Micrometabolic Imprinting in Infancy:
Microflora, Probiotics & Chronic Disease

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ABSTRACT: Colonization of an infant's gastrointestinal tract begins at birth. The acquisition and normal development of the neonatal microflora is vital for the healthy maturation of the immune system. Through a process called micrometabolic imprinting, intestinal microflora progression has profound effects on health. An infant's gastrointestinal microflora may be disrupted by a variety of factors such as cesarean section and formula-feeding. Infants born by cesarean section are usually colonized by hospital microorganisms and are at increased risk for asthma, allergies, and gastroenteritis. Breastfed infants have a microflora dominated by Bifidobacterium species. Formula-fed babies have low numbers of Bifidobacterium and a haphazard microflora. Breastfeeding reduces the risk of allergies, obesity, inflammatory bowel disease, celiac disease, diabetes mellitus, and childhood cancer. The gastrointestinal microbiota engendered by breastfeeding may be responsible in part for these benefits. Neonatal probiotics can supply Lactobacillus species essential for normal immunological development and establish a Bifidobacterium dominance in babies born by cesarean section or formula-fed. Altered intestinal microflora is well documented in atopic eczema and other allergies in infancy and childhood. Selected probiotics such as L. rhamnosus and B. lactis have been shown to reduce the risk of allergic eczema in infants and, when present, reduce its clinical severity. Probiotics selected for infants should ideally be dairy-free and produce only the L(+) form of lactic acid. An optimal infant probiotic should contain age appropriate Bifidobacterium species, Lactobacillus species important for immune system maturation, and Lactobacillus species normally found in breast milk. The potential of infant probiotics to reduce the risk of acute diseases in infancy and chronic disease in later childhood and even adulthood remains to be fully explored.

INTRODUCTION

Humans live in beneficial symbiosis with the vast numbers of microorganisms colonizing the gastrointestinal tract.[1,2] The intestinal microflora thrives in a nutrient-rich, sheltered, anaerobic environment. In return, a balanced microbial community protects against infection, promotes normal bowel function, provides energy and nutrition, and maintains normal immune function.[3] Sterile before birth, an infant's gastrointestinal tract is rapidly colonized in a progression that begins with delivery.[4] This microbial colonization is vital for normal neonatal gastrointestinal and immune system development.[3-5] Through a process of micrometabolic imprinting, normal intestinal microflora progression has profound implications not only for well-being during infancy, but for long-term health.[6] Many factors may disrupt an infant's normal acquisition and sustenance of a healthy, balanced gastrointestinal microcommunity. These factors include maternal microflora, maternal diet and medications, manner of delivery, breast- or formula-feeding, antibiotic use, and toxin exposure. Disruption of the normal intestinal microflora, known as dysbiosis, leads to proliferation of pathogenic microorganisms and impaired immune system development. Intestinal dysbiosis predisposes to infections and allergies during infancy. Long-term consequences of neonatal intestinal dysbiosis may include allergies, asthma, increased susceptibility to infections, inflammatory bowel diseases, diabetes mellitus, obesity, and colon cancer.[5] Probiotic administration during infancy and childhood offers a means of correcting intestinal microflora imbalances and favorably modulating immune and gastrointestinal system maturation.[4-8] Probiotic consumption during infancy and childhood has established health benefits[6,8] and may support long-term well-being.[9,10]

GUT MICROFLORA & IMMUNE SYSTEM MATURATION

Infants are born with an immature immune defense system.[11] The gastrointestinal tract is the largest bodily surface to come in contact with the outside world. The gastrointestinal mucosal immune system must learn to distinguish foe from friend; combat pathogenic microorganisms and accept the multitude of commensal bacteria. It must recognize and defend against toxins and allergens while simultaneously becoming tolerant of the plethora of ingested dietary antigens. The gastrointestinal mucosal immune system is linked to mucosal immune systems in the mouth, nasopharynx, lung, breast, and genitourinary tract in a common mucosa-associated lymphoid tissue.[12,13] Although the components of the mucosal immune system are in place after full gestation, bacterial colonization of the intestinal tract is important for stimulating full development and mediating maturation of the gut-associated lymphoid tissue (GALT), the largest collection of lymphoid tissue in the human body.[11,14,15] The acquisition of a normal, balanced gastrointestinal microflora is essential for the success of GALT, and indeed the entire common mucosa-associated lymphoid tissue, in carrying out their vital immune functions.[16,17] Studies involving germ-free animals have shown that without an intestinal microbiota, intestinal lymphatic tissue develops poorly,[18] exhibits fewer numbers of specialized ileal lymphoid follicles called Peyer's patches,[19] and produces intraepithelial lymphocytes with no lytic activity compared to normal colonized animals.[20] Germ-free rabbits have been found to lack natural bactericidal and hemolytic antibodies and are either unresponsive or poorly responsive to *Escherichia coli* antigens.[21] Studies suggest intestinal microbial colonization is responsible for differentiation and maturation of Peyer's patches.[19] The follicle-associated epithelium lining Peyer's patches of germ-free rats contains mainly immature epithelial immune dendritic cells with few B and T cells. After bacterial colonization, these rats develop increased numbers of T cells and immune stimulated CD86+ B cells in the follicle-associated epithelium while dendritic cells disappear. This demonstrates that acquisition and development of the gastrointestinal microflora is responsible for organizing the spatial relationships of B cells, T cells, and dendritic cells in Peyer's patches. In the absence of a developed GALT, animals have increased susceptibility to infections by *Salmonella enteritidis*, *Listeria monocytogenes*,

Clostridium difficile, and *Helicobacter pylori*.[11] Studies are confirming the same findings for humans with disruptions in the normal microflora increasingly implicated in the etiologies of many complex, chronic disorders including allergies, asthma, inflammatory bowel disease, obesity, diabetes mellitus, rheumatoid arthritis, and other autoimmune diseases.[16,17] The acquisition and developmental progression of the neonatal microflora are a period of vulnerability that may leave long-lasting impressions on a baby's immune function through a process of micrometabolic imprinting. This neonatal microimprinting may well determine a baby's susceptibility to childhood and adult diseases; emphasizing the importance of neonatal intestinal microflora acquisition and development.

NORMAL NEONATAL INTESTINAL MICROFLORA ACQUISITION AFTER BIRTH

The fetal gastrointestinal tract is completely free of any microbes.[4] Acquisition of the infant microflora begins during birth. The mother's intestinal and genital microflora, maternal diet and medications, manner of delivery, and birthing environment all influence its initial development.[3,5] The infant is exposed to microorganisms colonizing the mother's cervix, vagina, and perineum during passage through the birth canal.[3] *E. coli* serotypes isolated from mothers' feces and infant mouths just after vaginal delivery are identical.[22] Bacterial cultures of gastric contents from 5-10 minute old newborns are similar to the maternal cervical microflora.[23] The nasopharynx of most vaginally delivered newborns contain bacteria similar to those of the mother's vagina immediately before delivery.[24] However, the maternal vaginal microflora does not usually colonize the baby's digestive tract.[3] The maternal gastrointestinal microbiota is the normal source of the neonate's developing intestinal microecology.[3] *E. coli* and streptococci are the microbes most frequently cultured from the upper digestive tract immediately after birth.[4] Within hours of delivery, enterococci, staphylococci, steptococci, and enterobacteria are present in the gastrointestinal tract of newborns.[3,25] By the second day of life, all infants are usually colonized with *E. coli*. The early colonizing aerobic microorganisms consume intestinal luminal oxygen and lower intestinal pH and redox potential creating conditions favorable for anaerobic microbial colonization. Bifidobacteria may be found in low numbers on the first day after delivery, but generally *Bifidobacterium* and other anaerobic species, such as *Lactobacillus* and *Bacteroides*, do not appear in the infant intestines until several days after birth when a favorable microenvironment has been created.[3] It is well established that probiotics consumed by pregnant women will colonize the newborn gastrointestinal tract following birth.[26] Consumption of a *Lactobacillus rhamnosus* strain by pregnant mothers and continued by the infants up to 6 months has been shown to decrease the risk of eczema by about 50% in the children at 2 and 4 years of age.[27,28] Conversely, it is likely that maternal gastrointestinal dysbiosis may lead to abnormal infant microflora development, but this possibility has not been studied. However, maternal vaginal dysbiosis (bacterial vaginosis), in which normal vaginal lactobacilli have been replaced with pathogens, has significant implications for pregnancy and delivery and is associated with premature rupture of the membranes, preterm delivery, and low infant birth weight.[29,30] Oral probiotics have been shown to re-establish and support a normal vaginal microflora.[31,32]

CESAREAN DELIVERY AND NEONATAL INTESTINAL MICROFLORA ACQUISITION

Cesarean delivery significantly alters neonatal microflora acquisition. The infant does not pass into the world through the birth canal and so is deprived of the initial exposure to maternal vaginal and fecal microflora. Infants born by cesarean section are not usually colonized by maternal microorganisms, but rather by microbes from the hospital environment.[3] Prophylactic antibiotics administered to mothers prior to cesarean section may further compromise the acquisition of a normal neonatal intestinal microflora. Often, cesarean delivery is mandated by fetal distress or maternal complications and is associated with neonatal intensive care further depriving the infant of maternal microflora colonization and exposing the newborn to pathogenic hospital bacteria. In a study of neonatal *E. coli* colonization when first maternal-infant contact after delivery ranged from 8 to 72 hours, only 14% of hospitalized infants shared *E. coli* or other *Enterobacteriaceae* strains with their mothers.[33] Normal mothers and infants share identical *E. coli* strains. Hospital strains were the majority of acquired *E. coli* including a strain carrying the K1 capsular antigen virulence factor that led to hospital acquired urinary tract infections in two babies. Studies of infants delivered by cesarean section have consistently found that establishment of an intestinal microflora is delayed and imbalanced.[3,34-36] *Bifidobacterium* species are only intermittently cultured. Strains from the *Bacteroides fragilis* group are nearly always lacking, and the pathogenic *Clostridium perfringes* is the anaerobic microbe most commonly isolated. The reduction in *Bacteroides fragilis* colonization in infants delivered by cesarean section has been found to persist for 6 months after birth.[34] In another report, at 7 years of age, children delivered by cesarean section had fewer gastrointestinal *Clostridium* species than did children delivered vaginally.[37] This was associated with an increased incidence of asthma. Children born by cesarean section, especially a repeat cesarean section, are well-established to have an increased risk of asthma, allergic rhinoconjunctivitis, allergies, and gastroenteritis requiring hospitalization from 1 year of age up to age 10.[37-41] It is becoming increasingly clear that the neonatal period of intestinal microflora acquisition represents a time of vulnerability. Disruptions of microbial colonization and the associated micrometabolic imprinting carry long-term health consequences. Probiotics have been clearly documented in infants to decrease allergic inflammation and to favorably modulate extraintestinal immune responses resulting in a reduction in allergic symptomatology.[27,28,41] It is possible that the use of probiotics in infants delivered by cesarean section may support the establishment of a more normal intestinal microecology leading to both short- and long-term benefits.

BREASTFEEDING AND THE TRANSFER OF MATERNAL INTERNAL MICROFLORA

Neonatal intestinal microflora development is profoundly influenced by the infant's diet. There are significant differences in microflora composition depending on whether a baby is breastfed or formula-fed.[4,7,42] In addition to unparalleled nourishment, breast milk provides the newborn with factors protective against disease as the neonatal immune system begins to mature. These factors include large amounts of secretory immunoglobulin A (sIgA), white cells, antimicrobial enzymes, lactoferrin, immune-enhancing nucleotides, and oligosaccharides that impart immunologic and antimicrobial protection to the infant.[7,43] Lesser known, but equally important components of breast milk are commensal bacteria from the maternal gut microflora.[44,45] Maternal commensal bacteria in breast milk are important for infant immune system development.[42] Just how important is amply illustrated by the specific mechanism evolved to insure that a nursing mother supplies her own intestinal flora to her baby by means of her milk. The transfer of maternal bacteria from her intestines to her breast milk is mediated by intestinal dendritic cells, immune cells with branched protrusions that process pathogens and present them to T cells, B cells, and other lymphoid cells. Dendritic cells penetrate the intestinal epithelium with their projections and take up intact commensal bacteria from the gut lumen.[46] Instead of

destroying these microorganisms and degrading their proteins, dendritic cells migrate to the gut-associated lymphatic tissue where they transfer the living commensal bacteria to GALT-associated lymphocytes. These lymphocytes containing maternal intestinal microflora then travel to other mucosal surfaces including the lactating mammary gland where the maternal microflora is secreted into the milk for uptake by the infant.[45] Among the lactic acid bacteria normally found in breast milk are probiotic species *Lactobacillus gasseri*, *Lactobacillus fermentum*, and *Enterococcus faecium*.[45]

BREASTFEEDING AND NEONATAL INTESTINAL MICROFLORA DEVELOPMENT

Within the first week after vaginal birth, breastfed infants develop a gastrointestinal microflora dominated by bifidobacteria.[42,47,48] In large part this is due to prebiotic factors such as galactooligosaccharides in breast milk that stimulate bifidobacterial growth. Less prevalent species found in a nursing infant's gut include *Bacteroides*, *Lactobacillus*, and *Streptococcus*. During this same period, formula-fed infants have no predominant gut microbial population. Instead, they possess a more haphazard microbiota that includes *Bacteroides*, staphylococci, *E. coli*, clostridia, and bifidobacteria.[3,42] The intestinal bifidobacteria population in formula-fed infants is approximately one-tenth that of breastfed infants.[49] The stools of breastfed infants have a significantly lower pH than formula-fed infants although it is unclear whether this is a consequence of altered microbial populations or contributes to the disturbed microflora.[42] One month after birth, breastfed babies exhibit a stable flora clearly dominated by bifidobacteria.[3] Populations of enterococci, enterobacteria, clostridia, and *Bacteroides* are suppressed and can only be isolated in relatively low numbers. After 3 months a slight reduction in bifidobacterial populations appears.[3] At 1 and 3 months of age, no microorganism predominates in the formula-fed infant intestine. Formula-fed infants have higher numbers of facultative and obligate anaerobic bacteria compared to breastfed infants.[3]

BREASTFEEDING HEALTH BENEFITS AND THE INFANT INTESTINAL MICROFLORA

Breastfeeding is associated with unequivocal benefits for the infant. Perhaps the most widely appreciated benefit is protection against infection.[45] Breastfeeding not only confers protection against gastrointestinal infections and diarrheal diseases, but has been clearly shown to reduce the incidence of extraintestinal infections such as otitis media, acute lower respiratory diseases, urinary tract infections, and septicemia.[50-54] The protective effects persist for months and even years after weaning.[55] Breastfeeding provides many other health benefits beyond protection from infection. These benefits include a reduction in the risks of allergies and atopic diseases,[56] obesity,[57] inflammatory bowel disease,[58] celiac disease,[59] and diabetes mellitus.[60,61] Breastfeeding has been associated with a reduction in the risk of childhood cancers.[62] While breast milk contains numerous factors that undoubtedly combine to protect the infant from neonatal and childhood infection and reduce the risk of chronic disease in adulthood, the effect of breastfeeding on the development of the infant gastrointestinal microflora may be pivotal. In turn, the normal acquisition and development of the neonatal predominant bifidobacterial microflora is critical to the maturation and optimal performance of the immune system.[11] Disordered neonatal microflora, often manifested as higher numbers of *Bacteroides*, *E. coli*, and *Clostridium* with lower numbers of bifidobacteria, compromises normal immunological development.[16] It is becoming apparent that a neonatal microflora composed of specific lactobacilli and bifidobacteria species is needed to regulate dendritic cell differentiation, induce regulatory T cells, and facilitate the attainment of oral tolerance which enables the body to distinguish friend from foe and dangerous toxin from benign food protein.[63,64] This is precisely the type of microflora engendered by breastfeeding.

PROBIOTICS AND INFANCY

When the normal neonatal microflora does not develop properly or an infant's microflora is disrupted by antibiotics or other toxin exposures, probiotics become important tools to restore intestinal microbial balance. Probiotic supplements containing bifidobacteria may increase gastrointestinal bifidobacteria populations and reduce facultative and obligate anaerobic bacteria in formula-fed infants, creating a microflora more similar to healthy breastfed infants. Probiotics can also supply essential *Lactobacillus* species such as *L. casei*, critical for proper dendritic cell differentiation, and *L. rhamnosus*, shown to enhance bifidobacteria diversity in infants and increase populations of *Bifidobacterium breve*.[65] The use of probiotics in infancy to address a disorder associated with altered intestinal microflora has been best studied in children with allergies and atopic eczema. Atopic eczema is often the first manifestation of allergies in infants and its incidence, along with that of other allergic diseases, has been dramatically increasing in industrialized countries over the past four decades.[16,66] Altered intestinal microflora has been well documented in children with atopic eczema and other allergies.[67] The alterations consist primarily of reduced numbers of bifidobacteria, altered bifidobacteria populations with isolation of *Bifidobacterium* species more commonly found in adults, and larger numbers of *Clostridium* species.[67] These alterations in the infant microflora precede the onset of atopic disorders and are regarded as important causative factors.[68] In 1996, Sütas and coworkers in Finland hypothesized that probiotics might be effective therapy for atopic disorders.[69] In 2000, these investigators reported on their experience treating infants suffering from early onset atopic eczema with a probiotic formulation consisting of *Bifidobacterium lactis* and *Lactobacillus rhamnosus* GG (amount ranged from $3x10^8$ to $1x10^9$ CFU per gram of formula).[41] Infants given probiotics had a significant improvement in the severity and extent of their eczema. Improved laboratory markers of allergic inflammation included reduction in the serum levels of CD4 T cells and drops in urinary eosinophilic protein X concentrations. This was followed by a randomized, placebo-controlled clinical trial in which *Lactobacillus rhamnosus* GG was administered to pregnant women with a partner or first degree relative with an atopic disorder.[27] If the women were nursing their infants after delivery, they continued to receive the probiotic postnatally for 6 months. If the mothers did not nurse, then the infant was directly given the probiotic for 6 months. The probiotic intervention reduced the incidence of atopic eczema by 50% in these high-risk babies (23% vs. 46%). Administering *L. rhamnosus* to the mother was just as effective as giving it directly to the infant indicating how efficiently a nursing mother transmits her bacteria to her baby. Care must be taken to select appropriate probiotic organisms for infants. In one trial, the administration of *Lactobacillus acidophilus* to infants at high risk for developing atopic eczema had no benefit in reducing its incidence, but appeared to be associated with an increased risk for developing sensitivity to cow's milk.[70] As most probiotics, including *L. acidophilus*, are cultured using dairy products, dairy antigens within the probiotic may have been responsible for the increased risk rather than the *L. acidophilus* itself. While *L. acidophilus*, alone and in probiotic combinations, has been used safely in infants short-term for the treatment of diarrheal diseases,[71] there appears to be limited if any rationale for its use to restore normal infant intestinal microecology and modify allergic responses. Select probiotics are now

widely recognized to be an effective intervention in the prevention and treatment of allergic diseases in infancy and childhood.[68] Probiotic formulations have also been shown to be effective in treating viral diarrhea and antibiotic-associated diarrhea in infancy as well as in reducing the risk of necrotizing enterocolitis in premature and low birth weight infants.[8] While available research documents the safety of probiotics during infancy and offers guidance on what disorders may favorably respond to probiotics, much more research needs to be done to establish which strains or combination of species offer the greatest efficacy, at what doses, and in which conditions.

PROBIOTIC SELECTION FOR INFANTS

Probiotic selection for infants should be premised on data that support efficacy at creating an intestinal microflora similar to that of a healthy, vaginally delivered, breastfed baby. It is disruptions of this normal neonatal microbiota that lead to acute, short-term disorders such as diarrhea and allergies and may well be a significant causative factor in long-term chronic diseases such as obesity, inflammatory bowel disease, and diabetes. Restoring normal neonatal microbial equipoise would appear to be a desirable goal worthy of clinical testing. Additional factors may include the metabolic activities of different probiotic strains such as those species that produce the D(-) isomer of lactic acid during fermentation. Such organisms include *L. acidophilus*, *L. brevis*, *L. bulgaricus*, *L. helveticus*, and *L. plantarum*. Infants are unable to metabolize D(-)-lactic acid and theoretically, exposure could result in D(-)-lactic acidosis.[72] While no case of D(-)-lactic acidosis has been reported in an infant given probiotics and D(-)-lactic acid producing *Lactobacillus* species have been safely used in neonates, it seems prudent to avoid them. Many of the *Lactobacillus* species normally found in breastfed babies cannot be cultured without the use of dairy products which may limit probiotic options in dairy sensitive infants. Fortunately, the important *Bifidobacterium* species *B. breve*, *B. infantis*, *B. lactis*, and *B. longum* can be cultured dairy-free as can the highly important *L. casei* and *L. rhamnosus*. Probiotic dosing is important. Low doses may be ineffective as illustrated by two studies of probiotics for the prevention of necrotizing enterocolitis. One study,[73] which used a dose of 60 million CFU of *L. rhamnosus* GG, failed to find a benefit while the second study, which used a formulation containing 10 billion CFU per capsule administered with breast milk, found that the probiotic formulation significantly reduced the incidence and severity of necrotizing enterocolitis.[74] The *L. rhamnosus* may not have been beneficial in the first study because too low of a dose was used. Underdosing is a recurrent problem in probiotic research design. In terms of how much probiotic can be safely tolerated by infants, one study that assessed the efficacy of *B. lactis* to restore microbial balance in infants with atopic eczema established that doses up to 110 billion CFU per kilogram of body weight were safely tolerated by the babies.[75] A rational probiotic formulation for infants would include the age-appropriate bifidobacteria, lactobacilli important for antigen processing and dendritic cell differentiation, and lactobacilli normally found in the healthy infant intestines.

CONCLUSION

Sterile before birth, an infant's gastrointestinal tract begins to be colonized during passage through the birth canal. The normal populations of enteric microflora are acquired primarily from contact with the mother and during breastfeeding. An infant should have a microflora characterized by a predominance of particular *Bifidobacterium* species, the presence of specific lactobacilli, and low numbers of *Bacteroides*, clostridia, staphylococci, and enterobacteria. The normal infantile microbial population patterns appear to be crucial to the healthy maturation of the gastrointestinal and immune systems. The composition of the infant's microflora is so important that mothers have specific immunological mechanisms to ensure the transfer of their own enteric bacteria to their babies through breast milk. Breast milk also contains numerous factors such as prebiotic galactooligosaccharides that stimulate the growth of bifidobacteria. Factors such as cesarean section, formula feeding, and antibiotic and other toxin exposures have been shown to disrupt the normal infant microflora. Disruptions of the infant intestinal microflora have been shown to result in acute short-term disorders such as diarrhea and allergies. Because of the micrometabolic imprinting of the normal infant microflora on the immune system, disruptions of the microbiota during infancy may be related to the risk of chronic diseases in later childhood and in adulthood. Select probiotics have been shown to restore balance in the infant microflora. This restoration appears to effectively reduce the incidence of viral and antibiotic-associated diarrhea and to prevent and treat atopic diseases in infancy. The potential impact of probiotics in reducing the risk of certain chronic diseases in later years remains to be evaluated. Probiotics have been shown to be safe and well tolerated by infants. The precise strains and combinations of probiotics that offer the greatest benefits require further study. A rational basis for probiotic formulations for infants is to provide species that restore the intestinal microflora of a healthy, vaginally delivered, breastfed baby.

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