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Vaccine Adjuvant Properties of Probiotic Bacteria

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Abstract: Vaccine-preventable diseases are still responsible for the deaths of more than 1 million children under the age of 5 years annually, mostly in developing countries. A substantial number of these deaths are due to pneumococcal bacteria and infections with rotavirus. Important issues faced by the WHO, governments, vaccine manufacturers, and international organizations such as UNICEF and the Global Alliance for Vaccines and Immunization (GAVI) are the cost-effective introduction of these life-saving vaccines in resource-poor countries where there is a considerable disease burden, and achieving high rates of completion of vaccination schedules remains elusive. Problems with vaccine coverage and vaccine delivery in these regions are significant, as in some cases large proportions of the target population do not receive adequate vaccination. Consequently, there is a need to develop more effective vaccination strategies that can provide adequate protection with reduced schedules. To date, emphasis has been placed on identifying novel vaccine antigens and adjuvants that induce stronger protective immune responses, as well as developing mucosally-administered vaccines. These approaches would have enormous benefits in allowing safe administration of vaccines in remote areas and may overcome the necessity for multiple doses. In this regard, the use of probiotic bacteria as novel mucosal adjuvants to enhance existing vaccine specific-immune responses offers an exciting new approach. In this review, we discuss the evidence for the role of probiotics in enhancing vaccine responses and provide justification for further investigation into their clinical effects and mechanisms of action.

The Success of Vaccination

Vaccination is the most cost-effective health care intervention tool (Del Giudice, 2003). At the time when the Expanded Program on Immunization (EPI) was launched in 1974, less than 5% of the world's children were vaccinated against diphtheria, tetanus, pertussis, measles, polio, and tuberculosis; today, global vaccination coverage for these infectious diseases is over 80% (Clemens and Jodar, 2005; Ritvo *et al.*, 2005). Current vaccines prevent more than three million deaths each year worldwide and with the introduction of new-generation vaccines directed against the pneumococcus and rotavirus, this number continues to rise (Ehreth, 2003).

The efficacy of vaccination relies on the host generating heightened immune responses to the vaccine antigen that confers protection against natural infection with the organism. Antigen specificity and immunological memory are fundamental to long-term protective immunity in vaccinated populations (Beverley, 2002). In addition, the herd effects of vaccination support protection in unvaccinated individuals through reduced transmission of the organism between vaccinees and their contacts (Isaacman *et al.*, 2008).

Development of Optimal Vaccination Strategies for Resource-poor Settings Where Disease Burden Is Greatest

Despite the overwhelming success of vaccination in protection against infectious disease, significant progress is still required as millions of children, mostly in developing countries, still die every year from vaccine-preventable diseases such as pneumonia and diarrhea (Greenwood *et al.*, 2011). The WHO reports that 1.4 million children under 5 years of age die each year from vaccine-preventable diseases where existing vaccines are available (WHO, 2002). Access to life-saving vaccines in these regions is a major problem owing to their considerable cost as well as difficulties in feasibility of vaccine delivery. The importance of vaccine delivery in these regions is significant since most of the current immunization practices involve administering multiple doses which can be difficult to achieve. Indeed, vaccination dropout rates are as high as 70% in some developing countries, which is likely to result in sub-optimal protective immunity (Wilson-Welder *et al.*, 2009). Immunization coverage data in the Australian indigenous population who are at high risk of pneumococcal disease, shows that less than 50% of infants at 7 months have received the full three-dose pneumococcal conjugate vaccine schedule given at 2, 4, and 6 months (O'Grady *et al.*, 2009). Low vaccine coverage in high burden of disease areas is a major reason for continued susceptibility to these life-threatening infections.

Over the last 10 years, vaccine coverage has steadily increased through the efforts of the Global Alliance for Vaccines and Immunization (GAVI), a public-private consortium with an annual budget of US$1 billion aimed at countering the inequality in health care between rich and poor nations. It is estimated that over 5 million lives have been saved by this program, achieved through the implementation of the six standard vaccines against diphtheria, tetanus, polio, hepatitis B, *Haemophilus influenzae* type b, and yellow fever (Isaacman *et al.*, 2008). Nevertheless, significant challenges remain for developing nations that are most affected by infectious disease childhood morbidity and mortality, particularly in relation to funding of newer-generation vaccines for pneumonia, meningitis, and rotavirus. For example, the recently licensed higher valency (i.e., 10 or 13 serotypes) pneumococcal conjugate vaccines are estimated to provide up to 80% coverage against disease-causing vaccine serotypes in developing countries of Asia and Africa (compared to approximately 50% for PCV7); however, these vaccines are relatively costly (PneumoADIP, 2008). Furthermore, substantial efforts are ongoing to develop more effective vaccines against malaria, HIV, and tuberculosis (Johansson *et al.*, 2011). It is envisaged that a combination of improved vaccine coverage as well as the continued development of novel vaccines and adjuvants that can more efficiently target systemic and mucosal immune responses will go a long way to achieve more effective protection against preventable infectious diseases in these regions.

Approaches to Improve Mucosal Immune Responses to Vaccine Antigens

In general, vaccines in use today are described as live attenuated, killed/inactivated, or subunit (recombinant) in nature and are mainly administered parenterally (Tang *et al.*, 2010). However, problems with some of these vaccines include poor immunogenicity and a limited ability to induce mucosal and cell mediated immunity. Enhancing mucosal immunity offers an effective strategy for preventing pathogen adhesion to host tissues (colonization), which is a requisite precursor to invasive disease caused by the pneumococcus (Bogaert *et al.*, 2004). Furthermore, as discussed above, since most current vaccines require multiple doses, vaccine delivery can be more difficult in developing countries, and a mucosally-administered vaccine would improve feasibility of vaccine administration. This is particularly relevant as the greatest disease burden lies in these developing regions where barriers to effective delivery of vaccination programs are greatest. In developing countries, mucosal vaccines that can be administered via the oral or nasal route would be favored over parenteral injection due to their ease of administration and minimal risk of disease transmission (Davis, 2001).

Strategies to enhance current immunization regimens have focused on several key areas — modification of the vaccine antigen, varying the number and timing of doses, alternate routes of administration, use of enhanced vaccine delivery systems and/or adjuvants to increase immunogenicity — all have been explored and suggested to improve the efficacy of vaccines. The importance of improving mucosal immune responses to vaccine in particular is increasingly recognized. Pathogens such as *Streptococcus pneumoniae*, *Corynebacterium diphtheriae*, *Bordetella pertussis*, influenza, enterotoxigenic *E. coli*, and rotavirus all enter the host via the respiratory or gastrointestinal mucosa. Significant attention has been placed on identifying approaches to improve mucosal immune responses to vaccine antigens, including the development of new-generation mucosal vaccines. Mucosal immunity against pathogens involves the production of secretory IgA (sIgA) and the generation of T cell immunity against the pathogen (Wickens *et al.*, 2008). IL-17 production by T helper 17 (Th17) cells has recently been recognized as a possible new marker of mucosal immunity as IL-17 has been shown to increase sIgA levels as well as to protect against *S. pneumoniae* infection (Lawson *et al.*, 2011; Malley, 2010). Moreover, mucosal immunization at one site can induce protective antibody responses at distant mucosal sites as well as systemically (Morisset *et al.*, 2011). However, despite the significant advantages offered by mucosal vaccines, it has proven difficult to stimulate long-lasting sIgA responses in this way and only very few mucosal vaccines have been approved for human use.

The Use of Vaccine Adjuvants to Enhance Immunogenicity

A major barrier to the development of effective mucosal vaccines has been the limited immunogenicity of this approach. Sub-optimal vaccine formulations that have reduced bioavailability or failed to adequately activate innate and adaptive immune responses may lead to inadequate protection (Larsen *et al.*, 2011). Vaccine adjuvants are commonly utilized to enhance vaccine immunogenicity. Traditionally, rational vaccine design has involved the co-presentation of an adjuvant with a vaccine antigen to enhance specific immunity to that antigen (Bramwell and Perrie, 2005; Mahon, 2001; Schijns, 2000). An adjuvant — from the Latin word '*adjuvare'* meaning 'to help' — modulates the humoral or cellular immune response to the vaccine antigen and is an integral component of existing parenteral and mucosal vaccines (Schijns, 2003b). Although the precise mechanisms by which adjuvants enhance immune responses to co-presented vaccine antigens remain to be fully elucidated, it is considered that the adjuvant improves antigen presentation and antigen-antibody complex formation (depot effect), and also confers immunomodulatory effects (Isolauri *et al.*, 2000; Kukkonen *et al.*, 2008; O'Hagan and De Gregorio, 2009).

A broad range of compounds such as mineral salts, saponins, liposomes, and particulate compounds have all been considered as potential vaccine adjuvants (Cox and Coulter, 1997; Singh and O'Hagan, 1999; Stertman *et al.*, 2004). Alum, a term describing aluminium-based compounds, is the most commonly used vaccine adjuvant in humans and has been in use since 1926 (Mbow *et al.*, 2011). Only recently, alum was shown to interact with DCs to promote CD4+ T cell responses (Baba *et al.*, 2009). Nevertheless, alum is a weak adjuvant for recombinant proteins and is also poorly effective at inducing mucosal responses; therefore, the search for new-generation vaccine adjuvants has become a priority (Zinkernagel, 2003). Moreover, identification of vaccine adjuvants that can enhance local mucosal immune responses would assist the rapid introduction of existing vaccines in areas at high risk of disease (Del Giudice *et al.*, 1999). However, to date, adjuvants capable of augmenting mucosal immune responses have had variable and limited success. The most studied of these, the mutant cholera and *E. coli*-derived toxin molecules, have been shown to promote elevated IgA responses to vaccine antigens but are associated with toxicity despite recent attempts to address this (Holmgren *et al.*, 2003; Lawson *et al.*, 2011; Skene and Sutton, 2006; Sugai *et al.*, 2005; van Ginkel *et al.*, 2000a).

Bacterially-derived vaccine adjuvants such as those described above are thought to have the most potential. However, the balance between immunogenicity and toxicity remains an issue; for example, lipopolysaccharide (LPS), an outer membrane component of Gram-negative bacteria, is a potent adjuvant but is associated with adverse effects, whereas alum is regarded as safe but fails to elicit robust mucosal immune responses to vaccine antigen (Tang *et al.*, 2010). One of the advantages of using bacterial compounds as adjuvants is their ability to directly interact with and signal to the innate and adaptive immune systems via specific Toll-like receptors (TLRs). In particular, TLR ligands have shown promise as effective mucosal vaccine adjuvants. The TLR4 agonist, monophosphoryl lipid A (MPL), derived from *Salmonella minnesota*-isolated LPS, combined with alum in the AS04 formulation, is currently the only vaccine adjuvant with demonstrated ability to enhance mucosal immune responses to vaccine antigen that is licensed in the U.S. for use in the human papillomavirus vaccine (Evrard *et al.*, 2011; Konieczna *et al.*, 2011). The TLR9 ligand, CpG, has also been shown to induce potent mucosal T helper 1 (Th1) cell responses in animal studies, but this has yet to be demonstrated in humans (Kukkonen *et al.*, 2010). The potential for bacterial compounds to induce strong mucosal immune response has given rise to the idea that other sources of bacteria may also have adjuvant properties in relation to enhancing mucosal immune responses. One example of this is probiotic bacteria which have a history of use as immunomodulatory agents and have demonstrated effects on other healthy intestinal microorganisms as well as direct effects on mucosal immune cells.

Probiotic Bacteria Have Potent Immunomodulatory Actions

Probiotics are defined as live microorganisms that, when administered in appropriate amounts, confer a health benefit to the host (Tang *et al.*, 2010). Probiotics have been used throughout civilization but it wasn't until almost 100 years ago that Elie Metchnikoff discovered the health benefits of probiotics. Regarded by many as the father of probiotics, Metchnikoff attributed the long life of Bulgarian peasants to their consumption of the probiotic species, *Lactobacillus* (Rizzardini *et al.*, 2011; Youngster *et al.*, 2011). It is noteworthy to mention also that Metchnikoff, together with Paul Ehrlich, shared the Nobel Prize for their contribution to the discovery of what we now know as the innate and humoral immune systems (Johansson *et al.*, 2011). Since that time, probiotics have been demonstrated to have pleiotropic effects on innate and adaptive immune responses *in vitro* and *in vivo*. Probiotics can mediate immunological effects directly through their interaction with intestinal immune cells and epithelial cells and/or indirectly through modulation of the intestinal microbiota (Hajela *et al.*, 2010; Oelschlaeger, 2010). Furthermore, the role of probiotics in maintaining health is suggested to be a result of their combined effects on gut microbiota, epithelial barrier integrity, and immune modulation. It is

recognized that interactions between the microbiota and immune system are important for the development of healthy immune responses. Numerous studies have demonstrated that intestinal dysbiosis can lead to chronic inflammatory conditions such as allergic disease and inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis) possibly as a result of aberrant immune regulation (Johansson *et al.*, 2011; Westerholm-Ormio *et al.*, 2010; Youngster *et al.*, 2011).

Importantly, probiotic effects are species- and strain-specific, and immune effects can vary depending on the selected probiotic. To date, the most extensively studied probiotic bacteria in animal models and clinical trials are the *Lactobacilli* and *Bifidobacteria* species. The immunomodulatory potential of probiotic bacteria has been highlighted by the beneficial effects of some probiotic strains in the prevention of allergic disease (Tang *et al.*, 2010). The intestinal bacteria (whether pathogenic or commensal) interact with the gastrointestinal mucosal lymphoid system (GALT) through Pathogen Recognition Receptors (PRR) expressed on specialized epithelial M cells and DCs, and antigen presenting cells in turn direct host immune responses (Amdekar *et al.*, 2010; Tang, 2009). These signaling events are central to the maintenance of intestinal immune homeostasis, allowing host protection against intestinal pathogens while at the same time preventing unwanted immune activation through induction of tolerogenic responses. In relation to vaccine development, the role of the human microbiome in inducing systemic and mucosal immune responses has become increasingly evident, with probiotics as a potential source of novel vaccine adjuvants (Ferreira *et al.*, 2010).

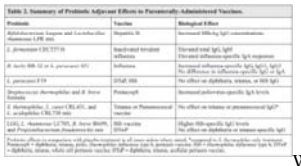
Probiotics as Novel Vaccine Adjuvants

While the immune suppressive effects of select probiotic strains have been applied to the prevention or treatment of allergic diseases, probiotic strains with immunostimulatory effects could be harnessed to enhance antigen-specific immune responses as novel vaccine adjuvants. Several features of probiotics — described below — make them ideal candidates as mucosal vaccine adjuvants. As we have previously discussed, the factors required of a promising adjuvant include the ability to target specific immune cell types, promote mucosal sIgA responses as well as having an acceptable safety profile. Probiotics can modulate DCs, T and B cell populations, as well as antibody and cytokine responses (Baba *et al.*, 2009; Evrard *et al.*, 2011; Konieczna *et al.*, 2011; Kukkonen *et al.*, 2010). Moreover, the widespread availability and consumption of probiotics in foods and health supplements highlights the general acceptance of their safety, and this is supported by clinical trials demonstrating that probiotics are safe when given to infants and adults (Tang, 2009).

The ease of administration of probiotics is another advantage for the implementation of improved vaccines. Immunostimulating probiotics may be able to enhance immune responses to a vaccine antigen in the setting of reduced vaccination schedules, thereby conferring protection against infectious diseases where vaccine coverage is low. In the case of *S. pneumoniae,* nasopharyngeal carriage of the organism occurs very early in life and can impact on vaccine efficacy in high risk populations (Klugman, 2011). Consumption of probiotics (or possibly intranasal treatment) may elevate mucosal sIgA and IgG responses following immunization or natural infection, as well as prevent nasopharyngeal colonization by pathogenic bacteria by directly modulating the airway microbiome.

Evidence from Human Clinical Trials of Probiotic Effects on Vaccine Immunity

Randomized, placebo-controlled clinical trials (RCTs) examining the effectiveness of probiotics in stimulating both mucosally- and parenterally-administered vaccine-specific immune responses have been promising (summarized in **Tables 1-2**). Specifically, *Lactobacilli* are reported to enhance the effectiveness of several candidate mucosal vaccines for malaria, HIV, and infantile diarrhea but these have predominately been examined in preclinical studies involving experimental animals (Amdekar *et al.*, 2010). Serum levels of rotavirus-specific IgA and IgM but not IgG following an oral rotavirus vaccine were higher (but not significantly) in infants given *L. casei* GG compared to placebo (Isolauri *et al.*, 1995), with a significant 8-fold higher rotavirus-specific IgM antibody secreting cell (ASC) response also observed. In a small RCT, adults given either *L. rhamnosus* GG (LGG) or *L. paracasei* CRL431 orally for five weeks and immunized with a live attenuated oral poliovirus vaccine (containing serotypes 1, 2, and 3) had significantly higher serum neutralizing antibody titers to poliovirus serotypes 1 and 2 (for LGG) and to serotype 3 (CRL431) (de Vrese *et al.*, 2005). Furthermore, LGG significantly elevated polio-specific IgA levels to serotype 1 compared to placebo-treatment while *L. paracasei* increased polio serotype 2-specific IgM levels (de Vrese *et al.*, 2005). In another study, LGG increased protective hemagglutinin inhibition titers in more adults than placebo following immunization with a live attenuated nasal influenza vaccine (Davidson *et al.*, 2011). In contrast, adults treated with LGG or *L. lactis* for seven days and immunized with an oral *Salmonella typhi* Ty21a vaccine exhibited no significant changes in total or *S. typhi*-specific IgG, IgM, or IgA ASCs although LGG did stimulate *S. typhi*-specific IgA ASCs in a greater number of subjects than *L. lactis* or placebo (Fang *et al.*, 2000). Moreover, neutrophil CR3 expression was upregulated by *L. lactis*, suggesting that this probiotic enhances innate rather than adaptive immunity. Similarly, adults treated with one of seven different probiotic strains (*B. lactis* Bi-07 and Bl-04*, L. acidophilus* La-14 and NCFM*, L. plantarum* Lp-115*, L. paracasei* Lpc-37*,* and *L. salivarius* Ls-33) had no effect on antigen-specific IgA or IgM levels following oral *Vibrio cholera* vaccination, while a trend towards higher cholera-specific IgG levels was observed (Paineau *et al.*, 2008). Taken together, results from these RCTs provide some evidence that probiotics stimulate mucosal vaccine responses in both infants and adults.



While enhancing mucosal vaccine responses may be a goal for future vaccine development, most vaccines are currently administered via the parenteral route either intramuscularly or subcutaneously. Therefore, probiotics also need to be able to enhance parenteral vaccine responses if they are to be of clinical benefit. Indeed, supplementation with a *Bifidobacterium longum* BL999 and *Lactobacillus rhamnosus* LPR mix to infants during the first six months of life doubled the serum anti-HBsAg IgG concentrations compared to placebo following a standard three-dose hepatitis B vaccination schedule, although this difference was not statistically significant (Soh *et al.*, 2010). However, when adults were given *L. fermentum* CECT5716 and an inactivated trivalent influenza vaccine, a significantly higher TNF-α, total IgG and IgM, as well as influenza-specific IgA responses were observed compared to placebo-treatment (Olivares *et al.*, 2007). In a larger RCT, treatment of adults with *B. lactis* BB-12 but not *L. paracasei* 431 significantly elevated influenza-specific IgG, IgG1, and IgG3 levels while both probiotics induced similar influenza-specific salivary IgA responses to placebo (Rizzardini *et al.*, 2011). A similar effect was observed in another study in infants fed a fermented formula containing *Streptococcus thermophilus* and *B. breve* for the first four months of life (Mullie *et al.*, 2004). In this study, increased poliovirus-specific IgA levels in the feces were detected following Pentacoq® vaccination [diphtheria, tetanus, polio, *Haemophilus influenza* type b (Hib), and pertussis vaccines] compared to placebo treatment, although the authors did not examine the adjuvant effect for the other administered vaccines.

From the above data, it is clear that the timing of probiotic administration is an important parameter to consider when evaluating their adjuvant effects. In particular, maternal (prenatal) treatment is suggested to be more effective as it provides added advantages to the infant via breast-feeding at a critical time when the neonatal immune system is rapidly developing. Daily administration of a probiotic combination containing LGG, *L. rhamnosus* LC705, *B. breve* Bbi99, and *Propionibacterium freudenreichii* to mothers in the last four weeks until delivery and to their infants (together with a prebiotic, galacto-oligosaccharides) for the first six months of life resulted in higher serum levels of Hib-specific IgG in infants as compared to placebo-treated infants (Kukkonen *et al.*, 2006). However, this probiotic treatment did not alter serum IgG levels against diphtheria toxoid or tetanus toxoid following immunization with the DTwP vaccine. The authors also reported that this probiotic mix induced protective Hib antibodies (≥1μg/mL) in a greater proportion of the probiotic-treated infants as compared to placebo-treated infants suggesting that a combination of probiotics may offer different outcomes to individual probiotic bacteria. In contrast to this, the daily treatment of infants with *L. paracasei* F19 between 4 and 13 months of age had no effect on antigen-specific IgG responses to Hib, diphtheria toxoid, or tetanus toxoid following immunization with DTaP and Hib-conjugate vaccines, at any of the post-vaccination time points studied compared to placebo-treated infants (West *et al.*, 2008). Similarly, a four month treatment in children aged 9 months to 10 years with a mixture of *Streptococcus thermophilus, L. casei* CRL431, and *L. acidophilus* CRL730 and the prebiotics, oligofructose, and inulin, did not affect antigen-specific IgG levels to tetanus toxoid or pneumococcal antigen post-vaccination in comparison with *S. thermophilus* only treatment (Perez *et al.*, 2009). This result may not be surprising given that both arms of the study were given some form of probiotic and the response was not compared to placebo treatment.

On the basis of the data above, probiotics may be a novel strategy to enhance the protection afforded by current vaccines, particularly in developing countries where the disease risk is greatest. Their capacity to modulate parenteral and mucosally-delivered vaccines is of significance as this may offer substantial advantages in terms of reduced dose schedules, vaccine coverage, and ease of administration. Importantly, single dose vaccines have been the focus of intense research and debate by the WHO, vaccine manufacturers, and academia given the existing issues with vaccine delivery. Therefore, probiotics may provide the necessary adjuvant qualities to enhance vaccine responses in this context.

How Might Probiotics Modulate Mucosal Immune Responses?

While the precise mechanisms by which probiotics mediate adjuvant activity are unknown, animal studies have shown that *L. rhamnosus* HN001 delivered together with a cholera toxin vaccine induced significantly increased phagocytic cell activity and gut mucosal anti-cholera specific antibodies compared to a placebo treatment (Gill and Rutherfurd, 2001). The effect of probiotics may be to influence innate immune cells such as intestinal macrophages or DCs, which in turn results in enhanced antigen presentation and promotes preferential differentiation of mucosal lymphocytes towards the production of protective antibody. This represents a critical step in the initiation of immune responses, particularly at sites such as the GALT where antigen encounter frequently occurs. Probiotic bacteria such as *L. reuteri, L. johnsonii,* and *L. gasseri* were all demonstrated to upregulate the expression of human myeloid DC markers such as HLA-DR, CD80, CD83, and CD86 as well as induce the secretion of Th1 cytokines (IL-12, IL-18, IFN-γ) but not Th2 (IL-4, IL-13) or IL-10, indicating preferential Th1 polarization (Mohamadzadeh *et al.*, 2005). Such responses are important for healthy immune development. Furthermore, consumption of *L. acidophilus* Lafti L10 by healthy adults was shown to promote Th1 responses and in particular, upregulate IFNγ-associated chemokines and gene expression in intestinal biopsy samples (Rizzardini *et al.*, 2011). A more detailed understanding of the mechanisms of actions for probiotics is therefore required in order to more effectively target the critical immune pathways that drive vaccine-induced protection.

An intriguing phenomenon common to many of these studies is that the vaccine and probiotic adjuvant do not need to be co-administered unlike existing vaccines. The ability to augment systemic vaccine-specific immunity using this two-site approach has been demonstrated previously with a novel plant-derived adjuvant (Licciardi and Underwood, 2010; Underwood *et al.*, 2009). Indeed, it has been postulated that the combined approach of both mucosal and systemic vaccination may be critical to induce both mucosal and systemic immunity in order to provide optimal protection against infectious disease (Larsen *et al.*, 2011).

In addition to their immune-adjuvant effects, probiotics are also demonstrated to have effects on the microbial composition (microbiome) of the intestinal and respiratory tracts in animals and humans. Such effects have been thought to be important in the context of allergic disease and other chronic inflammatory conditions where dysbiosis is common (Thomas and Greer, 2010). More crucially, serious infections such as those caused by *Streptococcus pneumoniae* are associated with increased nasopharyngeal carriage of pneumococcal bacteria (Brandtzaeg, 2011). In animal studies, *L. fermentum* treatment reduced colonization of pneumococcal serotype 6A in the nasopharynx in a challenge model (Cangemi de Gutierrez *et al.*, 2001). More recently, microbiota-influenced changes to inflammasomes have been found to regulate lung IgA immunity (Ichinohe *et al.*, 2011), suggesting a potential mechanism by which probiotics mediate their effects. Indeed, consideration of the microbiome when developing novel vaccines could improve their protective efficacy against multiple infections (Ferreira *et al.*, 2010). Therefore, manipulation of the microbiome by probiotics is becoming an attractive approach for the prevention of infectious disease.

Conclusions

Vaccine-preventable diseases such as those caused by the pneumococcus, rotavirus, and others are still a significant cause of morbidity and mortality in children less than 5 years old, particularly in developing countries. Access to vaccines in these regions is difficult and many infants do not receive complete vaccination schedules which can further increase susceptibility to disease. Novel approaches that can enhance mucosal and systemic immune responses to current vaccines may provide a significant benefit in these populations. Probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* species have important immunomodulatory properties that can be exploited for use in the prevention and treatment of human disease. Further studies in humans using large randomized, placebo-controlled trials are warranted to investigate the effectiveness of probiotics as novel vaccine adjuvants.

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