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Science Letters

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Immunomodulatory Effects of Probiotics: An Update

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ARTICLE INFO

Article History:
Received 15 November 2019
Revised 01 Janaury 2020
Accepted 4 January 2020
Available Online 7 January 2020

Keywords:
Probiotics,
Immune Response,
Pattern Recognition Receptors,
Lactobacillus,
Microbiome,
immunomodulation

ABSTRACT

Probiotic microorganisms have generated a great interest in the scientific community to understand the complex mechanisms underlying their beneficial effects. Probiotic commensal organisms are recognized to be modulators of innate and adaptive immune responses. They work by activation of immune response and yet are able to suppress the over-inflammatory responses.The signalling mechanisms usually involve the innate pattern recognition receptors such as Toll-like receptors which recognize the microbe-associated molecular patterns leading to activation of immune signalling. Moreover, probiotic microorganisms also modulate the intestinal barrier function by inducing defensin production and regulating the immune signalling. Increasing evidence also suggests that induction of epithelial signalling by intestinal microbiota can modulate barrier functions, defensin production and regulate inflammatory signalling. Probiotics also act by activating the cell mediators of innate immunity such as dendritic cells, T effector cells and macrophages. The present review focuses on the immunomodulatory role of probiotics.

1. Introduction

Probiotics are now one of the popularly accepted functional foods which have been established to a have a diverse beneficial effect on health, immunity and the longevity of the individual. The benefits of probiotics in gastrointestinal disorders have been documented in many path-breaking scientific discoveries. The scientists have also established profound immunomodulatory effects of probiotic microflora. The purposes of the present review the recent scientific discoveries that establish the effect of probiotics on immune system.

In oral cavity, it was shown that *Bifidobacterium* strains (*B. adolescentis*) compete with *Porphyromonasgingivalis* and reduce vitamin K (growth substrate) concentration (Hojo *et al*., 2007).

Repair of intestinal tissues is facilitated by several mechanisms (i) secretion of organic acids like lactic, formic and phenylacetic acid that lower the pH and inhibit growth of harmful microorganisms (ii) production of short chain fatty acids that show benefits such as reduction of obesity in mice (iii) Production of antimicrobial compounds such as bacteriocins and hydrogen peroxide (Choi & Chang, 2015; Hassan *et al*., 2012; Macouzet *et al*., 2009; Ouwehand & Vesterlund, 2004; Tharmaraj & Shah, 2009; Zyrek *et al*., 2007) (iv) Inhibition of proinflammatory cytokines by probiotics result in suppression of inflammation and stimulate immune response (Gill *et al*., 2000, 2001; O'Hara *et al*., 2006; Sheil et al., 2004; So *et al*., 2008). These effects can be summarized as: (i) competition with pathogens for nutrients (ii) production of vitamins (iii) fermentation of sugars (iv) production of anti-microbial inhibitory compounds (v) competition for binding receptors (vi) improving epithelial barrier (vii) decreasing inflammation (viii) activation of immune response. Adhesion of probiotic micro biota initiates the repair of the barrier function by secreting antimicrobial substances or proteins, promote mucous secretion through the exclusion of pathogens (Gómez-Llorente *et al.*, 2010; Hirano *et al*., 2003; Lebeer *et al*., 2010; Perdigon *et al*., 2002; Schiffrin *et al*., 1997). All these mechanisms are

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DOI: 10.46890/SL.2020.v01i01.003

inter-related and help in elimination of pathogens. Probiotic microorganisms modulate innate as well as adaptive immune responses of the host by adapting the functions of dendritic cells, macrophages, and T and B lymphocytes (Vanderpool *et al*., 2008; Yan & Polk, 2011). Recent reports give strong evidence for role of probiotic microorganisms in conservation of intestinal homeostasis. This effect has been proposed to be the result of down-modulation of immune response and inducing the growth of T regulatory cells (Tregs).

2. Modulation of Innate Immunity

Probiotic microorganisms stimulate innate immunity through activation of intestinal epithelial cells (IEC) and dendritic cells (DCs). Pattern Recognition Receptors (PRRs) such as toll-like receptors (TLRs) attach to pathogen-associated molecular patterns (PAMPs) that are found in a vast majority of pathogens (Gómez-Llorente *et al.*, 2010; Lebeer *et al.,* 2010). This binding results in activation of nuclear factor- κB (NF-κB) system. NF-κB system triggers the production of cytokines, chemokines, and effectors of innate immunity (Lee *et al*., 2006; Rakoff-Nahoum *et al*., 2004). Some microbial strains induce the production of antimicrobial peptides such as human defensin-1 and 2. Defensins are known to possess chemo-attractant activity for cells expressing chemokine receptor-6, such as DCs. Therefore these could serve as a link between the innate immunity at the intestinal mucosa and subsequent adaptive immune responses.

Parenteral administration of *Lactobacillus casei* has shown to confer protection against *Listeria monocytogenes* infection. It has also been shown to illustrate anti-tumour action against MethAfibrosarcoma by activation of innate immunity (Kim *et al*., 2006). Alternatively, a substantial amount of evidence has been found describing the role of *Lactobacillus sp*. in the induction of cytokine production such as TNF-α, IL-12, IL-18, and IFN-γ in human peripheral blood mononuclear cells *in vitro*(Haller *et al*., 2000; Hessle *et al*., 1999, 2000; Miettinen *et al*., 1998). Microfoldcell (M cells) has been shown to bind and transport bacterial antigens in cytoplasmic vacuoles and could therefore present the putative antigen to gastrointestinal lymphocytes situated in the Peyer's patches. The oxidative changes induced by the binding of the bacterial metabolites on cell surface are proposed to activate the innate immunity (Dugas *et al*., 1999). These bacterial metabolites may affect the production of nitric oxide, which is known to be an important immunoregulatory molecule(Dugas *et al*., 1995; Kolb & Kolb-Bachofen, 1998).

It has recently been reported that cellular components of *Lactobacillus casei*(Shirota) induces NF-κB activation in human embryonic kidney 293T cells transfected with a combination of cluster of differentiation (CD)-14 and TLR2 (Matsuguchi *et al*., 2003). *Lactobacillus rhamnosus* has been shown to stimulate NF-κB, STAT1, and STAT3 DNA-binding activity in human macrophages. These outcomes clearly suggest that specific *Lactobacillus* strains can directly activate host immune component cells (Miettinen et al., 2000). Foregoing studies have established the essential role of NF-κB and Mitogen-activated protein kinase (MAPK) activation in the innate immune response to a number of microbial stimuli (Sweet & Hume, 1996). (Figure 1)

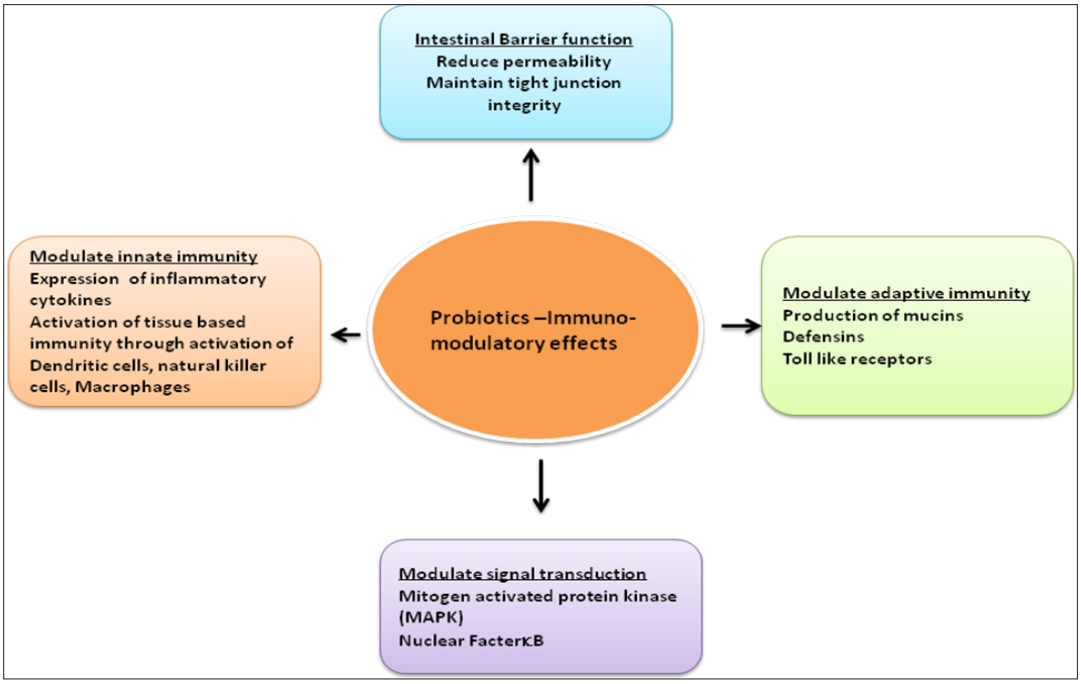

Figure 1. *Immunomodulatory effects of probiotics*

The fight of the host against *Listeria monocytogenes* at the premature stage of infection involves interaction of macrophages with NK cells by way of IL-12, which activates NK cells to produce IFN-γ(Bancroft, 1993; Trinchieri, 1995; Unanue, 1997). IFN-γ then synergizes with bacterial products such as listeriolysin O and cell wall constituents to maximally activate macrophage for secretion of inflammatory cytokines. Moreover, these cytokines also influence the cells of innate immunity. For *e.g.* IL-12 signalling through TLRs and Nod Like Receptors (NODs) results in upregulating the expression of a number of genes associated with NF-κB, which plays a pivotal role in adapting innate immunity (Jiang *et al.*, 2003; Li & Verma, 2002; Sakurai *et al.*, 2003; Schmitz *et al.*, 2001; Vermeulen *et al*., 2003). The p38 MAPK pathway has been shown to play a crucial role in the post-transcriptional regulation of innate cytokines(Clark *et al*., 2003; Kotlyarov & Gaestel, 2002; Mavropoulos *et al*., 2005; Salomonsson *et al*., 2002; Stokoe *et al*., 1992; Takanami-Ohnishi et al., 2002).

Innate immunity exerts protective function in host homeostasis in part by priming adaptive immune responses which also induce inflammation. On the other hand, the unbalanced immune response leads to harsh inflammation and unrestrained tissue damage and disease(Vanderpool *et al*., 2008). The most fascinating aspect of these protective mechanisms lies in maintaining the balance or homeostasis. The microbiota presents a challenge to the adaptive immune system because it contains an enormous foreign antigenic burden, which must be either ignored or tolerated to maintain health. The immune response must not be triggered by the probiotic bacteria and the inflammatory response must be restricted (Jang *et al*., 2004). The adaptive immune system effectively discerns between self and foreign antigens and mounts an appropriate response to clear invading pathogens by recognizing non-self-molecules (Delcenserie *et al*., 2008). One hypothesis describes that this occurs as in "immunologic ignorance," whereby spatial separation of bacteria from the immune system or down-modulation of innate immunity prevents over inflammation(Hooper, 2009). This concept rests on the inability of the innate immune system to distinguish pathogens from symbionts because they distribute similar molecular patterns such as TLR ligands. Rather than ignorance, tolerance could also be induced by the microbiota, given the capacity of gut bacteria to induce Treg lineage differentiation. Molecules produced by our microbiome may be considered "self," because inflammatory bowel disease is thought, in part, to involve a loss of tolerance to antigens of the microbiota. Therefore, it appears that we may tolerate the microbiota in the same way that we tolerate antigens encoded by our own genome(O'Mahony *et al*., 2005; Round & Mazmanian, 2010).

D4+ Foxp3+ regulatory T cells have now been reported to have distinct role in immune tolerance. In an interesting study, probiotic mixture consisting of *Lactobacillus acidophilus*, *L. casei*, *L. reuteri*, *Bacillus bifidium*, and *Streptococcus thermophiles* stimulated regulatory DCs that express high levels of IL-10, tumor growth factor-β, cyclooxygenase-2, and indole amine 2,3-dioxygenase, which in turn stimulated the production of CD4+ Foxp3+ regulatory T cells (Tregs) from the CD4+CD25– population and increased the suppressor activity of naturally occurring CD4+CD25+Tregs. Additionally, this probiotic mixture induced both T-cell and B-cell hypo-responsiveness and downregulated Th1, Th2, and Th17 cytokines without inducing apoptosis. *In-vivo* studies have discovered that this mixture suppressed 2,4,6-trinitrobenzenesulfonicacid-induced intestinal inflammation, which was associated with enrichment of CD4+Foxp3+ Tregs in the inflamed regions. Thus, probiotics that enhance the generation of regulatory DCs to induce Tregs represent a potential therapeutic approach for inflammatory disorders(Kwon *et al*., 2010). It can be envisioned that binding the ability of the microbiota to induce the tolerance through Treg cells may provide new treatments for autoimmunity by improving immunologic imbalances found in an evolving adaptive immune system(Lee & Mazmanian, 2010).

3. Conclusion

Recent studies have highlighted the immune – modulating function of probiotic commensal microorganisms. However, several mechanisms are still unclear. The balance of immune-modulatory activity is important in maintaining homeostasis. The increasing evidence points to development of effective preventive or treatment strategies for several pathological and infectious diseases involving probiotics.

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