friendly' bacteria in facilitating the proper functioning of physiological systems has been fully appreciated. This is no more apparent than in the case of the immune system, where it has been demonstrated that an impoverished or absent gastrointestinal (GI) tract microflora can lead to a deficient oral tolerance induction mechanism, which is sufficient to promote systemic-level atopic hypersensitivity as a consequence [2–4]. Further, both experimentally and clinically, it has been shown that re-colonisation of the GI tract with appropriate strains of orally delivered microbes can restore tolerance induction and can regain the subsequent development of a balanced immune phenotype [2,5].

These observations indicate two important principles. First, that while the gut microflora is undoubtedly important in supporting a functional yet balanced immune system, the processes that lead to this balance can be emulated by transiently colonising the GI tract with appropriate strains of microbes – most commonly Gram-positive lactic acid bacteria (lactobacilli or bifidobacteria) – that are delivered orally as probiotics. And secondly, that the functioning of the immune system, at the systemic as well as local (GI tract) level, can be influenced by signals provided by these de novo colonisers. This latter point has been the subject of intense research interest over the last few years, and supports the notion that bacteria which reside in the GI tract are far from inert commensals, but actively communicate with the immune system. Recent studies have indicated that surveillance cells of the GI tract immune system routinely sample the intestinal microflora [6]; that components of the Gram-positive bacterial cell wall can transduce nuclear factor-κB- and STAT-mediated signals through leucocyte pattern-recognition receptors [7–9]; and that the host responds to such stimuli by the release of pro-CMI/pro-inflammatory cytokines (such as tumour necrosis factor (TNF)α, interleukin (IL)-12 and interferon (IFN)γ) or the production of anti-inflammatory/regulatory cytokines (such as transforming growth factor-β and IL-10), dependent on the strain of bacterium [10–13]. An important consideration in this regard may be the ability of some strains of lactobacilli to intermittently translocate across the intestinal mucosa without causing infection [14], whereupon they can inter-

* Corresponding author. Fax: +64 (6) 350 5446.
E-mail address: m.l.cross@massey.ac.nz (M.L. Cross).

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act with leucocytes subsequently entering circulation (via lymphatic drainage and thoracic duct channelling) and can thus influence systemic immune events. Conversely, there is also evidence that some strains of lactobacilli can directly stimulate the immune system at the gut mucosal surface via localised GI tract lymphoid cell foci [15]; can increase lymphocyte populations and cell surface receptor expression in the GALT environment [16,17]; and can facilitate increased immunoglobulin output into the intestinal lumen [18,19].

While the intricacies of immune signals generated in the GI tract by de novo bacterial colonisation remain to be fully elucidated, the irrefutable fact remains that orally delivered (probiotic) lactobacilli and bifidobacteria have the capacity to modulate systemic-level immune phenotype expression. This has been utilised in health care research, where (for example) pro-CMI-promoting strains (such as *Lactobacillus casei* Shirota) have been shown to induce IL-12 and IFNγ expression [20], to activate NK cell tumouricidal activity [21], and to limit tumour growth in certain forms of non-metastatic carcinoma [22,23]. In contrast, those strains capable of increasing the expression of regulatory cytokines (such as IL-10) have shown promise as both prophylactic and therapeutic agents for the prevention of immune hypersensitivity/atopy [5,24] and for the alleviation of inflammatory bowel disease and colitis [25,26]. And further, probiotic strains that are capable of increasing antibody production in the GI tract have been postulated as potential adjunct therapies to boost immune responsiveness to oral vaccination [27].

A further employment for the use of immunomodulatory probiotics in health care is in the control of microbial pathogens. While evidence has existed for several years that orally delivered probiotics can combat infectious diseases (for review, see Elmer [28] and Reid and Burton [29]), several potential mechanisms have been proposed to support this phenomenon, including: that localised lactic acid production by probiotics in the GI tract can limit pathogen growth; that anti-pathogen substances secreted by the probiotics (e.g. bacteriocins) are directly microbicidal; that seeding the gut mucosa (albeit transiently) with de novo 'friendly' bacteria can limit pathogen attachment (i.e. competitive exclusion); or that immunomodulatory signals generated by probiotics can stimulate host immunity sufficiently to afford a degree of enhanced protection against pathogens. While none of these theories is mutually exclusive, a thorough discussion of all of these possibilities is beyond the scope of this review. Instead, I will focus on the supportive and suggestive evidence of a role for probiotic-mediated immunomodulation in the control of microbial pathogens. Further, the review will specifically consider the use of Gram-positive lactic acid bacteria (principally lactobacilli, but also some species of bifidobacteria) as probiotics; however, it is important for the reader to appreciate that other taxa have been utilised as probiotics in this field, including Gram-negative enterobacteria and yeasts (for review, see Alvarez-Olmos and Oberhelman [30]).

2.1. *Animal model studies of immunomodulation and protection against pathogens (summarised in Table 1)*

Several studies have investigated the use of immune-modulating probiotics for the control of microbial enteropathogens by utilising rodent infection/challenge models (although it must be emphasised to the reader that such models are often atypical representations of true human enteropathogen infection). A large volume of research in this area has focussed on the use of *Salmonella typhimurium* as a model pathogen in mice. An initial study by Perdigon et al. [31] involved feeding mice with diets containing milk that had been fermented by *Lactobacillus acidophilus* or *L. casei* (CRL strains), or a combination of both lactobacilli, prior to oral challenge with *S. typhimurium*. Results indicated that while either *Lactobacillus* species could effectively reduce pathogen translocation to the spleen and liver (and consequently increase survival of challenged mice) the greatest degree of protection was recorded in mice fed the combination of the two lactobacilli – 100% of mice surviving *S. typhimurium* challenge compared to $<20\%$ survival in non-probiotic-fed mice. Both serum and GI tract mucosal anti-*Salmonella* antibody titres were noticeably elevated in the mice fed combined *L. acidophilus*/*L. casei*, leading the authors to conclude that probiotic-mediated enhancement of the acquired immune response likely contributed to the protective effects. Similar protective effects of probiotic feeding have been demonstrated subsequently using other strains of *Lactobacillus* [32,33] or *Bifidobacterium* [34], delivered as single-strain dietary supplements in normal or fermented milk prior to *Salmonella* challenge. But in addition to enhanced pathogen-specific antibody production, these later studies have also indicated that non-specific in vivo phagocytic activity of peritoneal macrophages [32] and ex vivo phagocytic capacity of peritoneal macrophages and blood-borne neutrophils [33,34] was increased by probiotic feeding, suggesting that enhanced cell-mediated immunity may also contribute to increased protection. Importantly, the study by Shu et al. [34] provided some clear evidence that immune stimulation conferred by the probiotic *Bifidobacterium lactis* (strain HN019) might indeed be a major contributing factor to enhanced anti-microbial protection in these mice, since statistical analyses indicated positive correlations between the degree of protection and both cellular and humoral immune responses.

Further studies involving oral challenge with enteropathogens have investigated the protective effects of probiotic feeding against *Salmonella*, *Escherichia coli* and *Shigella sonnei* in mice. Perdigon et al. [35,36] demonstrated that mice fed *L. casei* (CRL strain) prior to *S. typhimurium* or *E. coli* oral challenge exhibited increased protection against either pathogen, and that this effect was concur-

Table 1
Animal model studies involving dietary supplementation with lactobacilli or bifidobacteria that have shown enhanced immune response and protection against enteric pathogen challenge

Authors	Pathogen and challenge route	Probiotic treatment	Consequence of probiotic feeding and immune correlate
Perdigon et al. [31] and Macias et al. [37]	Oral challenge of mice with *S. typhimurium* or *S. sonnei*	8 days pre-feeding of milk fermented with *L. casei* and *L. acidophilus* prior to pathogen challenge	Increased survival, decreased pathogen translocation to the spleen and liver; increased serum and gut mucosal anti-*Salmonella* antibody titres
Gill et al. [33] and Shu et al. [34][a]	Oral challenge of mice with *S. typhimurium*	7 days pre-feeding of milk containing *L. rhamnosus* (strain HN001) or *B. lactis* (HN019)[a]	Increased survival, reduced cumulative morbidity index, and decreased pathogen translocation to the spleen and liver; increased serum and gut mucosal anti-*Salmonella* antibody titres and increased ex vivo phagocytic capacity of blood neutrophils and peritoneal macrophages
Paubert-Braquet et al. [32]; Perdigon et al. [35,36]	Oral challenge of mice with *S. typhimurium* or enteropathogenic *E. coli*	2 or 7 days pre-feeding of *L. casei* (CRL or Danone strains) prior to pathogen challenge	Increased survival, reduced infection levels of both pathogens; increased serum total IgA levels and in vivo phagocytic activity [32]; increased gut mucosal pathogen-specific IgA antibody titres [35,36]
de Waard et al. [38]	Oral challenge of rats with *Listeria monocytogenes*	3 days pre-feeding of *L. casei* (Shirota strain)	Decreased pathogen burden in GI tract tissues and visceral organs; increased pathogen-specific in vivo DTH response
Qiao et al. [40]	Oral infection of neonatal mouse pups with rotavirus	Feeding of two *Bifidobacterium* strains (*B. bifidum* and *B. infantis*)	Delayed onset and reduced duration of severe diarrhoea; increased serum and GI tract rotavirus-specific IgA levels
Shu et al. [41]	Environmentally acquired diarrhoea in weaning piglets	7 days pre-feeding of milk containing *B. lactis* (strain HN019)	Reduced cumulative morbidity index; maintenance of higher food conversion efficiency; decreased faecal shedding of *E. coli* and rotavirus; increased GI tract (faecal pellet) pathogen-specific antibody titres; increased phagocytic capacity of blood neutrophils and increased mitogen-induced proliferation of blood T cells
Shu and Gill [42]	Oral challenge of mice with enterohaemolytic *E. coli* strain O157	7 days pre-feeding of milk containing *B. lactis* (strain HN019)	Reduced cumulative morbidity index; decreased pathogen load in somatic tissues among translocation-positive animals; increased gut mucosal anti-*E. coli* IgA antibody titres, increased phagocytic capacity of blood neutrophils and peritoneal macrophages
Ogawa et al.[43]	Oral challenge of infant rabbits with enterohaemolytic *E. coli* strain O157	2 days pre-feeding of milk containing *L. casei* (Shirota strain)	Reduced incidence of diarrhoea, and decreased pathogen load in GI tract tissues; reduced faecal shedding of Shiga toxin and increased GI tract IgA anti-*E. coli* and anti-Shiga toxin antibodies

[a]Statistically significant correlative link between degree of immune enhancement conferred by *B. lactis* HN019-feeding and degree of protection against *Salmonella*.

Table 2
Dietary supplementation studies in humans that have demonstrated probiotic lactobacilli with immune-enhancing and anti-microbial properties

Authors	Clinical situation	Probiotic treatment	Consequence of probiotic feeding and immune correlate
Kaila et al. [44] and Majamaa et al. [45]	Children, aged 4 months to 3 years, presenting acute gastroenteritis due to rotavirus infection	5 days consumption of *L. rhamnosus* (strain GG) following oral rehydration	Reduced severity and duration of diarrhoeal episodes; increased frequency of blood-borne immunoglobulin (IgG, IgM and IgA)-secreting cells during diarrhoeal phases, and increased serum rotavirus-specific humoral and cell-secreted IgA during the convalescent phase
Araki et al. [46]	Infants attending a residential institution	28 days consumption of *B. breve* (strain YIT 4064)	Reduction in the incidence of rotavirus shedding in stool samples, and a tendency toward increased rotavirus-specific IgA class antibodies in the stools
Isolauri et al. [47]	Infants, aged 2–5 months, who received a reassortant live oral rotavirus vaccine	Simultaneous administration of *L. rhamnosus* (strain GG) with the vaccine	Increased frequency of rotavirus-specific IgM class antibody-secreting cells; increased incidence of rotavirus-specific IgA antibody seroconversion
Link-Amster et al. [48] and Fang et al. [49]	Healthy, adult volunteers who received oral vaccination with live attenuated *S. typhi* Ty21A	Administration of *L. acidophilus* (strain La1) and bifidobacteria for 3 weeks; or *L. rhamnosus* (strain GG) for 7 days	Statistically significant increase (*L. acidophilus*/bifidobacteria) and a non-significant trend toward increase (*L. rhamnosus*) in pathogen-specific IgA class serum antibodies

Table 3
Animal model studies involving dietary supplementation with lactobacilli or bifidobacteria that have shown enhanced immune response and protection against respiratory tract pathogen challenge

Authors	Pathogen and challenge route	Probiotic treatment	Consequence of probiotic feeding and immune correlate
Alvarez et al. [50]	Aerosol challenge of mice with *P. aeruginosa*	2 days pre-feeding of *L. casei* prior to pathogen challenge	Increased rate of clearance of *P. aeruginosa* from the lungs; increased phagocytic activity of alveolar macrophages and increased levels of IgA in BAL fluid
Yasui et al. [51]	Intra-nasal challenge of mice with influenza virus following experimental oral vaccination	Pre-feeding of *B. breve* (strain YIT4064) for 3 months prior to challenge	Increased survival rate and reduced viral infection of the lower respiratory tract; increased incidence of virus-specific IgG antibody seroconversion
Hori et al. [52]	Intra-nasal challenge of mice with influenza virus	Pre-feeding of *L. casei* (Shirota strain) for 4 months prior to challenge	Reduced viral titre in nasal washings; increased NK activity of splenocytes and nasal tract mononuclear cells; increased IFNγ and TNFα production by mitogen-stimulated nasal lymphocytes

rent with increased levels of pathogen-specific IgA antibodies in intestinal fluid samples of these animals. A similar study by the same team investigated the protective effects of probiotic feeding against oral challenge with *S. sonnei* [37]. Mice fed milk fermented by CRL strains of *L. acidophilus* and *L. casei* (combined) showed increased survival against challenge and reduced pathogen translocation to the spleen and liver, compared to mice that were not fed probiotics prior to challenge. Similarly to that team's previous studies on *Salmonella*, probiotic-fed mice were shown to exhibit increased anti-*Shigella* antibody titres in the serum and GI tract, once again suggesting that augmentation of the acquired immune response may have contributed to increased protection. Stimulation of the acquired immune response, as a possible means of providing enhanced immune protection against enteropathogens, has also been demonstrated by probiotic feeding against *Listeria* infection. De Waard et al. [38] demonstrated that rats fed *L. casei* (Shirota), prior to oral challenge with *Listeria monocytogenes*, showed reduced pathogen burdens in several excised GI tract tissues (including the stomach and small intestine) and lower pathogen translocation to the spleen and liver, in comparison to non probiotic-fed control mice; additionally, the *Listeria*-specific DTH response was elevated in the probiotic-fed mice, indicating an increased level of in vivo lymphocyte sensitisation to microbial antigens in these mice.

In terms of disease protection, a few studies have investigated the protective effects of immune-modulating probiotic bifidobacteria against rotavirus-induced diarrhoea in neonatal animals. Yasui et al. [39] demonstrated that feeding rotavirus-vaccinated mouse dams with *Bifidobacterium breve* (strain YIT4064) could increase virus-specific IgA levels in maternal milk, and this conferred an increased degree of passive protection to nursing pups against rotavirus diarrhoea. A more recent study has shown that direct oral administration of two strains of bifidobacteria (*Bifidobacterium bifidum* and *Bifidobacterium infantis*) to rotavirus-infected mouse pups could increase virus-specific IgA levels in serum and the GI tract [40], and this treatment both delayed the onset of diarrhoea and reduced the period of severe diarrhoeal disease in probiotic-treated animals compared to controls. Protective effects against diarrhoeal disease have also been demonstrated in a large animal neonatal model by feeding immune-modulating bifidobacteria. A study of environmentally acquired diarrhoea (due mainly to non-specific rotavirus and *E. coli* infection) was undertaken in weaning piglets by Shu et al. [41]. They showed that pre-feeding piglets with *B. lactis* (strain HN019), prior to weaning in conditions that would pre-dispose the piglets to environmental pathogen exposure, could effectively reduce the cumulative morbidity index in these animals and, as a consequence, the probiotic-fed animals maintained a greater rate of food intake and exhibited a higher feed conversion efficiency compared to non probiotic-fed con-

trol animals. Interestingly, the piglet study by Shu et al. [41] provided further evidence that pertinent cellular and humoral immune parameters could be increased by probiotic feeding, since both serum and GI tract pathogen-specific antibody titres, as well as blood-derived neutrophil phagocytic capacity and T cell proliferative responsiveness to Concanavalin A mitogen, were significantly elevated in *B. lactis*-fed animals.

Further to studies of diarrhoea-causing enteropathogens, two animal model studies have focussed on the protective effects of probiotic feeding against enterohaemolytic Shiga toxin-producing strains of *E. coli*. Shu and Gill [42] demonstrated that both BALB/c and C57BL/6 mice that were fed the probiotic *B. lactis* HN019 showed increased protection against oral challenge with *E. coli* strain O157:H7. *B. lactis*-fed mice maintained a greater rate of food intake, post-challenge, and exhibited a lower cumulative morbidity rate compared to control mice that were not fed the probiotic, while among a sub-sample of mice that were translocation-positive for *E. coli*, the mean visceral tissue pathogen burdens were reduced by more than 100-fold in the probiotic-fed mice; this enhanced protection was concomitant with elevated GI tract mucosal anti-*E. coli* IgA antibody titres and increased non-specific phagocytic activity in blood and peritoneal leucocytes. In another study, Ogawa et al. [43] studied the protective effects of feeding *L. casei* (Shirota) to infant rabbits against oral challenge with *E. coli* O157:H7; the probiotic-fed animals exhibited a lower incidence of severe diarrhoea, as well as reduced pathogen burdens and lower levels of Shiga toxins 1 and 2 in GI tract tissues, and in addition the anti-*E. coli* and anti-toxin IgA levels were higher in GI tract tissue homogenates prepared from probiotic-fed mice compared to controls.

2.2. Immunomodulation and increased protection against pathogens in humans (summarised in Table 2)

The range of clinical studies in which probiotics have been trialed for their potential enhancement of immune-mediated protection is lower than for experimental, animal models. Obviously, pathogen challenge studies are not feasible in human subjects, and hence reports in the literature have been limited to probiotic intervention trials among at-risk groups. Several studies over the last decade have investigated the prophylactic or therapeutic effects of probiotics against diarrhoeal infections among hospitalised children, overseas travelers or subjects undertaking antimicrobial chemotherapy (reviewed in [28] and [30]) but relatively few of these have reported concomitant measures of immune status in the subjects. Research has been undertaken using *Lactobacillus rhamnosus* (strain GG) as a probiotic to combat rotavirus gastroenteritis among infants and children admitted to paediatric clinics. Kaila et al. [44] and Majamaa et al. [45] conducted randomised, placebo-controlled dietary supplementation trials in which infants aged up to 3 years, who were diagnosed with acute gastroenteritis and diarrhoea, were given liquid suspensions containing *L. rhamnosus* GG. Compared to placebo or non-probiotic microbial supplements, *L. rhamnosus* GG treatment successfully reduced the duration of diarrhoeal episodes among these subjects by approximately 1 day and lessened the subsequent recovery period. Immunological analyses of blood samples indicated that, during the acute phase of infection, probiotic treatment was associated with a significant increase in the frequency of circulating immunoglobulin-secreting cells (of the IgG, IgM and IgA classes) [44]; and that during the convalescent phase, probiotic treatment promoted a significant increase in the frequency of antibody-secreting cells producing rotavirus-specific IgA class antibodies, and an increase in serum rotavirus-specific IgA titres [45]. A similar study undertaken using *B. breve* (strain YIT4064) demonstrated that the frequency of rotavirus shedding among infants attending a residential institution could also be reduced by *B. breve* treatment [46], concomitant with a non-significant trend toward increased titres of rotavirus-specific IgA class antibodies in the stool samples of these subjects.

Although pathogen-challenge experiments are not possible in humans, a few studies have investigated pertinent immune responses following exposure to attenuated or non-virulent strains of enteric pathogens. Isolauri et al. [47] reported that infants who were given a reassortant live oral rotavirus vaccine in conjunction with *L. rhamnosus* (strain GG) developed a higher frequency of blood-borne rotavirus-specific IgM class antibody-secreting cells, and exhibited an increased incidence of rotavirus-specific IgA antibody class seroconversion, in comparison to placebo subjects. Further studies on adult human subjects, given the attenuated Ty21A strain of *Salmonella typhi* as a live oral vaccine, have indicated that 21 days pre-feeding with *L. acidophilus* (strain La1) plus bifidobacteria could significantly increase the serum IgA class pathogen-specific antibody response [48], while 7 days pre-feeding with *L. rhamnosus* (GG) induced a non-significant trend toward an increase in the same parameter [49].

2.3. Recent advances: probiotic-mediated immune protection at distal mucosal sites (summarised in Table 3)

Although the majority of research concerning probiotic-mediated enhanced immune protection has focussed on GI tract pathogens, a few recent studies have begun to consider the possibility that probiotics might stimulate the common mucosal immune system sufficiently to provide increased protection to other mucosal sites as well. Again, the bulk of this work has been conducted in animal models where it is permissible to undertake challenge infection studies. Alvarez et al. [50] looked at the ability of feeding mice with *L. casei* (strain CRL 431) to provide enhanced protection against aerosolised challenge with *Pseudomonas*

aeruginosa. Results showed that the clearance rate of *P. aeruginosa* from the lungs was markedly increased by probiotic feeding, and that this effect coincided with an up-regulation of the non-specific phagocytic capacity of alveolar macrophages, and an increase in total serum and broncho-alveolar lavage fluid IgA levels. Other studies of respiratory tract pathogens have focussed on influenza virus. Yasui et al. [51] investigated the ability of feeding mice with *B. breve* (strain YIT4064) to augment the antibody response to oral influenza virus vaccination and thus affect protection against intra-nasal virus challenge; they showed that *B. breve*-fed/virus-vaccinated mice showed a markedly increased survival rate against influenza challenge compared to control-fed/virus-vaccinated mice, and that a significantly greater number of these animals developed high serum anti-influenza virus-specific IgG class antibody titres. A recent study by Hori et al. [52] has shown that feeding mice with *L. casei* (Shirota) prior to intra-nasal influenza virus challenge can confer a significant increase in protection to the upper respiratory tract. Post-challenge influenza virus titres in nasal washings were significantly reduced in *L. casei*-fed mice compared to controls, and this protection was concomitant with local and systemic indices of enhanced CMI by the probiotic treatment, including increased production of IFNγ and TNFα by mitogen-stimulated nasal tract lymphocytes, and increased NK activity against YAC-1 target cells by splenic and nasal tract mononuclear cells.

Although no human studies have yet been undertaken that link probiotic-mediated immune enhancement with increased protection against respiratory tract pathogens, a 2001 dietary intervention trial hinted at this. Hatakka et al. [53] conducted a randomised study in which children aged 1–6 years who were attending day care centres consumed milk supplemented with *L. rhamnosus* (GG) or unsupplemented milk as a placebo. Over a period of 7 months, the subjects who consumed *L. rhamnosus* GG reported a significantly lower incidence of respiratory tract infections and a trend towards less frequent antibiotic treatment for respiratory complications, compared to the control group. Although concomitant indices of immune status were not recorded in this study, the authors contend that stimulation of the immune system by the probiotic was a likely contributing factor to this enhanced protection.

3. Overview and future perspectives

Probiotic feeding represents a safe and non-pharmaceutical means of combating microbial pathogens. While definitive proof that modulation of the immune system is the major contributor to this effect (above or in addition to other modes of anti-microbial protection afforded by probiotics), a growing body of evidence links increased anti-microbial protection with the enhancement of pertinent immunoresponses by probiotics. Interestingly, a large body of both animal model and clinical studies has focussed on the enhancement of GI tract antibody responses as the primary immune correlate of probiotic-mediated enhanced protection. Certainly, it is well-established that certain strains of lactobacilli and bifidobacteria can effectively increase mucosal antibody production [18,27], yet for many microbial pathogens the contributing role of enhanced cellular immune responses has been unjustly neglected. For example, while several animal studies have reported enhanced pathogen-specific antibody responses in probiotic-fed mice infected with *S. typhimurium*, fundamental immunological studies suggest that cell-mediated immune responses (such as IFNγ production and subsequent activation of phagocytes) play the predominant role in control of this pathogen [54]. In this regard, studies such as those reported by Gill et al. [33] and Shu et al. [34] that report probiotic-mediated enhancement of leucocyte phagocytosis responses (as well as antibody production) in *S. typhimurium*-infected mice offer a more thorough insight into the contributing role of immunomodulation in enhanced protection. Similarly, experimental and clinical studies of enteric viral infections have focussed on the enhancement of humoral immunity as the major read-out of immune status [44–47], yet relatively few studies have reported underlying cellular responses that are pertinent to the control of viral pathogens [41]. In the case of enteric pathogens (such as *Listeria*) where protective immunity is thought to be solidly dependent on cell-mediated mechanisms, there is good evidence that probiotic feeding can indeed enhance pertinent in vivo cellular responses [38], yet even here the underlying patterns of cytokine production that drive these responses have not been reported. Given that certain strains of probiotic lactobacilli (such as *L. casei* Shirota) have been shown to be potent inducers of pro-CMI cytokines (IL-12, TNFα and IFNγ), and can activate strong cellular effector mechanisms (such as NK activity [20–23]), there is the obvious possibility to investigate the potential role of these probiotics for activating cellular immune effector responses that are pertinent to the control of intracellular pathogens.

Although the studies reviewed here have reported the potential for probiotic-mediated immune-modulation to confer enhanced protection to immunocompetent animals and humans, there is also some evidence of probiotic efficacy in immuno-compromised or -deficient hosts. Studies have shown protective effects of probiotic feeding against *Cryptosporidium parvum* [55] and *Candida albicans* [56] in mice immunocompromised by leukaemia virus or corticosteroids (respectively), although concurrent measures of immune responsiveness in these studies did not indicate strongly that immune modulation by the probiotics per se was a contributing factor to increased protection. However, in other studies of *C. albicans* infection, Wagner et al. [57,58] investigated protective effects of probiotic feeding in NK cell-deficient gnotobiotic mice, whose GI tracts

had been colonised with *C. albicans* or *C. albicans* plus probiotic lactobacilli; they showed that the probiotic-fed/*C. albicans*-infected mice mounted stronger localised inflammatory responses, produced heightened pathogen-specific splenic lymphoproliferative and GI tract IgA antibody responses, and exhibited reduced GI tract tissue pathogen burdens and systemic pathogen dissemination compared to their non-probiotic-fed counterparts. Clearly, there is potential to investigate the use of immunomodulatory probiotics as protective agents against opportunistic pathogens in further studies of immunocompromisation.

Recent research in probiotics has begun to think beyond enhanced protection at the GI level, and has instead moved to investigation of enhancement of protective responses at distal mucosal sites. In particular, it is now apparent that probiotic feeding can influence immunoresponses in the respiratory tract tissues [50–52], and this effect has been shown sufficient to afford increased protection against bacterial and viral respiratory tract pathogens. However, this research has been far from exhaustive, and we can expect further more detailed studies of both the mechanisms of immune activation at distal mucosal sites, and reports of protection against a wider range of mucosal pathogens. In the latter regard, a recent report has indicated that direct administration of the probiotic *Lactobacillus fermentum* to the nasal tract of mice can increase pulmonary cellular immune activity and provide enhanced protection against an intra-nasal challenge of *Streptococcus pneumoniae* [59]. There is the possibility also that immunomodulating probiotics, delivered orally or locally [60,61], could provide enhanced protection against mucosal pathogens of the urogenital tract, and a recent experimental study has shown that direct intra-vesicular administration of *L. casei* (Shirota) can induce potent cytokine- and CMI-activation in the bladder mucosa of mice [62]. Clearly, research in this field is far from exhaustive, and as contemporary research increases into mechanisms of infection by – and protection against – microbial pathogens, we can expect to see further more detailed research into the potential employment of immunomodulating probiotics in this area.

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