The Biotics Family in Early Life

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Disclaimer
Any information provided herein regarding infant dietary supplementation is intended to serve an informative purpose only, and should not take the place of careful and appropriate clinical judgment. Guidelines and recommendations may vary between countries.

Glossary

2'-FL	2'-fucosyllactose
3'-GL	3'-galactosyllactose
AD	atopic dermatitis
ESPGHAN	European Society for Pediatric Gastroenterology, Hepatology and Nutrition
FAO	Food and Agriculture Organization of the United Nations
FOS	fructo-oligosaccharides
GOS	galacto-oligosaccharides
HMOs	human milk oligosaccharides
Ig	immunoglobulin
ISAPP	International Scientific Association for Probiotics and Prebiotics
lcFOS	long chain fructo-oligosaccharides
NEC	necrotizing enterocolitis
QPS	qualified presumption of safety
SCFA	short chain fatty acid
scGOS	short chain galacto-oligosaccharides
Th	T helper (cell)
TLR	toll-like receptor
WAO	World Allergy Organization
WHO	World Health Organization

Table of contents

Chapter 1

Introduction

The first 1000 days, from conception to around a child's second birthday, represent a critical period of growth and development that can shape a child's future health and wellbeing.[1,2] It is widely recognized that nutrition during early life can significantly impact growth as well as immediate and future health, and that breastfeeding and/or nutritional intervention during this critical window can help avert both infectious and non-communicable disease risk during childhood and later life (**Figure 1**).[1]

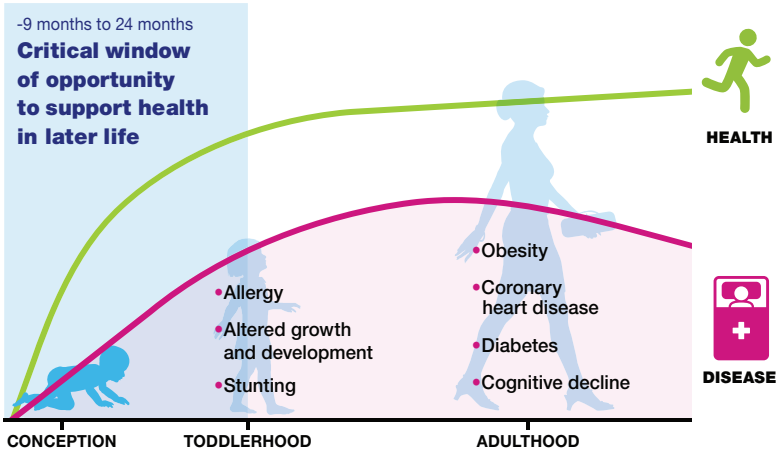

Figure 1. Nutrition during early life; critical window of opportunity

This Essential Knowledge Briefing series discusses various aspects of health in early life. The books are intended to be used as a practical guide for healthcare professionals working with infants and their families. **Book 1** highlighted the gut microbiota and its importance for infant and future health. **Book 2** focused on functional gastrointestinal disorders and

digestive problems in pregnant women and infants. In **Book 3**, we discussed the impact of fetal and infant nutrition on growth.

In this fourth Essential Knowledge Briefing, we present more information on immunity, specifically with regard to the influence of the gut microbiota on immune function. Human milk is the gold standard for infant nutrition. Besides nutritional compounds, human milk contains many bioactive compounds (for example, human milk oligosaccharides [HMOs], long chain polyunsaturated fatty acids [LCPUFAs], microRNA, leptin, insulin, and insulin-like growth factors), as well as bacteria and immune cells. These all play a key role in supporting the development of a healthy, balanced gut microbiota and immune system.[3,4]

In this book, we discuss these concepts, and how active modulation of the gut microbiota through the use of dietary 'biotics' in non-exclusively breastfed infants, including those with dysbiosis, may help optimize health outcomes and help to reduce the risk of disease in later life.

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Chapter 2

The infant gut and immune system

The human intestine is more than simply a digestive, absorptive, and waste-eliminating organ. It is a highly sensory organ harboring a complex enteric nervous system that communicates with the brain; it also contains 70%–80% of the body's immune cells, and hosts a huge microbial ecosystem.[1] This ecosystem is collectively called the 'gut microbiota', and comprises an ecological community of commensal, symbiotic, and pathogenic microorganisms including bacteria, archaea, fungi, and viruses. The gut microbiota interacts in complex ways with the immune, metabolic, and nervous systems in the host, and helps protect the body from pathogenic and chemical insults through its ability to modulate the gut barrier and immune responses (see **Chapter 2**).[2–4]

As such, the gut represents the largest interface between the host and the external environment, and shows complex and highly integrated responses to environmental signals and changes in its luminal contents.[1]

The gut barrier: mucosal defense

The intestinal barrier is composed of the epithelium and underlying *lamina propria*, as well as extracellular mucus layers.[5] Collectively, these represent a physical and chemical barrier to protect the host from attack by potentially harmful microorganisms and other environmental threats.[5,6]

Between the epithelial cells, tight junction proteins form a continuous intercellular barrier that acts as a permeable seal,

to selectively regulate the trafficking of important macromolecules, and exclude toxins.[5,7]

Within the gut lumen and on the epithelial surface, 'commensal' (i.e. normally non-harmful, resident) gut microbes appear to contribute to the development and strengthening of the gut mucosal barrier through various mechanisms, including promotion of epithelial cell maturation and tight junction integrity.[8]

The *lamina propria* acts as an important interface between the environment and the gut immune system, facilitating activation of an immune response if antigens or pathogens cross the epithelial layer.[5]

Gut microbiota composition and activity

At birth, an infant transitions from an environment with limited exposure to microbes in the amniotic fluid, to an environment with widespread and continuous exposure to air-, skin-, and surface-borne microbes.[9] The infant gut, with its nutrient-rich and temperature-stable environment, nurtures colonization by beneficial bacteria – including *Bifidobacterium, Lactobacillus*, and *Bacteroides* species – allowing the development of a unique 'gut microbiota' or 'microbiome'.[9,10] Early environmental exposures and microbial colonization are believed to 'set the scene' for the long-term health of the gut mucosa and immune system.[11]

Microbiota colonization and composition

Gut colonization and establishment of the microbiota is a dynamic process through the first three years of life.[12] Bifidobacteria are among the first beneficial microbes to colonize the gastrointestinal tract of a newborn infant, and are present as the predominant bacteria in the intestinal tract of breastfed infants.[13] These bacteria are usually transmitted to the infant via the mother and the surrounding environment.[14,15] Other common 'pioneer' microbes include those from the genera *Bacteroides, Clostridium,* and *Eubacterium*.[2,16]

Bifidobacteria produce antimicrobial substances such as short chain fatty acids (SCFAs) (e.g. acetate and lactate) through anerobic fermentation of HMOs. These acidic compounds help to inhibit the growth of potentially pathogenic bacteria.[17]

Gradually, as the infant matures, the gut microbiota diversifies through colonization with a variety of microbes, and reaches a stable, balanced microbial community around three years of age.[12]

Many factors appear to shape the development of the gut microbiota during early life, including genetics, pregnancy factors, mode of delivery (cesarean vs. vaginal), gestational age, dietary exposures (human vs. formula milk), antibiotic use, other drug use (e.g. proton pump inhibitors and non-steroid anti-inflammatory drugs), and other early environmental exposures.[2,10,18,19] Early life nutrition has a

major impact on the process of colonization and microbiota composition and function.[10]

Functions of the gut microbiota

The gut microbiota has a profound influence on the maturation and functional development of the intestinal immune system during the first 1000 days of life, playing a vital role in normal gut function and maintenance of health (**Figure 2**).[6,10]

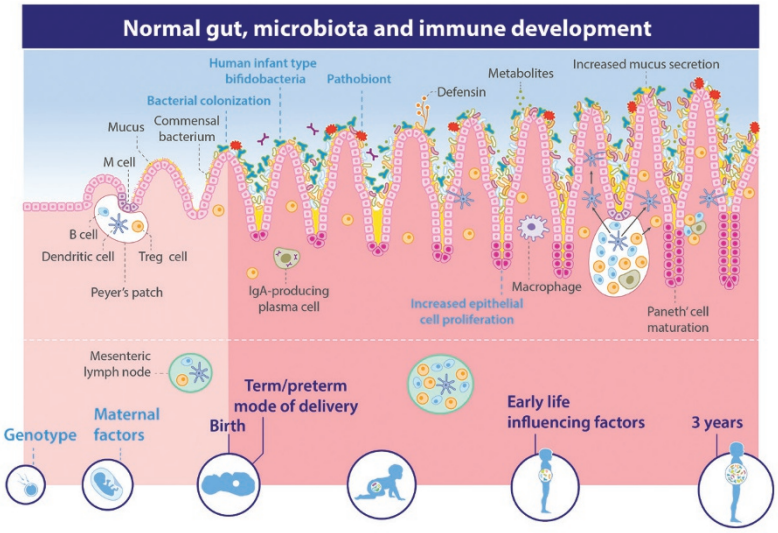

Figure 2. The first 1000 days of life: a crucial period for the development of immunity through the gut[10]

The microbiota plays a beneficial role for the host in a variety of ways, including nutritive, immunological, and nervous system benefits (**Figure 3**):[2–4,9,10,16,20–22]

- Facilitation of **efficient digestion** (e.g. fermentation of dietary fiber; pre-digestion of some nutrients), and **nutrient absorption**

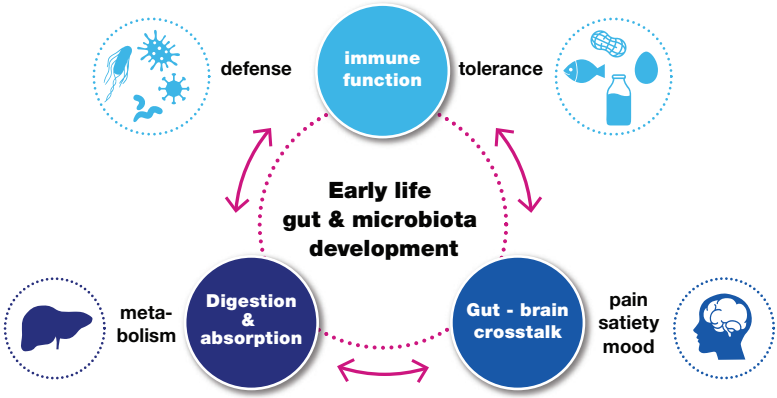

Figure 3. Vital role of the gastrointestinal tract and gut microbiota[9,10,20,21]

- Maintenance of **intestinal homeostasis**
- **Stimulation of gut development**
- Maintenance of **epithelial barrier function**
- **Protection from pathogens** ('colonization resistance'), through competition for nutrients and adhesion sites, and production of antimicrobial peptides
- Development and functioning of the **mucosal immune system**
- **Modulation of immune and inflammatory responses**
- **Regulation of the enteric nervous system**
- Influences **neurodevelopment** (gut–brain 'crosstalk')

Evidence supporting the wide-reaching benefits of the gut microbiota in both short- and long-term human health and disease is rapidly expanding.[20,23]

Immune function and its link to the gut

The immune system collectively comprises the organs and physiological processes that provide protection for an individual against harmful infections and toxins. The mucosal immune system is the largest immune component in the body.[2] A complex interplay of innate and adaptive components takes place as the body responds to diverse environmental and microbial challenges in order to maintain homeostasis.[20]

Healthy immunity relies upon balance; to function properly, the immune system must be able to detect a pathogen or toxin, distinguish it from the body's own normal tissue, and provide the appropriate response.[24] Pathogens or damaged cells need to be destroyed, foreign elements that are beneficial need to be tolerated, and healthy cells must continue to be accepted (**Figure 4**).

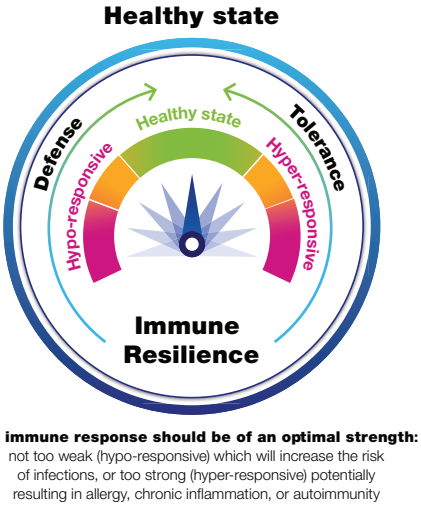

Figure 4. Immune system balance

'Resilience' means the ability of a system to withstand changes in its environment while still functioning properly. The term 'immune resilience' refers to an individual's ability to adapt to immunological challenges by regulating an appropriate immune response. In the short term, immune resilience has implications for food tolerance/allergy and infection; in the longer term, there are implications for the development of other non-communicable diseases, like autoimmune disorders.[24–26]

Innate and adaptive immunity

Infants are at continual risk of infectious and inflammatory diseases.[27] It is vital that the body's gut-associated lymphoid tissues are able to provide effective and appropriate immune responses when necessary.[24,28]

As a first line of defense, the commensal bacteria in the gut provide important protection against pathogens by, for example, promoting mucus production, lowering the pH of intestinal contents, secreting antimicrobial substances that inhibit the adhesion and growth of harmful bacteria, or by competing with invading organisms for binding sites and nutrients.[2]

Innate immune responses form a second line of defense. While commensal bacteria are non-invasive and do not trigger inflammatory responses, other microorganisms such as pathogens and soluble toxins readily penetrate the epithelium, where they are immediately recognized by specialized cells and receptors that form the innate immune system, initiating a non-specific effector response.[2,28]

As a third line of defense, the adaptive (acquired) immune system involves functional properties of B and T lymphocytes, and their antigen-specific surface receptors. Adaptive immunity is characterized by a long-lasting 'immunological memory' after initial response to an antigen, leading to enhanced responses with subsequent exposures to the same antigen.[29] This reaction is mediated by secretory immunoglobulins (Ig) (antibodies), triggering a complex cascade of events resulting in destruction of the antigen.[2]

Tolerance is the normal physiologic response to innocuous (harmless) ingested antigens.[25] Early exposure to such potential antigens is necessary for immune system 'training' in early life, to promote appropriate effector responses and the development of oral tolerance.[30] A breakdown in, or over-reaction of, the immunological response at the cellular and molecular level can lead to sensitization and allergic disease after antigen exposure, through inappropriate activation of the adaptive immune system.[26,29]

The gut microbiota and immune function

Despite the ever-building volume of literature in the field of microbiomics (i.e. the study of communities of microbes in the human body), the molecular mechanisms underlying the bi-directional interaction between the gut microbiota and the immune system – including allergy development – are not fully understood.[31]

However, it is becoming widely acknowledged that the

establishment of an optimal microbial community after birth, and the maintenance of a balanced gut microbiota, can have a profound effect on the development of both the innate and adaptive immune systems.[2,4,24,32] A healthy gut microbiota is resilient and supports immune resilience by promoting the development of appropriate immunologic regulatory networks, both in the local intestinal immune system but also with regard to systemic responses. Aberrations in the composition of the microbiota, known as dysbiosis, can thus result in impaired or overactive immune responses.[20,24]

An example of a common and early-onset immune disorder is allergic disease. The modern epidemic of allergic diseases points to vulnerability of the immune system to modern environmental change. Multifactorial gut microbiota imbalance (dysbiosis) can be a major underlying factor.

The gut–brain–immune axis

The gut is a highly sensory organ containing millions of neurons and 70%–80% of the body's immune cells.[1] Sensory neurons, endocrine cells, and immune cells enable signaling to modulate gut motility, tissue defense, vascular perfusion, and functions of other organs; and signals are also sent to the central nervous system to influence feeding behavior.[1] In this way, the gut influences the brain, and in turn, the brain influences the gut by way of the 'gut–brain axis'.

Brain development appears to be partly modulated by the gut microbiota. The complex microbiota–gut–brain communication

is driven by a variety of pathways including barrier function, hormonal and neural regulation, as well as via immune and metabolic pathways.[33] Bacterial metabolites such as SCFAs are able to cross the blood–brain barrier and can directly affect learning and memory. The blood–brain barrier plays a vital role in brain development by protecting the brain from external harm. In cases where the development of the gut microbiota is disrupted during the first 1000 days, brain development may be impacted, which can translate into complications that can last into adulthood.[33]

Dysbiosis and immunity

The concept of 'dysbiosis' refers to a state of imbalanced proportions and function of commensal, beneficial, and potentially harmful pathogenic bacteria, largely due to environmental influences and exposures.[10,34]

As one important example, cesarean-born infants show delayed colonization by *Bifidobacterium* and *Bacteroides* and enrichment of other species, compared with those delivered by vaginal birth, due to lack of normal exposure to bacteria through the birthing canal (**Figure 5**).[35,36] Acquisition and colonization of commensal gut bacteria may also become delayed or disrupted in infants born prematurely.[9] This may be due to cesarean birth, and/or antibiotic use, use of other pharmaceuticals, exposure to hospital-acquired infections, delayed enteral feeding, or lack of human milk feeding.

Because the gut microbiota helps shape the immune system

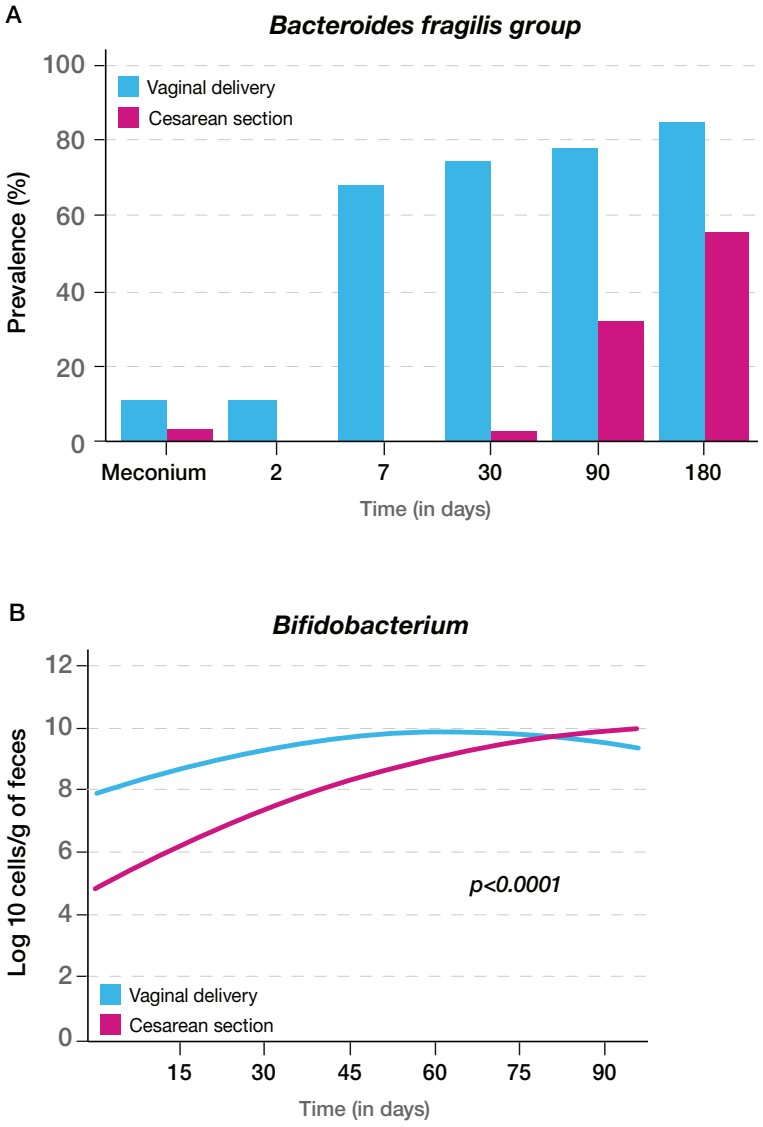

Figure 5. Prevalence of (A) *Bacteroides fragilis* and (B) bifidobacteria in fecal samples from infants born by cesarean versus vaginal delivery

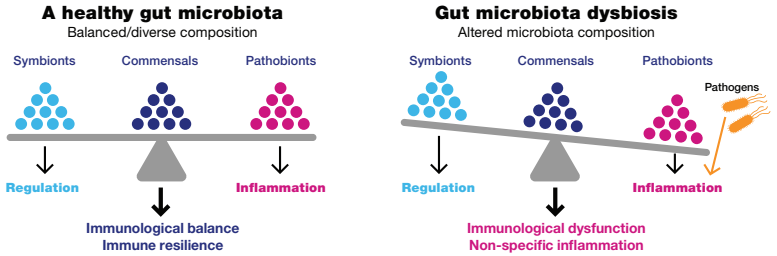

Figure 6. Dysbiosis and its potential immune consequences

in early life (**Figure 6**),[2] dysbiosis can be associated with both short- and long-term consequences related to immunity. Several preclinical studies and clinical trials of pre- and/or probiotics have yielded promising results in the restoration of a normal microbiota composition (see **Chapters 3–6**).[23]

For more information on the gut microbiota and the health implications of dysbiosis, please refer to *Significance of the Gut Microbiota and Nutrition for Development and Future Health*.*

Immune benefits of human milk

The WHO recommends exclusive breastfeeding for the first six months of life, and introduction of complementary feeding while breastfeeding is continued up to the age of two.[37] Breastfeeding provides a unique opportunity for ingestion of nutritional compounds and functional/bioactive agents to support gut maturation and optimal growth and development.

*https://www.essentialknowledgebriefings.com/downloads/gut-health-in-early-life-significance-of-the-gut-microbiota-and-nutrition-for-development-and-future-health/

Human milk composition is extremely complex, and naturally provides thousands of different nutritive and protective compounds that interact with each other in a unique way and are specifically tailored to the infant's needs. Human milk consists of 88% water and major compounds such as lactose (53–61 g/L), lipids (30–50 g/L), HMOs (12–15 g/L) and proteins (8–10 g/L). Immune cells, stem cells, bacteria, hormones, vitamins, minerals, nucleotides and other bioactive compounds are also present in human milk as minor compounds.

Human milk contains a variety of potentially health-promoting microbes and metabolites produced by and/or derived from these beneficial bacteria,[38] the most common being from the Firmicutes and Actinobacteria phyla.[36] There is high variability in the composition and number of bacteria in human milk among mothers, and in some cases even within mothers at different time points during the lactation period. It has been estimated that human milk contains between 10^3 and 10^6 bacterial cells/mL,[39,40] with some of this number being made up of non-viable bacteria.[39] Not only the bacteria in human milk, but also their metabolites (e.g. cell wall components and various bacterial metabolites) are anticipated to stimulate a healthy gut microbiota, immune functioning, and gut development.[39]

In addition, human milk contains many immune cells and other bioactive compounds such as HMOs. These play important roles in the development of a healthy immune system by supporting a

balanced gut microbiota, and providing anti-infective and immune development stimulating properties.[39,41–43] Human milk is thus considered to provide the best immune training opportunity for an infant.[20,43,44]

HMOs are one example of naturally occurring 'prebiotics' (see **Chapter 3**). They comprise a structurally diverse group of molecules with a size distribution of short chain HMOs and long chain HMOs.[45] Most HMOs escape digestion in the small intestine and progress to the colon where they are metabolized, acting as 'food' for the commensal gut bacteria.

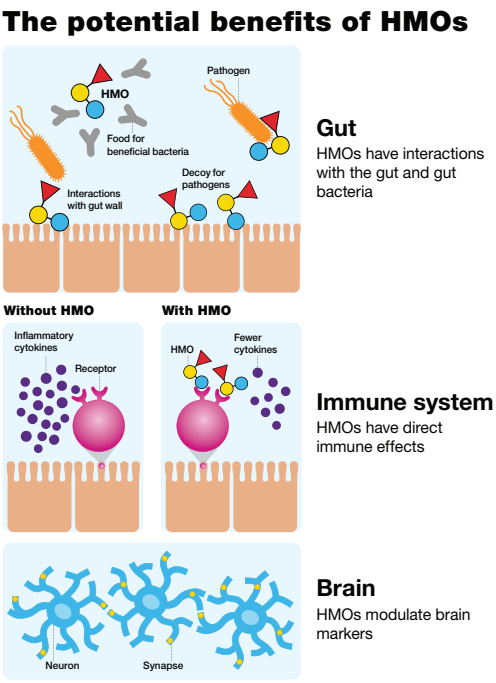

Figure 7. Human milk oligosaccharides and the gut–brain–immune axis[44,46–52]

This results in production of beneficial compounds such as SCFAs. HMOs thus play an important role in the 'feeding', nurture, and development of an infant's gut microbiota, intestinal barrier function, and immune system (**Figure 7**).[44,46–48]

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Chapter 3

The biotics family: Supporting immunity through the gut

What are biotics?

The term 'biotic' is derived from the Greek word *biōtikós*, meaning 'pertaining to life', and essentially refers to the biological ecosystem made up of living organisms together with their physical environment.[1] In the nutritional sense, biotics are a group of nutritionally active components that, when consumed, can confer a health benefit on the host.

The study of the microbiota has recently spurred remarkable scientific, commercial, and public interest in the use of nutritional biotics to modulate the gut microbiota to support human health.[2] There is rapidly increasing awareness among healthcare professionals around the beneficial effects of prebiotics and probiotics in human health – particularly for infants and children.[3]

Prebiotics and probiotics may be used in combination, as 'synbiotics'. Finally, the latest member of the biotics family, postbiotics, refers to bioactive compounds produced during a fermentation process, that may confer health benefits on the host (**Figure 8**).

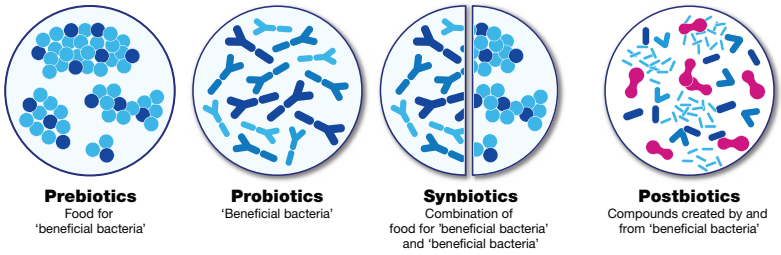

Figure 8. Prebiotics, probiotics, synbiotics, and postbiotics: definitions and functions

Subsequent chapters discuss definitions, examples, benefits, and safety of each of the four members of the biotics family.

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Chapter 4

Prebiotics

Definitions

The International Scientific Association for Probiotics and Prebiotics (ISAPP) recently reviewed the definition and scope of prebiotics, and produced a consensus statement on the definition of as illustrated below:

> *A prebiotic is a substrate that is selectively utilized by host microorganisms, conferring a health benefit*[1]
>
> ISAPP consensus statement, 2017

Note that the ISAPP consensus statement focuses on the importance of *selectivity*; as different prebiotics pass through the gastrointestinal tract to the colon, they are selectively fermented by specific health-promoting bacteria.[2–4]

Examples of prebiotics in infant formulas

It is well established that HMOs play an integral role in the nurture and development of the infant gut microbiota.[4,5] Therefore, when breastfeeding is not possible, addition of specific oligosaccharide mixtures to infant formulas is one strategy used to help promote the growth of beneficial gut microbes, particularly bifidobacteria.[4]

The most frequently used and well-studied oligosaccharides are galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS).[2,4] A specific combination of short chain GOS/long chain FOS (scGOS/lcFOS) in a 9:1 ratio in some infant formulas aims to mimic the function and size

distribution of non-digestible oligosaccharides in human milk.[6–8] The beneficial effects of the prebiotic mixture scGOS/lcFOS (9:1) are supported by a large number of clinical and preclinical studies.

Other prebiotics that could potentially be used in infant formula include inulin and polydextrose.[2] To date, there are limited data describing specific benefits of these compounds.

More recently, 2'-fucosyllactose (2'-FL) and lacto-N-neotetraose (LNnT) – two of the most abundant HMOs in human milk – have been used in infant formulas.[9,10] Both have been shown to promote the growth of *Bifidobacterium* in preclinical models.[10]

2'-FL has been described to have beneficial effects on the immune system,[11] gut microbiota,[12–16] gut barrier,[17] and brain development.[18] 2'-FL may be used with or without LNnT and with or without GOS. In a recent clinical study, supplementation of infant formula with both 2'-FL and LNnT was found to be safe and well-tolerated, and to result in lower morbidity and antibiotic use compared with unsupplemented control formula.[10]

Known and possible benefits of prebiotics such as scGOS/lcFOS

Evidence for the health benefits of prebiotics is rapidly evolving[1,19] (**Figure 9**).

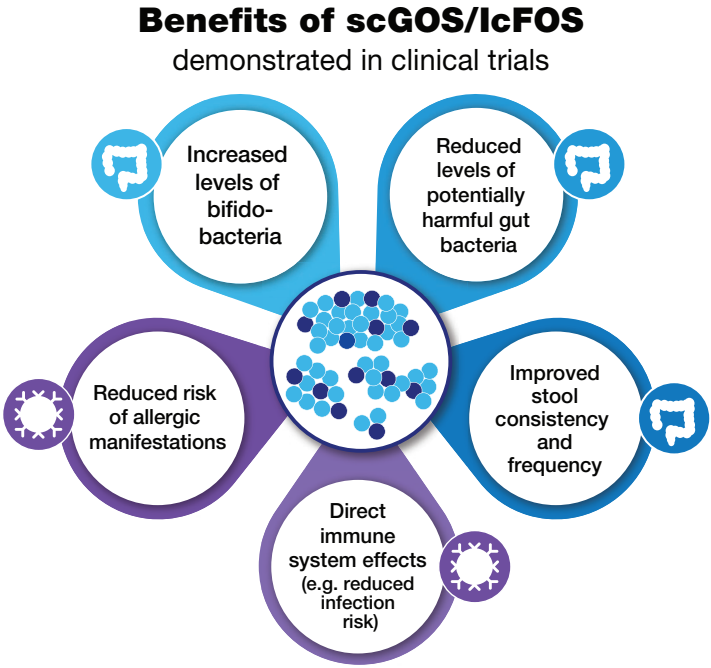

Figure 9. Benefits of scGOS/lcFOS prebiotic mixture demonstrated in clinical trials[4,7,20-35]

Specific benefits of prebiotics in infants and young children may include the following:

Modulation of the gut microbiota

Prebiotics have been shown to stimulate the growth and/or activity of important beneficial bacterial populations in the gut. For example, prebiotic supplementation of infant formulas with scGOS/lcFOS has been shown to increase levels of fecal bifidobacteria in a dose-dependent manner,[25,31,33,36] resulting in a microbiota composition closer to that of breastfed infants.[22,29]

In addition, this specific prebiotic mixture has been shown to reduce a wide array of clinically relevant pathogens.[28] This finding suggests that the bifidogenic effects of prebiotic oligosaccharides may help protect against infections.[24,27,28,32,33,37] Indeed, bifidobacteria are known to selectively metabolize non-digestible oligosaccharides and HMOs, resulting in the production of SFCA and a low fecal pH that inhibits pathogens.[4,7,32]

A recently published review of 14 studies showed a trend toward increasing mean stool pH in healthy breastfed infants between 1926 and 2017, from pH 5.0 to pH 6.5. This trend mirrors the recent reported decreases in *Bifidobacterium* abundance and associated dysbiosis in infants in developed countries.[38]

Immunomodulatory effects

Preclinical and clinical evidence indicates that non-digestible oligosaccharides may have direct immunomodulatory and anti-inflammatory effects at the cellular level.[39] A large, randomized study showed a reduced risk of infection following consumption of young child formula supplemented with scGOS/lcFOS/n-3 LCPUFAs.[35]

scGOS/lcFOS supplementation has also been shown in some studies to reduce the incidence and duration of acute diarrhea and antibiotic use in infants.[19,40] In addition, a protective effect against upper respiratory tract infections has been observed in infants fed a formula

supplemented with scGOS/lcFOS (**Figure 10**).[21,40] However, statistically significant reductions in infectious morbidity in prebiotic-supplemented infants have not consistently been demonstrated.[19,41]

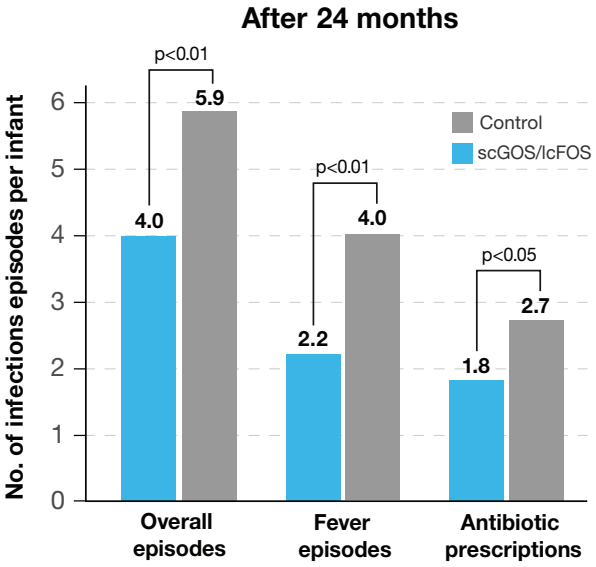

Figure 10. Significant reduction in infectious episodes between infants fed scGOS/lcFOS supplemented formula versus control formula. Data from Arslanoglu et al. *J Nutr.* 2008;138:1091–5.[21]

With regard to allergic manifestations, emerging evidence suggests that prebiotic supplementation to infant formula may have a protective effect. scGOS/lcFOS has been shown to reduce food allergy related symptoms such as atopic dermatitis (AD) (**Figure 11**), urticaria, and rhinoconjunctivitis in infants,[21,31,34,42] and to decrease the need for anti-inflammatory and antihistamine treatment.[42] One study showed that immune markers (sIgA, lysozyme, α-defensins)

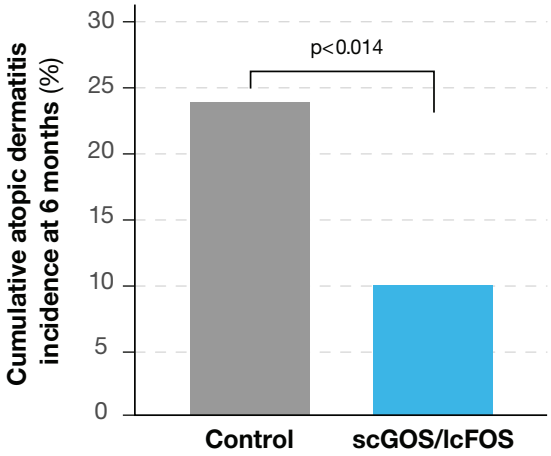

Figure 11. Decreased cumulative incidence of atopic dermatitis at 6 months in infants fed formula with scGOS/lcFOS versus formula with placebo. Adapted with permission from BMJ Publishing Group Limited. [*Arch Dis Child*. **Moro et al. 2006;91:814–9, copyright 2006**][31]

in scGOS/lcFOS-supplemented infants mimicked those of a breastfed control group.[34]

Based on the available evidence, the World Allergy Organization (WAO) recommends that prebiotics may be beneficial in allergy prevention after exclusive breastfeeding has been completed.[43]

Improved gut motility and stool characteristics

Administration of prebiotics to infants has been shown in randomized trials to improve gut motility, gastric emptying, and stool softness (including in infants with hard stools), mimicking the effects of human milk, and resulting in improved feeding tolerance.[7,23,26,30,32,44] However, some studies also included partially hydrolyzed whey protein, making it difficult to draw firm conclusions on the effect of scGOS/lcFOS specifically.

Safety of prebiotics

Prebiotic administration to infants is considered generally safe and well tolerated, with no concerns regarding age-appropriate growth or adverse effects.[7,19] One study showed non-inferior weight gain in infants fed oligosaccharide-containing formula, compared with standard formula;[30] another showed no significant difference in standard growth measurements between infants fed prebiotic/probiotic-enriched formula versus standard formula or versus human milk.[45]

No serious adverse effects of prebiotic use have been reported; however, prebiotic intake is known to be associated with mild gastrointestinal side effects in some cases, such as bloating, flatulence, and diarrhea. This effect generally resolves in short time due to adaptation of the gut.[2,3]

A 2018 systematic review concluded, in line with an earlier statement by the Committee on Nutrition of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN),[46] that specific oral prebiotic supplementation may confer favorable effects in some infants, but that further research is warranted.[19]

The European Commission specifically allows the use of scGOS and lcFOS with a specific ration of 9:1 and a maximal concentration of 0.8g/dL. This is included in the European Directive 2006/141/EC on infant and follow-on formulae. Furthermore, the safety of scGOS/lcFOS is endorsed by the

European Food Safety Authority (EFSA) and it is an approved ingredient, notified as Generally Recognized as Safe (GRAS; US FDA GRAS Notice [GRN] No. 477).

Summary

Clinical evidence
• Improved stool characteristics (softer stools[7,21,25,32])
• Lower pH[4,7,34]
• SCFA pattern similar to breastfed infants[24,31]
• sIgA levels similar to breastfed infants[47]
• Bifidogenic effects[27,33,35,38]
• Protection against infections[26,29,30,34,35,37,39]
• Reduced incidence and duration of diarrhea episodes[21]
• Reduction of allergic manifestations[22,33]
• Possible improvement in colic symptoms[27]
Preclinical evidence
• Direct immune system effects[41]
• Promotion of intestinal barrier integrity[48]

Table 1. Summary of potential beneficial effects of prebiotics

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Chapter 5

Probiotics

The term 'probiotic' was derived from a Greek word meaning 'for life'.[1] Probiotics facilitate the fermentation process in the colon, and play an important role in digestive, immunological, and respiratory health.[2]

Definition

The definition of probiotics is based on a 2001 FAO/WHO expert group consensus statement:

> *Probiotics are 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host'*[2,3]
>
> FAO/WHO Expert Group (2001); endorsed by ISAPP (2014)

Examples of probiotics

Numerous probiotic organisms have been researched in infants, including preterm infants, at various dosages and for different durations. Probiotic products may contain one or more bacterial strains.[1] The two most frequently studied bacterial probiotic species are from the *Bifidobacterium* and *Lactobacillus* genera.[1,4]

Ideally, probiotics should be well characterized, and known to be non-pathogenic, genetically stable, robust, and able to survive processing/storage conditions and gut transit.[1] Additionally, the health effects should be demonstrated in human studies.

Benefits of probiotics

Probiotics appear to have certain beneficial class effects on the host infant/child,[3] although the mechanisms by which probiotics confer these benefits remain largely unclear.[5]

Probiotic effects are highly strain-specific, and cannot be generalized for the most part;[1,3,5] however, potential common effects may include protection against infection, immune system benefits, and synthesis of important nutritional elements such as some vitamins.[5] These are described in more detail below.

A FAO/WHO Expert Consensus document states that '*adequate scientific evidence exists to indicate that there is potential for the derivation of health benefits from consuming food containing probiotics*', but that further evidence is needed to confirm a number of these health benefits.[2]

Normalization of a perturbed microbiota

One of the main advantages of probiotics is in their ability to normalize the gut microbiota, conferring important benefits as described below. Probiotics multiply and colonize the intestinal tract of the host, helping ensure a proper balance between pathogens and commensal bacteria, and shifting the balance toward that found in breastfed infants.[6]

Competitive inhibition of pathogens

Probiotics such as *Bifidobacterium lactis* Bb12 and *Lactobacillus rhamnosus* GG have been shown to effectively inhibit

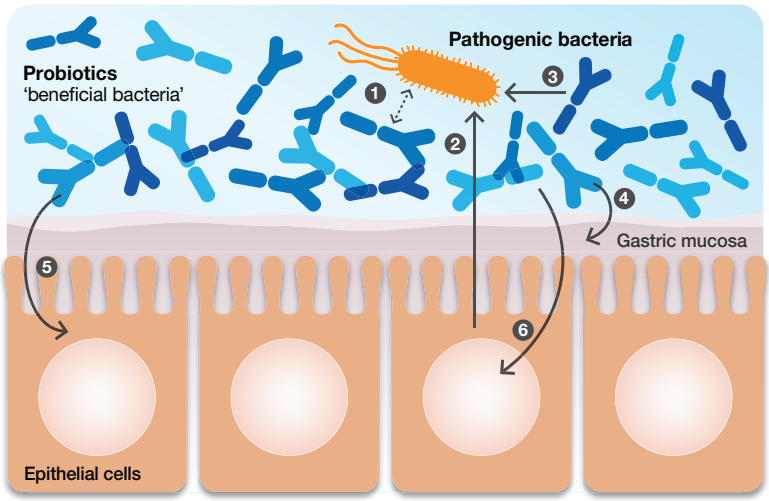

Figure 12. Competitive exclusion of pathogenic bacteria by probiotics. Adapted with permission from S. Karger AG, Basel [*Dig Dis*. Girardin & Siedman 2011;29:574–87]

colonization by pathogenic bacteria (**Figure 12**),[4] including various species/strains of *Clostridium*, *Campylobacter*, *Salmonella*, *Escherichia coli*, *Shigella*, *Staphylococcus*, and *Yersinia*.[6,7] Lactic acid bacteria and bifidobacteria have also been shown to inhibit viral pathogens.[5] It has been reported that certain probiotics (e.g. *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, and *L. rhamnosus* GG) could play a significant role in preventing or alleviating gastrointestinal conditions such as *Clostridium difficile*-associated diarrhea, and *Helicobacter pylori* infection.[2,8,9] The anti-infective benefits of probiotics may also extend to prevention of respiratory tract infections.[10]

Probiotics directly protect against pathogen colonization and infection in various ways, including:[3–5]

- Competing with pathogens for nutritional sources and adhesion sites
- Stimulation of mucus secretion to prevent pathogen adhesion
- Secretion of antimicrobial substances
- Supporting the integrity of the epithelial barrier function
- Modulation and regulation of immune responses

Regulation of gut motility

As with prebiotics, placebo-controlled trials have shown improvement in gut motility and gastric emptying in infants fed probiotic-containing formula (*Lactobacillus reuteri* DSM 17938), mimicking the gut motility of breastfed infants.[11] This effect may help improve feeding tolerance.

Improvement of colic symptoms

A 2018 meta-analysis, which included data from four randomized trials involving 345 infants with colic, demonstrated that the administration of *L. reuteri* DSM 17938 significantly reduced crying and/or fussing time in breastfed infants with infantile colic, but its role in formula-fed infants was less clear.[12]

Immune-modulating effects

Some probiotics, including specific *Lactobacillus, Bifidobacterium,* and *Streptococcus* strains, appear to have immunomodulatory properties, with beneficial effects on cell-mediated immunity and inflammation.[1,5] Modulation of host immunity with probiotic therapy thus represents a promising

area of research,[2] particularly in infants where the most pronounced immune-modulating effects are observed.[13]

The mechanisms of these immune function effects are complex and not well understood, but appear to involve both the innate and adaptive immune systems,[8,14] particularly inhibition of the production of immunoglobulin (Ig)E.[15] The effects are largely strain- or species-specific.[13,16] Direct effects may include secretion of factors that mediate host immune responses and immune cell signaling,[1,2,8,17,18] as well as regulation of inflammatory pathways.[4,8,18] Indirect effects include enhancement of the intestinal epithelial barrier, stimulation of mucus production, and competitive inhibition of pathogenic bacteria.[18]

The immunomodulatory effects of probiotics have been demonstrated in various preclinical studies. For example, *Bifidobacterium breve* M-16V demonstrated significant suppressive effects on allergic responses and reduced skin reactivity in murine models, with corresponding reductions in serum markers of sensitization (IgE and IgG) and suppressive effects on T-helper type 2 immune responses.[19,20]

Prevention and/or reduction of infectious or antibiotic associated diarrhea

Data supporting the use of probiotics such as *L. rhamnosus* GG and *Saccharomyces boulardii* as an intervention in cases of acute infectious diarrhea are reasonably well documented.[21–24]

Based on the available evidence, the ESPGHAN Working Group on Probiotics recommends the use of probiotics such as *L. rhamnosus* GG and *S. boulardii* in the management of acute gastroenteritis and for prevention of antibiotic-associated diarrhea in infants and children.[21,23,25,26] The use of *L. rhamnosus* GG may be recommended for the prevention of nosocomial diarrhea.

Prevention of allergic manifestations

The immunomodulatory effects of probiotics also appear to translate to a reduction in the risk and severity of allergic diseases,[6,27,28] although the evidence is somewhat inconsistent and requires further confirmation.[14,28] Several small trials have demonstrated allergic symptom improvement with a *Bifidobacterium* strain administered to infants/children with AD.[29,30] In addition, it has been shown that the immune-related negative effects of not breastfeeding may be mitigated by inclusion of *B. lactis* Bb12 in infant formula.[31] Despite low levels of evidence, World Allergy Organization (WAO) guidelines suggest a likely net benefit from administering probiotics to infants at high risk of developing allergy, primarily in terms of eczema prevention.[32] However, it requires specific studies to support the benefits of the specific strains.

WAO guidelines also suggest a possible benefit from using probiotics prenatally, in pregnant women at high risk of giving birth to an allergic child.[32] However, currently, the evidence is not strong,[32] and it remains unclear which strain to use.

Note that the data supporting the benefits of probiotics for allergy prevention are frequently reported with probiotics administered as part of a synbiotic group (see **Chapter 3**).

Prevention of necrotizing enterocolitis in preterm infants

Evidence suggests that abnormal development of the gut microbiota, resulting in dysbiosis, may contribute to the pathogenesis of nectrotizing enterocolitis (NEC) in preterm and other high-risk infants.[33] NEC is a leading cause of neonatal morbidity and mortality.

While the evidence for *pre*biotics for the prevention of NEC is not compelling,[34] the data supporting the use of specific *pro*biotics is robust and dates back at least three decades.[35,36] Clinical trials and well conducted meta-analyses suggest a highly important role for probiotics – particularly some *Lactobacillus* and *Bifidobacterium* species – in the prevention of NEC;[4,37] however, further strain-specific research is needed.[37]

Nutritional benefits

Non-clinical studies suggest some nutritional benefits of probiotics. Probiotic organisms, such as *L. reuteri, Lactobacillus plantarum, Bifidobacterium adolescentis* and *B. pseudocatenulatum*, are active producers of B group vitamins (B1, B2, B3, B6, B8, B9, and B12).[6] Some *Lactobacillus* and *Bifidobacterium* species have also been shown to enhance the absorption of vitamins and minerals from the gut, and stimulate the generation of amino acids and SCFAs, and produce important digestive enzymes (e.g. lipase, esterase).[6,38,39]

Other effects

Other possible (strain-specific) effects of probiotics may include some neurological and endocrinological effects,[3] but these have not been described specifically in infants and children.

Safety of probiotics

Probiotics are generally well tolerated in infants.[1]

With qualified presumption of safety (QPS)-approved probiotics, there is very little risk with regard to inducing, or being associated with the etiology of, disease.[2,40] However, a Joint FAO/WHO Expert Consultation document suggests that there is a need to establish clear guidelines based on practical criteria, to ensure safety with probiotics.[2] This document recommends that probiotic bacteria containing transmissible drug resistance genes are not used in foods.[2] There have been no pathogenic or virulence properties found for *Bifidobacterium, Lactobacillus*, or *Lactococcus*.[2]

Despite the positive clinical data supporting the benefits and safety of live probiotics in preterm infants, concerns around safety and dosing in infants with an immature gut epithelial barrier or impaired immune defenses have limited their use.[1,4,41] Interest is therefore increasing with regard to the use of pre- and postbiotics in infants born prematurely.[4,41]

Quality control

Quality control is important to ensure the safety of probiotic-containing products. The ESPGHAN Working Group on

Probiotics recently evaluated the available data and suggested a more stringent quality control process to ensure that the probiotic content shown on the label meets the actual content throughout the shelf life of the product, with no contamination present.[42]

Summary

Clinical effects
• Normalization of perturbed microbiota[6]
• Protection against pathogenic bacteria[3,5]
• Stimulation of the immune system[1,5]
• Reduction in allergy risk[6,13,27,43]
• Prevention/improvement of diarrhea[10,21,24]
• Protection against NEC[4,33,35,36,44,45]
Preclinical effects
• Modulation of intestinal barrier function[18]
• Synthesis of vitamins[6] and other nutritional elements[6]

Table 2. Summary of potential benefits of probiotics

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Chapter 6

Synbiotics

Probiotics in combination with prebiotics are being referred to in the scientific literature as 'synbiotics'.

In some cases, synbiotics can have a greater, or additive, effect, over and above that which can be achieved with either constituent alone. Such an effect can be due to the way prebiotics support the growth and survival of probiotics – especially with regard to bifidobacteria.[1]

Definition

The term 'synbiotic' refers to the combination of prebiotics and probiotics.[2]

> *Synbiotics are 'a mixture of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract of the host'*
>
> FAO/WHO Expert Consensus[3]

Selectivity

With synbiotics, the prebiotic compound selectively stimulates the colonization and activity of the probiotic and other bifidogenic bacteria.[4] Prebiotics can help improve the survival of probiotics during their transit through the upper intestinal tract, stimulate their growth, and/or activate their metabolism.[1,2] Probiotics and prebiotics may complement each other's beneficial effects, or a complementary or synergistic effect may be achieved by the combination.[5]

Selectivity is a key consideration when developing synbiotics; combinations must be judiciously selected based on knowledge of the specific carbohydrate utilization patterns of different bacterial species and strains.[2] It is hoped that further understanding of the complex mechanisms involved will lead to more successful synbiotic combinations.[2]

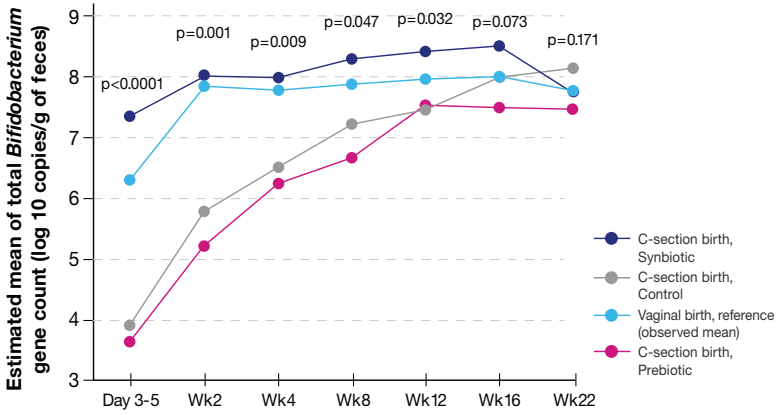
Benefits and uses of synbiotics

The benefits of pre- and probiotics have been previously described. Administration in combination, as a synbiotic mixture, may enhance some of these benefits.

Bifidogenic effects

Clinical data indicate that synbiotic-supplemented formula (bifidobacteria/lactobacilli + oligosaccharides) significantly increases bifidobacteria gut colonization compared with non-supplemented formula, supporting the development of a robust host–microbiota mutualism.[6]

In a multinational, double-blind trial, 183 healthy, full-term, cesarean-delivered infants were randomized to prebiotic (scGOS/lcFOS 9:1), synbiotic (scGOS/lcFOS 9:1 + *B. breve* M-16V), or control formula groups; a vaginally-delivered group was used as a reference cohort. Synbiotic supplementation (but not prebiotic supplementation alone) showed a bifidogenic effect on the gut microbiota, restoring the *Bifidobacterium* colonization delay and dysbiosis characteristic of cesarean-delivered infants (**Figure 13**).[7]



Adapted from: Chua et al. JPGN2017;65:102-106

Figure 13. Early synbiotic intervention increases the *Bifidobacterium* count in cesarean-born infants, mimicking that of vaginally delivered infants[7]

As described in an earlier chapter, infants at risk of developing allergy (due to exposure to environmental factors such as cesarean section delivery, or antibiotic use), and those with established cow's milk allergy, can present gut microbiota dysbiosis in early life. Given that a bifidogenic environment in the gut microbiota is important for immune development in infants, restoring a challenged gut microbiota through the use of synbiotics may help maintain appropriate immune function.

Immune function and allergy prevention/management

Due to the known immunomodulatory effects of pre- and probiotics, synbiotic mixtures such as scGOS/lcFOS plus *B. breve* M-16V are an attractive therapeutic proposition for further enhancing immune function.[2] It has been suggested that the synbiotic concept may be involved in suppression of IgE-mediated immune responses.[8]

In murine models, synbiotic mixtures have demonstrated enhanced oral tolerance and reduced allergic effector responses,[9–11] which may have important implications for allergy prevention in humans.

Knowing that a bifidogenic environment in the gut microbiome is important in the immune development of an infant, restoring this dysbiosis is a key element for infants at risk for allergy, or for infants with an established allergy. Synbiotics have been shown to restore the delayed colonization of bifidobacteria in cesarean section delivered infants, bringing the levels closer to that of vaginally born and breastfed infants.[7] This is thought to potentially reduce the development of atopic dermatitis (AD)/eczema in these infants;[7] but the evidence is not consistent, reinforcing the fact that specific combinations of prebiotics and probiotics have specific individual capacities.[12]

In another example in infants with AD, a 12-week intervention with scGOS/lcFOS and *B. breve* M-16V restored the gut microbiota closer to the healthy breastfed profile,[13] and resulted in a lower prevalence of asthma-like symptoms and asthma medication use after one year of follow-up, suggesting long-term effects of nutritional interventions in early life.[14] In addition, this study has shown a significantly lower incidence of diaper dermatitis in infants receiving synbiotics, compared with those receiving standard formula.[13]

The use of synbiotics for the treatment of allergic disease has also received recent attention. A meta-analysis in 369 infants and children showed evidence supporting the use of synbiotics – particularly containing mixed strains of bacteria – for the treatment of AD. The results were most pronounced in children over 12 months of age.[12]

Summary

- Enhanced bifidogenic effects[6]
- Immunomodulatory effects[2,14]
- Enhanced SCFA production[13]
- Improved stool characteristics[15]
- Improved viability of probiotics[1,2]

Table 3. Potential clinical beneficial effects of synbiotics

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Chapter 7

Postbiotics

Unlike the probiotic concept, in which live bacteria must be ingested and produce beneficial bioactive metabolites in the host, the postbiotic concept comprises the set of metabolites and remaining non-viable microorganisms arising in the food product as a result of fermentation facilitated by bacterial metabolic activity. The bacteria can act as a 'microbial factory' to enrich the food matrix, thus conferring health benefits that do not require bacterial viability. It is well established that some products of bacterial fermentation and/or remaining non-viable bacterial materials possess bioactive properties. All these compounds are denoted as postbiotics.[1]

Since postbiotics do not require bacterial viability or colonization in the host,[1] they may have several advantages as nutritional compounds. Postbiotics do not contain potentially harmful bacterial components; in addition, they show relative stability during storage, and are unaffected by emerging antibiotic resistance.[1,2]

In contrast to the abundant data on pre- and probiotics, the field of postbiotics in food and infant formula is emerging and ongoing research is being performed in this area.

Definition

Fermentation by naturally occurring microorganisms can be regarded as a bioactive enrichment of food. Indeed, bacteria used during this process can be used to naturally enrich the food matrix with a broad range of bioactive compounds that may confer different health benefits.

As postbiotics is a relatively new term in the literature, there is no universally accepted definition as yet, although some have been proposed. Aguilar-Toalá and colleagues proposed that '*Postbiotics refers to soluble factors (products or metabolic byproducts), secreted by live bacteria, or released after bacterial lysis, such as enzymes, peptides, teichoic acids, peptidoglycan-derived muropeptides, polysaccharides, cell surface proteins, and organic acids*'.[1] This proposal has been further fine-tuned and made more specific in the context of food for human consumption.

We present below a working definition for the purpose of this book:

> *Postbiotics are bioactive compounds produced during a fermentation process (including microbial cells, cell constituents and metabolites) that support health and/or wellbeing*

Thus, postbiotics are usually presented in the food matrix that was fermented, but could also be derived from the fermentation media (**Figure 14**). It should be noted, however, that purified compounds synthesized by microorganisms such as antibiotics are not considered postbiotics.

The main bioactive compounds produced during fermentation are organic acids, microbial cell wall components, proteins, lipids, carbohydrates, vitamins, or other complex molecules (**Figure 15**).[1] Notably, selection of the right bacterial strains is crucial, since the ability of microbial cultures to produce

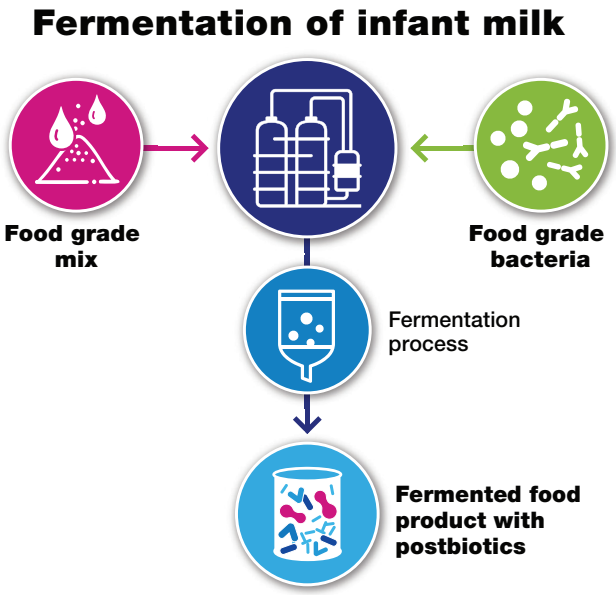

Figure 14. Fermentation of infant milk

bioactive metabolites is commonly a strain-dependent trait. Consideration should also be given to the optimum conditions required for fermentation to produce bioactive compounds. Postbiotics thus arise as a result of a specific food matrix, a particular bacterial strain, a unique fermentation process, and optimal conditions; hence, all postbiotics are different.

The range of mechanisms by which various postbiotics confer their unique benefits have not yet been fully elucidated. However, scientific data indicate that postbiotics have potential physiological functions at both the local and systemic level in the host. These functional properties can positively affect the microbiota homeostasis, host metabolic and immunological responses, and host resilience to detrimental changes (**Figure 16**).[1]

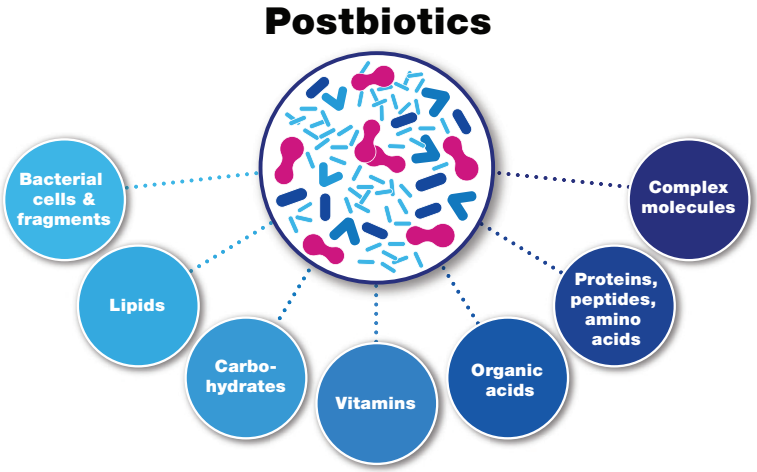

Figure 15. Examples of postbiotics[1,3–6]

Benefits of postbiotics

Along with pre- and probiotics, the postbiotic concept is emerging as a further source of support for host health through improvement of distinct physiological functions. Several studies have reported beneficial effects of specific bioactive metabolites produced by a number of microorganisms. Here we present a number of potential benefits of postbiotics, although these compounds would not all fall under the postbiotics definition proposed above. Not all are produced through food-grade fermentation processes and/or in their fermentation matrix.

In most reports, bioactive compounds produced by *Bifidobacterium* and *Lactobacillus* species have been studied.[1] Evidence suggests the potential of postbiotics to be systemically available and thereby act on different organs and functions beyond the gut. Some have been shown to

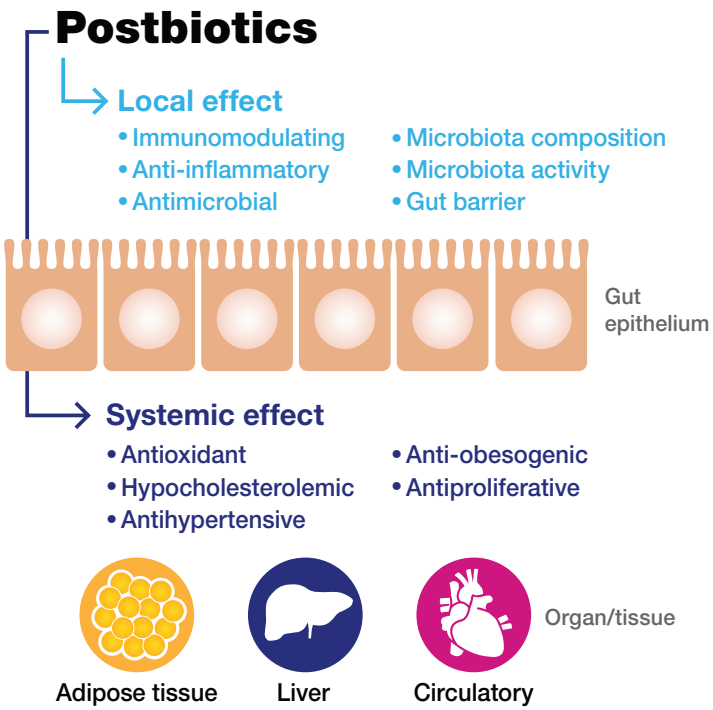

Figure 16. Local and systemic effects of postbiotics. Adapted from: Aguilar-Toalá et al. *Trends Food Sci Technol.* Accepted manuscript. 2018[1]

support intestinal barrier function[6], modulate inflammatory signaling pathways,[5,7] and confer antimicrobial and immunomodulatory effects in the gut (**Figure 16** and **Table 3**). These actions may positively impact gut microbiota homeostasis and host metabolic and signaling pathways, thus representing a highly promising opportunity in the field of functional foods.[1]

Local effects on the gut epithelium
Immunomodulating
Anti-inflammatory
Antimicrobial
Enhancement of intestinal barrier function
Systemic effects on organs/tissues
Antioxidant
Hypocholesterolemic
Antihypertensive
Anti-obesogenic
Antiproliferative
Anxiolytic
Antidepressant

Table 3. Summary of key potential effects of postbiotics[1]

Bioactive metabolites from *L. plantarum* strains RG11, RG14, RI11, UL4, TL1 and RS5, when combined with the prebiotic inulin, have been shown to inhibit proliferation of pathogenic bacteria. These antimicrobial properties may be attributable to the presence of specific compounds with antimicrobial activity.[8] In addition, effector molecules from *Lactobacillus* species were shown to be able to protect against the inflammatory properties of invasive *Salmonella* on healthy tissue and downregulate ongoing inflammatory processes in inflammatory bowel disease tissue.[9] Metabolites from *Lactobacillus casei* DG were shown to mitigate the inflammatory response in an *ex vivo* organ culture model of post-infectious irritable bowel syndrome patients.[10]

Specific postbiotics have been shown to help stimulate the growth and activity of specific components of the gut microbiota.[11–13] Some of these have been shown to directly inhibit pathogens such as *Listeria*, *Salmonella*, *Escherichia coli*, and *Enterococcus* strains.[1,14] For example, postbiotics

from *B. breve* C50 showed a reduction in pathogens including *Clostridium perfringens* and clostridial spores, a reduction in fecal pH, and an increase in the number of bifidobacterial species after seven days of consumption.[15]

Postbiotics in infant formula

In infant formulas, the concept of postbiotics is not widely used, although specific fermented infant formulas with postbiotics have been commercially available in Europe for decades. The postbiotics in fermented formulas are generally derived from fermentation of a milk matrix by food-grade bacteria such as *Bifidobacterium, Streptococcus,* and/or *Lactobacillus* strains.[1,14,16] Inactivation of the bacteria during post-fermentation production processes such as homogenization, pasteurization, sterilization, and/or spray-drying, ensures that few or no viable bacteria remain in the final product.[17]

Fermented formulas have the potential to improve some digestive symptoms, particularly lower gastrointestinal symptoms.[17,18] A systematic review of the available literature concluded that '*infants that could potentially benefit from fermented formulas are those with digestive discomfort (colics, bloating) and infants with diarrhea.*'[17] In addition, there is some rationale for harnessing the immunomodulatory activity of postbiotics to provide other benefits, such as improving AD symptoms.[1] Also, postbiotics have recently been suggested as a potential preventive strategy against NEC in preterm infants.[14]

Potential benefits of specific postbiotics in infant formula

Postbiotics derived from *Lactobacillus paracasei* CBA L74

Lactobacillus paracasei CBA L74 is used to prepare commercial fermented infant, follow on, and young child formula, by fermenting cow's skim milk. In the final product, non-viable bacteria and fermentation products are present, corresponding to 5.9 x 10^{11} CFU per 100g.[19]

Preclinical data

In preclinical research, postbiotics from *L. paracasei* CBA L74 have been reported to have anti-inflammatory effects on dendritic cells in response to the pathogen *Salmonella typhimurium*. The postbiotics inhibited pro-inflammatory cytokines, while not affecting IL-10. It was shown that this effect was not induced by the non-viable *Lactobacillus* cells and fragments, but rather by the metabolites produced. In the same study, the fermented milk displayed a protective effect against colitis and against an enteric pathogen infection (*S. typhimurium*) in a mouse model.[20]

Clinical data

In a clinical study in 377 healthy children aged 12–48 months who were attending daycare, dietary supplementation with the *L. paracasei* CBA L74 fermented formula prevented common infectious diseases, including upper respiratory tract infections and acute gastroenteritis. This preventive effect was accompanied by a reduction in

medication use (e.g. antibiotics, antipyretics, or steroids). In addition, an increase in fecal biomarkers of innate and acquired immunity were observed, and a negative association between these biomarkers and the occurrence of common infectious diseases was observed.[19]

Postbiotics derived from *B. breve* C050 and/or *Streptococcus thermophilus* 065

Bifidobacterium breve C50 and *S. thermophilus* 065 are both used in the preparation of commercial infant and follow on formula, in which a milk matrix is fermented. One of the postbiotics produced during this fermentation process is 3'-galactosyllactose (3'-GL), which is produced by the transglycosylation activity of *S. thermophilus* 065.[21] 3'-GL is also found in colostrum and human milk, and has been reported to have anti-inflammatory properties.[22–24]

Preclinical data

In a preclinical model, postbiotics derived from *B. breve* C50 induced prolonged dendritic cell survival and maturation, and induced high IL-10 production through TLR-2, suggesting immune regulatory functions.[25] Moreover, postbiotics from this strain when combined with postbiotics from *S. thermophilus* C65 have been reported to reinforce the intestinal barrier capacity and stimulate Th1 response in a mice model.[26]

Clinical data

The effects of an infant formula with postbiotics derived from *B. breve* C50 and *S. thermophilus* 065 was tested in newborn

infants. Eleven infants received the test formula, while nine controls received a standard infant formula without postbiotics. The microbiota of the active group showed a higher number of bifidobacteria and a decrease in the number of adult-like species, and the antipoliovirus IgA titers increased significantly after a challenge ($p<0.02$) in the active group versus the control group without postbiotics.[13]

In another clinical study involving 90 healthy term newborn infants, the fecal pH of those who used an infant formula with postbiotics was significantly lower compared to infants fed a standard formula ($p<0.05$), being similar to the faecal pH of human milk fed infants.[27] In the same study, it was shown that the infant formula with postbiotics from *B. breve* C50 and *S. thermophilus* 065 induced a significantly higher thymus size, which was closer to the thymus size of human milk fed infants.[27]

Another randomized, controlled trial investigated the incidence of acute diarrhea and its severity in healthy infants fed a formula containing postbiotics from *B. breve* C50 and *S. thermophilus* 065, compared to infants using a standard infant formula without postbiotics. Diarrhea incidence, duration of diarrheal episodes, and number of hospital admissions did not differ significantly between the groups. However, episodes of diarrhea were less severe in infants using an infant formula with postbiotics, indicated by fewer cases of dehydration, medical consultations, oral rehydration solution prescriptions, and changes to other formulas.[28]

Finally, a randomized trial has also demonstrated that consumption of an infant formula with postbiotics from *B. breve* C50 and *S. thermophilus* 065 decreased the incidence of potentially allergic adverse events, suggesting an improvement of oral tolerance to cow's milk in infants with high risk of atopy.[29]

Postbiotics derived from *B. breve* C050 and *S. thermophilus* 065 combined with prebiotics scGOS/lcFOS

Clinical data

A randomized, controlled, double-blind trial investigated the safety and efficacy of an infant formula with prebiotics scGOS/lcFOS 9:1 at a level of 0.8 g/100 mL and postbiotics derived from *S. thermophilus* 065 and *B. breve* C50, using the Lactofidus™ fermentation process.* The study included 432 healthy infants divided into four different groups, receiving formula with prebiotics and postbiotics (with two different levels of postbiotics), formula with prebiotics only, and formula with postbiotics only. The study showed that the combination of postbiotics and prebiotics was safe and well tolerated, and supported normal growth.[30] Also, the study demonstrated that the formula with prebiotics and postbiotics led to less crying and lower reported incidence of infantile colic.[31] Since infantile colic is correlated with low-grade systemic inflammation,[32] these findings may indicate an effect of the new nutritional concept on inflammatory immune regulation.

*A well-defined fermentation process in which the two strains *S. thermophilus* 065 and *B. breve* C50 are used to ferment a milk matrix.

A second randomized, controlled, double-blind clinical study investigated infant formula with postbiotics from *B. breve* C50 and *S. thermophilus* 065 and prebiotics scGOS/lcFOS (0.8 g/100 mL) in a 9:1 ratio, using the Lactofidus™ fermentation process (n=200). The control formula did not contain prebiotics and postbiotics. Breastfed infants were included as a reference group. The combination of specific prebiotics and postbiotics in infant formula was shown to be safe and well tolerated.[33] Compared to the control group, the composition and metabolic activity of the fecal microbiota among infants fed prebiotics and postbiotics was more aligned with that of breastfed infants. A lower pH, higher levels of acetic acid and sIgA, increased number of bifidobacteria, and decreased *C. difficile* occurrence were observed in the gut microbiota of infants using prebiotics and postbiotics.[34,35] Moreover, infants using prebiotics and postbiotics had significantly softer stools versus the control group.[36]

Safety of postbiotics in infant formula

No negative health effects of infant formulas with specific postbiotics have been documented,[17] and infant formulas with postbiotics are reported to support a normal growth trajectory. This was confirmed in a recently published systematic review by Szajewska et al,[17] in which data were analyzed from five randomized trials involving 1326 infants who received either formula fermented with *B. breve* C50 and *S. thermophilus,* or unfermented infant formula. Compared with infants receiving standard

formula, those receiving formula with postbiotics showed similar weight and length gains.[17]

Postbiotics have been suggested as a potential preventive strategy against NEC in preterm infants, to avoid the risk of administering live microorganisms that could translocate and cause infection. Well-designed trials investigating the efficacy and safety of postbiotics for the prevention or treatment of NEC should confirm this.[14]

As postbiotic signatures are dependent on bacterial strains and processes, the safety and suitability of specific postbiotics in infant formula remains to be confirmed.

Summary

Clinical effects
- Prevention of common infectious diseases, including upper respiratory tract infections and acute gastroenteritis[19]
- Increased fecal biomarkers of innate and acquired immunity[19]
- Increased poliovirus-specific intestinal antibody response[13]
- Less severe diarrhea[28]
- Similar thymus indexes as human milk-fed infants[27]
- Modulation of the gut microbiota with higher proportion of bifidobacteria and with fewer adult-like species[13]
- Upregulation of fecal secretory IgA in preterm infants[37]
- Lower incidence of infantile colic[31]
- Modulation of the fecal microbiota and activity towards infants fed human milk[34,35]

Preclinical evidence
- Anti-inflammatory properties[20]
- Protection against colitis and enteric pathogen infection (e.g. *S. typhimurium*)[20]
- Prolonged dendritic cell survival and maturation[20]
- High IL-10 production through TLR-2[25]
- Reinforcement of intestinal barrier capacity[26]
- Stimulation of Th1 response[26]

Table 4. Potential benefits of some specific postbiotics in infant formula

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Chapter 8

The future of biotics in infant health

Research is continuing into defining the concept of the healthy microbiota. The National Institutes of Health-supported 'Human Microbiome Project' (https://hmpdacc.org/) was established in 2008, with the purpose of characterizing microbial communities from hundreds of healthy individuals.[1] This project is expected to revolutionize future research into biotics and their applications, including infant health and development.[2]

Future biotics research

As discussed above, the efficacy of different biotics in preventing and treating some disorders such as allergies and gastrointestinal problems and infections is becoming well established. As more probiotic organisms – and the specific prebiotics that fuel them – are discovered, it is likely that strain-specific applications will continue to strengthen and expand.[3] Importantly, future research into prebiotics is expected to yield more compounds with structures identical to functional HMOs in human milk, improving the functionality of prebiotic-supplemented formulas.

Postbiotics research is still in its infancy, but is a highly promising area of discovery.[4] As noted in the previous chapter, there is a pressing need for an aligned consensus definition for postbiotics, and for well-designed trials evaluating specific fermentation processes and postbiotics, and their utility in infant formulas, including in high-risk preterm infants.[4] As new fermentation processes and formulations become available, including formulations also

containing added prebiotics, more studies evaluating the benefits of these modifications are planned.[5]

Other areas of research

On a practical note, different methods of biotics administration through functional foods and supplements are being investigated.[3] There has been some concern around the shelf life of live probiotic bacteria in foods, and poor survival during transit through the gastrointestinal system. Recent research efforts are continuing to focus on improving bacterial survival through technologies such as microencapsulation.[6]

Other research is focusing on the potential role for prebiotics and probiotics in the approach to overcoming global antibiotic resistance, with applications both in humans and in the food production industry.[2,7] In addition, while the evidence is not yet strong, there is also increasing rationale for the use of probiotics alongside antibiotics as standard practice, to help maintain a healthy gut microbiota composition.[8] At the same time, there is increasing interest in postbiotics for gut microbiota modulation and health promotion.

Concluding remarks

While the study of biotics in helping modulate the gut microbiota is warranted, it is important that such interventions are considered alongside other strategies that help address the cause of the dysbiosis in the first place, such as birth/delivery method, type of feeding, and environmental factors.[9]

Human milk will always remain the gold standard in infant nutrition. However, for infants who cannot be exclusively breastfed, pre-, pro-, and postbiotics, and combinations thereof, are promising bioactive compounds to mimic human milk functionality and support immunity through the gut in infancy. Further research is anticipated to strengthen the data around the use of these compounds, and it is expected that biotics will eventually become prerequisite ingredients in infant formulas.

Indeed, momentum continues to grow as the scope of preventative and therapeutic uses for biotics further expands.

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THE BIOTICS FAMILY IN EARLY LIFE is the fourth book in an educational series on the first 1000 days of life. This book examines the influence of the gut microbiota on immunity, and discusses the building evidence supporting the use of nutritional biotics in early life, to ensure the development of a balanced microbiota and normal immune function development.

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