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Probiotics as an Immune Modulator

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Summary Probiotics are nonpathogenic live microorganism that can provide a diverse health benefits on the host when consumed in adequate amounts. Probiotics are consumed in diverse ways including dairy product, food supplements and functional foods with specific health claims. Recently, many reports suggest that certain probiotic strains or multi strain mixture have potent immunomodulatory activity in diverse disorders including allergic asthma, atopic dermatitis and rheumatoid arthritis. However, underlying mechanism of action is still unclear and efficacy of probiotic administration is quite different depending on the type of strains and the amounts of doses. We and others have suggested that live probiotics or their metabolites could interact with diverse immune cells (antigen presenting cells and T cells) and confer them to have immunoregulatory functions. Through this interaction, probiotics could contribute to maintaining immune homeostasis by balancing pro-inflammatory and anti-inflammatory immune responses. However, the effect of probiotics in prevention or modulation of ongoing disease is quite diverse even within a same species. Therefore, identification of functional probiotics with specific immune regulatory property is a certainly important issue. Herein, we briefly review selection methods for immunomodulatory probiotic strains and the mechanism of action of probiotics in immune modulation.
Key Words probiotics, immune regulation, immune disorder

FAO/WHO defined for probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (*1*). Probiotics including *Lactobacillus* and *Bifidobacterium* produce different types of antibacterial substances and stimulate host immune system through diverse mechanisms (*2*). The immunomodulation activity of probiotics was also proposed in different diseases (*3*). For example, probiotic supplementation could improve and prevent symptoms of autoimmune disorders. However, not all the probiotics have identical positive effect and they are quite differ depending on strains within same species, amount of doses and treatment periods. In this review, we briefly summarized the mechanisms of probiotic actions and screening methods to identify immunomodulatory probiotics for immune disorders.

Selection of Anti-Inflammatory Probiotics

Selection of probiotic strain should be based on the probiotic activity. So far, however, most of the screening methods are based on the survivability of probiotics in the GI tract. To survive in passage from mouth to intestinal tract, resistance to gastric acidity and bile toxicity is essential ability of probiotics (*4*). Probiotic strains isolated especially from human showed a strong resistance to gastric acidity and bile toxicity. Probiotic strains that produce antimicrobial compounds including organic acids, fatty acids and hydrogen peroxide were primarily selected as a potential probiotic strains. Selected probiotic strains based on the criteria showed a good adherence to the intestinal mucosal wall and could strength the tight junctions. The enhancement of epithelial barrier is one of the key mechanisms of beneficial functions of probiotics, which significantly contribute the exclusion of pathogenic bacteria by the competition for nutrition and adhesion (*4*, *5*). Human intestinal cell, HT-29 and Caco-2 cells are used for in vitro attachment and mechanistic studies. *L. rhamnosus* GG was one of the most adhesive bacteria and *Lactobacillus* strains showed better adherence to the intestinal mucosal wall than that of *Bifidobacterium* strains (*6*, *7*). However, strong resistance to gastric acidity and bile toxicity has little relationship with immune regulatory functions. We have developed a screening method to identify anti-inflammatory probiotic strains using an ex vivo screening system (*8*). Candidate probiotic strains were co-cultured for 72 h with a mixture of immune cells isolated from mesenteric lymph nodes, and then the level of anti-inflammatory (IL-10) and pro-inflammatory (IL-12p70) cytokine. Candidate strains were selected based on its anti-inflammatory activity to induce IL-10^{high}/IL-12^{low} expression. Our ex vivo screening systems showed a close correlation between ex vivo anti-inflammatory activity and in vivo immunomodulatory efficacy. Through this system, we have selected IRT5 (*B. bifidum*, *L. casei*, *L. acidophilus*, *L. reuteri* and *Streptococcus thermophiles*) probiotics mixture (*8*). We believe that any screenings system that

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mimics in vivo GALT microenvironment might have better chance to identify anti-inflammatory probiotics. Although our ex vivo screening system works nicely but still needs further improvements such as avoid of using live animals at the preliminary screening stage. We are actively investigating to improve our screening system to mimic the GALT microenvironment by employing co-culturing of diverse immune cells together with candidate probiotic strains.

Probiotics Alters the Immune Responses in the Gut Associated Lymphoid Tissue

Orally administered probiotics could interact with gastrointestinal (GI) mucosa and gut associated lymphoid tissue (GALT) that more than 70% of immune cells are localized (*9*). Probiotics interact with intestinal epithelial cells (IECs), mucosal dendritic cells (DCs) and macrophages through diverse way. Pattern recognition receptors (PRRs) including Toll-like receptors (TLRs) play essential roles in recognition and delivery of signaling cascades, which mediate different gene expression profiles. DCs in lamina propria (LP) could contact with probiotic in gut lumen through the dendrites and then importing them into the lumen by M-cell mediated transcytosis. Depending on types of probiotic strains, they can either induce immune activation signaling by producing IL-12, IL-1β and TNF-α or trigger tolerance signaling by stimulating anti-inflammatory cytokines such as IL-10 and TGF-β levels. Under the IL-10/TGF-β enriched cytokine milieu, DCs and macrophages can enhance the generation of induced regulatory T cells (iTregs) that play key roles in maintaining peripheral immune tolerance by balancing the ratio of effector and regulatory T cell. We have demonstrated that administration of a rationally selected mixture of five probiotics (IRT5: *B. bifidum*, *L. casei*, *L. acidophilus*, *L. reuteri* and *Streptococcus thermophiles*) could up-regulate iTregs ($CD4^+Foxp3^+$) population through the generation of regulatory DCs (rDCs) in mesenteric lymph node (MLN) (*8*). The IRT5 probiotics was more potent to successfully induce $CD4^+Foxp3^+$ iTreg cells than that of single or three- and four-strain combination treatments.

The Role of Probiotics in Immune Disorders

Any mistakes in controlling hyper-immune responses could lead to develop hyper-immune disorders such as atopic dermatitis and colitis, and autoimmune disorders including rheumatoid arthritis (RA). T helper cells (Th) play important roles in the development of hyper-immune disorders. Th1/Th17 cells produce pro-inflammatory cytokines (IL-12, IFN-γ, IL-6 and IL-17, respectively) and mediate inflammatory disorders. Th2 cells produce IL-4, IL-5 and IL-13 cytokines, and mediate allergic disorders through activation and differentiation of B cells and mast cells. Treg cells can suppress the effector function of T cells (Th1, Th2 and Th17) and ameliorate the pathogenic symptoms of hyper-immune disorders. Although the etiological agents of hyper-immune disorders are unclear yet, increasing evidences suggest that changes in the composition and diversity of the intestinal microbiota are closely associated and restoration of imbalanced gut microbiota could ameliorate the symptoms (*10*). In this aspect, administration of probiotics for a certain periods of time might be helpful. Indeed, recent studies have shown the beneficial effect of probiotics in modulating diverse immune disorders. For example, administration of *L. rhamnosus* GG could prevent the development of atopic dermatitis in children at high risk of allergy (*11*). Inflammatory bowel disease (IBD) is characterized by an autoimmune or hyper-immune disease, in which the immune system attacks and damages its own tissues in the GI tract. The pathogenesis is still not clear, but the pro-inflammatory are the key pathophysiologic elements (*12*). Beneficial effect of probiotics was tested to treat IBD. *L. plantarum* or *B. infantis* were able to decrease the level of INF-γ in IL-10 knock out (KO) mice that spontaneously develop colitis (*13*, *14*). We also demonstrated that administration of *L. casei* or IRT5 probiotics could prevent or suppress diverse immune diseases (*15*, *16*). RA is a systemic autoimmune disease at the joints and an increase in the levels of pro-inflammatory cytokines mediate the disease pathogenesis. We proved that co-administration of *L. casei* with type II collagen (CII) inhibited the severity of ongoing RA by enhancing type-II collagen-mediated oral tolerance and levels of immunoregulatory cytokines (IL-10 and TGF-β) and $Foxp3^+$ Tregs cells (*15*, *16*). We also showed that co-administration of *L. casei* with CII and glucosamine could suppress Th1 effector functions and severity of joint pain in experimental OA (*15*). Administration of IRT5 probiotics mixture showed the positive effect on suppression of experimental autoimmune myasthenia gravis (EAMG) and experimental autoimmune encephalomyelitis (EAE) (*17*, *18*). MG is a T cell dependent-antibody mediated autoimmune disorder that acetylcholine receptor (AChR) at the neuromuscular junction (NMJ) is the major auto-antigen. Pretreatment of IRT5 probiotics decreased the development of EAMG through a decrease of AChR-reactive lymphocyte proliferation, anti-AChR reactive IgG levels and inflammatory cytokine levels (*17*). This effect is associated with the induction of rDC which could convert $CD4^+$ T cells into $CD4^+Foxp3^+$ Treg. This probiotic mixture can also suppress the disease onset of EAE by reducing production of pathogenic cytokines (IL-17, INF-γ and TNF-α) while increasing IL-10^+ and/or $CD4^+Foxp3^+$ Treg cells (*18*). Maintenance of immune balance between the effector and regulatory T cell (Treg) is crucial to suppress development and progression of immune disorders (*14*, *19*). Especially, maintenance of immune homeostasis is really critical at the mucosal sites where large amounts of antigens always encounter with host immune system. Breakdown of this immune homeostasis could lead to food allergy and hyper-immune responses to commensal bacteria. Based on our own studies and others, we believe that administration of probiotics may restore the immune tolerance by diverse mechanisms such as enforcement of barrier function of gut epithelial cells, restoration of gut microbiota composition and phenotypic alteration of immune

cells by enhancing iTregs generation.

Conclusion

Beneficial effects of probiotics to keep our body in good health are well documented. However, functional properties of probiotic activity are quite different even within a same species and identification of functional probiotic stain is a big challenge. So far, most of the probiotic screening systems are based on the measuring of resistance against gastric acidity and bile toxicity. However, this screening system has limitation to identify immunoregulatory probiotic strains. We believe that combination of screenings systems that mimics in vivo GALT microenvironment and experimental disease models might help to identify anti-inflammatory probiotics. If we can rationally select probiotic strains and elucidate the underlying mechanism of their activity, we can apply probiotics to treat inflammatory disorders as a complementary and alternative functional medicine.

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