

## REVIEW ARTICLE

# ROLE OF PROBIOTICS IN IMMUNOMODULATION AND ITS USEFULNESS IN MANAGEMENT OF INFANTILE COLIC

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### ABSTRACT

Probiotics have been part of our diet since eons. With emerging research work, it has been acknowledged that the gut microflora differs depending on the mode of delivery and the subsequent nutrition. These microbes play an important role in modulating the body events towards health or disease. The role of probiotics in modifying gastrointestinal disorders has long been contemplated. Several studies have utilized varied probiotics that have shown variable results. Some recent studies have investigated the role of *Lactobacillus reuteri* DSM 17938, the daughter strain of *L. reuteri* ATCC 55730 in controlling the symptoms of infantile colic. Results have shown improvement with reduced periods of crying throughout the treatment period with significant reduction by day 21 of the course.

### Development of the Gut Microbiome

The beneficial effects of the fermented foods are related to the microbes that colonize the food. These organisms were referred to as "probiotics", having been derived from the Greek words "pro bios", meaning "for life". (1) The gut of the new-born infant is sterile at birth, and colonization occurs depending on the route of delivery. Those infants born normally by vaginal delivery acquire the organisms from the maternal vagina that include more of *Lactobacilli* and *Streptococcus spp.* In case, the infants are born via caesarean delivery, the skin flora dominate as is seen by the growth of *Staphylococci spp.* Initial colonization occurs with facultative bacteria like *Enterobacteriaceae*, *Enterococcus* & *Streptococcus spp.*, which thrive on oxygen that is abundant in the gut. (2) With the passage of time, there is overgrowth of the anaerobic organisms like *Bifidobacterium*, *Bacteroides* and *Clostridium difficile* which then outnumber the aerobic organisms. Breastfed infants tend to have more amounts of *Lactobacillus rhamnosus*, *Staphylococcus spp* and *Bifidobacterium* compared with formula fed infants who would have more *Clostridium difficile*, *Bacteroides*, *Enterobacteriaceae* and *Enterococci spp.* in their gut. With the introduction of supplementation, the facultative species are overshadowed by 100-1000:1 and around the age of 1 year, the microbiome resembles that of an adult. (2)

### Probiotics

Probiotics are defined as live micro-organisms that when administered in adequate amounts, confer a health benefit on the host. (1) As per this definition, certain foods that contain live microbes have been shown to be associated with well-defined health benefits. (3) Much current research and studies have pointed to the myriad benefits that can be attributed to the consumption of probiotics. Studies from questionnaires have co-related the consumption of probiotic containing milk products during pregnancy and infancy with reduced incidence of atopic dermatitis, rhino conjunctivitis and asthma in a large cohort of children. (4)

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### Mode of Action of Probiotics

#### Gut priming

The new-born immune system is primed according to the exposure that it faces in utero as well as in the immediate new-born period. The gut microbiome too develops according to the organisms which are arrayed before it. The infant's immune system can develop in such a way as to have either a predominance of T helper cell type 2 (Th2) which is associated with more secretion of Interleukin (IL)-4, or develop a Th1 dominance that tends to support cytokines with interferon gamma (IFN- $\gamma$ ) production. (5) Much work has been done in an attempt to evaluate whether dietary manipulations can effectively avert early development of atopy. Prolonged breast feeding for at least 6 months, avoidance of allergenic foods during pregnancy and lactation and hypoallergenic formula foods have been thought to influence the development of atopy. However, the role of dietary manipulations in modulating the immune system to protect against disease has not received extensive attention.

#### Immunomodulation

Current research has attempted to focus on the immune modulatory role of prebiotics and probiotics. Studies have been done to expound the molecular basis of the effects of probiotics on the immune system. (6) The organisms studied included *Lactobacillus rhamnosus GG*, *Lactobacillus gasseri* (PA 16/8), *Bifidobacterium bifidum* (MP20/5) and *Bifidobacterium longum* (SP07/3). These live organisms were pre-incubated with the patient's peripheral blood mononuclear cells and then cultured. The Th1/Th2 cytokine production was measured. It was found that the live probiotic bacterial genomic DNA inhibited the staphylococcus enterotoxin and dermatophagoides pteronyssinus stimulated secretion of Th2 cytokines, IL-4 and IL-5. It stimulated the production of Th1 induced IFN Gamma in a dose dependent manner. These effects were found to be more pronounced in the healthy

subjects as compared to those with allergic tendencies. Pochard et al investigated probiotics *Lactobacillus spp*, (LAB) to determine their effects on the production of Type 2 cytokine which are characteristic of allergic disease. (7). This was done by collecting the peripheral blood monocytes, pre-incubating them with LAB and then stimulated for 48 hours with allergens from *Dermatophagoides* or *Staphylococcal spp* superantigen. The results showed inhibition of secretion of Th2 cytokines, IL-4, IL-5 in a dose dependent manner, irrespective of the LAB strain used. This inhibition mechanism was dependent on the antigen presenting cells as well as on the involvement of IL-12 and IFN- $\gamma$ , and hence LAB may be useful in prevention of allergic diseases. Some theories suggest that the early exposure to respiratory infections caused by respiratory syncytial virus may be associated with later incidences of asthma. (5)

The probiotics exert varied effects on the body that induce the immune functions. Studies have shown that probiotics exert direct immune-modulatory effect on the gut associated lymphatics (GALT) by both humoral and cellular immune stimulation and enhanced B cell mediated antibody production. (7,8) This effect would occur by cytokine production including IL-1, IL-2, TNF- $\alpha$  (Tumor necrosis factor -alpha) and IFN- $\gamma$ . There is also increased production of IFN- $\alpha$  in the macrophages which is mediated by the messenger m- RNA encoding IFN-gamma. Besides this, there is proliferation of the Peyer patch B Cells, enhanced antibody production and optimization of the cellular immune responses. IL-12 is reported as a key to cytokine in the initiation of IFN- $\gamma$  production in humans. This IL-12 production is stimulated by the LAB in large amounts by the peripheral monocytes which directly activate the signal transducer and activator of transcription (or STAT 4) that is essential for a direct activation of the IFN- $\gamma$ . (7) The indirect activation of STAT DNA -binding activity in the macrophages via cytokines has been proven to be possible by the *Lactobacillus GG* bacteria which in turn also stimulates the production of IFN- $\gamma$ . Some reports by Miettinen et al have shown that the *Lactobacillus GG* organisms indirectly act to increase cytokine activity which in turn induces STAT DNA -binding activity in the macrophages. (9) As a result of this there is increased IFN - $\gamma$  production that modulates the immune mechanism in the body. (7,8) The LAB reduce the Th2 cytokine production which are involved in the development of allergy.

The balance between the pro and anti-inflammatory cytokine secretion is augmented by the increased numbers of immunoglobulin (Ig) A, IgM and IgG, together with activation of macrophages, improvement of natural killer (NK) cell activity and increased fecal IgA levels, all of which are all increased following administration of probiotics. (10) The reduced fecal  $\alpha$ -1 antitrypsin, reduced urinary eosinophil protein X and TNF- $\alpha$  activity and increases in transforming growth factor  $\beta$  activity, are all pointers to the altered balance.

#### **Effects of probiotics on epithelial barrier function**

Probiotics play an important role in maintaining the epithelial barrier function. One mechanism of action involves an action via the Toll like receptors (TLR) 2 and TLR 4, which effect changes on the epithelial

cells. (11) The probiotics induce the production of protective cytokines like IL-6, KC-1 (keratinocyte derived cytokine) that are responsible for epithelial cell regeneration, reduction in epithelial cell ulceration and apoptosis and decreased production of TLR 9 which commonly occurs with pathogenic organisms. Some probiotics affect the epithelial barrier function in a slightly different manner. (11) The zonula-occludens-2 (ZO-2) has a vital role in maintaining the epithelial tight junction functions. Some probiotics, like *E.Coli Nissle* has the capacity to counteract the disruptive effects of the pathogenic organisms on the T-84 epithelial cell mono layers by altering the protein kinase C signalling and thus leading to increase in the expression of ZO-2. *Lactobacillus rhamnosus* has the capacity to activate the epithelial cell Akt, which inhibits TNF- $\alpha$  mediated cell apoptosis while favouring the growth of the colonic epithelial cells. (12)

#### **Role of Probiotics in Disease Reversal**

Much interest and research has been put in to gauge the beneficial effects of probiotics in its modulating capacity. It has been shown to be beneficial without doubt in the prevention of several disease conditions and pathological processes. The capacity to reverse the damage that has been caused by disease processes via interleukins and other mediators has been studied. In studies published by Resta-Lenert et al, they have shown that in case of inflammatory bowel disease, the changes in the epithelial permeability occurred due to the TNF- $\alpha$  and IFN- $\gamma$  that is secreted due to intestinal cell damages. (13) Incubation of the probiotics esp. *Streptococcus thermophilus*, *Lactobacillus acidophilus*, as well as, occasionally *Bacteroides thetaiotamicron*, with human intestinal epithelial cell lines like HT29 and Caco-2, resulted in prevention of the changes in the permeability that was responsible for the disease process, including alterations in the chloride secretion as well as the changes in permeability. This study threw up an amazing pointer that the probiotics and to a lesser extent the commensals were able to modulate directly the intracellular events induced by cytokines and thus modulate the deleterious effects of the pro-inflammatory cytokines on the intestinal epithelial cells. However this was not the only mechanism of signalling modulation that was discovered. Ko et al have successfully shown that probiotics like *Lactobacillus plantarum* when incubated with the intestinal Caco-2 cells, were able to reverse the IL-8 secretion and TNF- $\alpha$  induced increase in intestinal permeability, through the inhibition of NF- $\kappa$ B as well as the ERK inflammatory pathways. (14) These mechanisms are possibly related to fundamental alterations in cytokine signalling, and have immense potential for disease modification, even after the onset of inflammatory diseases, like Inflammatory Bowel Diseases.

#### **Probiotics and Infantile Colic**

Studies done by Savino et al have shown that those colicky infants who received *L. reuteri DSM 17938* had a significant reduction in the daily crying time as compared to the placebo group. (15) Early effects were noted by Day 7, while significant improvement was observed by Day 21. Another study was reported by Szajewska et al who studied 85 infants below

the age of 5 months with infantile colic. They were administered *L. reuteri* DSM 17938 at 108 CFU and watched for reduction in crying time. It was found that the responders were significantly higher in the treatment group on Day 7, 14, 21 and 28 as compared to those in the placebo group. (16) Urbańska and Szajewska conducted a meta-analysis of the various studies conducted on the use of *L. Reuteri* DSM 17938. (17) They located 1 systemic review and 14 randomised controlled trials (RCT's) which met the predefined criteria and all were placebo controlled trials. Of the various indications for use three RCT's were evaluated for the effect of *L.reuteri* DSM 17938 for the management of infantile colic. All the RCT's reported the pooled results of crying time on Day 21 and it was found in all of them that the administration of *L. reuteri* DSM 17938 reduced the crying time of Day 21 by approximately 43 minutes. Treatment success was defined as percentage of children achieving reduction in the daily average crying time  $\geq 50\%$ . A significant reduction in the median crying time was noted throughout the period of study together with an increased parental perception of colic severity.

### Conclusion

The gut microbiome is a dynamic living environment that offers exciting prospects in medicine. While the type of organisms depend on the in-utero exposure as well as the subsequent nutrition, it is liable to be altered based on the status of health or disease of individuals. Various methods and pathways of its actions are being elucidated. It is now found to produce favourable actions that would be useful in preventing disease. What is more exciting is the probability of using probiotics in reversing the effects of inflammation in the body. The role of probiotics in reducing the unconsolable crying time of infants has been studied by many. *Lactobacillus reuteri* DSM17938 has been found to be an effective formulation to reduce the persistent crying and bring relief to the infant. More studies are needed to determine its use as a preventive measure for infantile colic.

### Compliance with Ethical Standards

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**Conflict of Interest:** None

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