SMALL TALK

PAEDIATRIC NUTRITION UPDATE INTENDED FOR HEALTHCARE PROFESSIONALS ONLY | AUTUMN 2020

IMMUNITY SPECIAL EDITION

Microbiome and Immunity • Probiotics • LCPUFAs and the developing immune system

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CONTENTS

WELCOME

To our special shortened edition: spotlight on **IMMUNITY**

Autumn has arrived, bringing us multicoloured leaves, a cool chill and an awareness of the time we have been affected by the COVID-19 global pandemic. The country has set new restrictions, and the tireless efforts of NHS staff working hard throughout this pandemic has never been more apparent. The importance of understanding the factors affecting immunity is very clear in this current climate and we wanted to share some of the research in this area of Immunity and Nutrition for you in this small-er edition of Small Talk. We hope this edition offers informative content and an enjoyable read to support you with your ongoing CPD.

In this small-er edition, we have articles on the microbiome to immunity and the role of Long-chain polyunsaturated fatty acids (LCPUFAs) in the developing immune system. We hope you find these articles useful to support your knowledge and development, alongside some recent abstracts.

As always, if you would be interested in contributing to a future edition of Small Talk, or have any feedback on what you would like to see included please don't hesitate to get in touch..

Keep safe,

Emma

CONTACT POINT

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Early life microbiome and immunity

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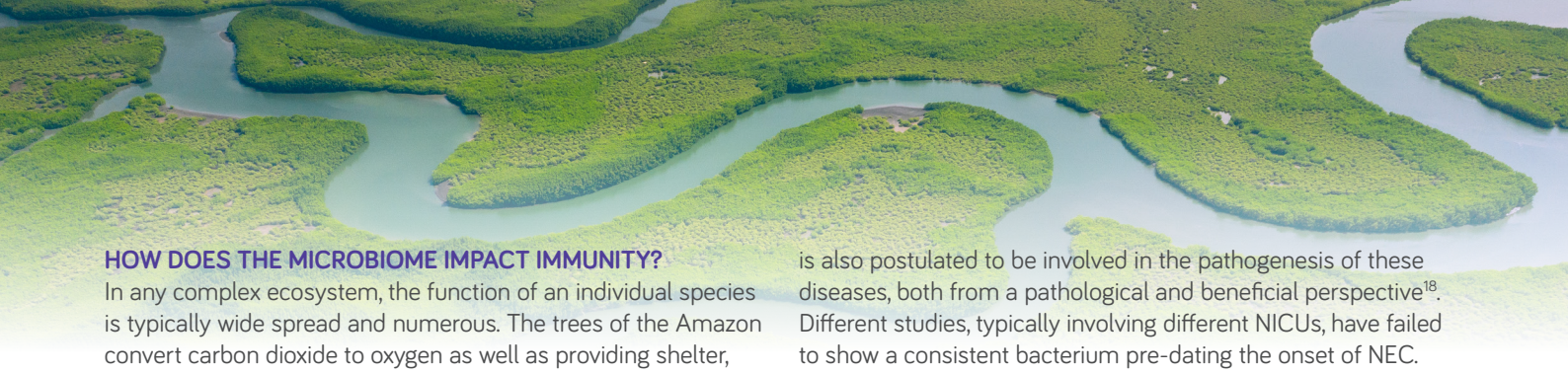
WHAT IS THE MICROBIOME?

The microbiome is a concept gaining rapid attention, owing in part to its proposed importance in health and a wide range of diseases, making it a tangible target for the development of new diagnostics and therapeutics. The microbiome is the collection of bacteria and other microorganisms (e.g. fungi and viruses) that exist within a given ecosystem. For the purposes of my work and this article in general, we will think about this ecosystem as the human body and, in particular, the gut. The gut contains the largest density and diversity of microorganisms of any body site, due to its continuous exposure to the environment and different foods, and the largely ideal conditions that support the growth of a vast number of microbes[1]. From a microbial perspective, one can think about the human body as analogous to planet earth – the numbers and types of plant and animal species which thrive in the humid and wet amazon are very different to those found in vast open sun-drenched African planes, which are completely different again to those found in wetlands and the oceans. On an even finer scale, the short distance from a shallow coral to deeper ocean results in a different ecosystem and different species adapted to survive there. A bacterium adapted to survive in anaerobic, warm, moist, nutrient rich conditions of the gut (think the 'amazon rainforest') may not survive on the skin ('desert planes') or the mouth ('ocean'). So too, on finer scales, the bacterial species which colonise the gut alter as the conditions change, with reduced oxygen, pH and motility resulting in higher bacterial density and diversity at the most distal sites.

HOW DOES THE MICROBIOME DEVELOP IN EARLY LIFE?

Following birth, the neonate is colonised by viable bacteria for the first time. Infants delivered vaginally are colonised by bacteria from the mother's vaginal tract, whereas C-section infants acquire their initial bacteria from the mothers skin and the surrounding environment[2,3]. In light of these findings and epidemiological reports that infants delivered by C-section have a higher risk of obesity, asthma, allergy, and diabetes, researchers have postulated that 'seeding' of C-section infants with vaginal microbes may reduce these diseases later in life. A small study demonstrated that C-section infants can be seeded with maternal vaginal microbes collected on a gauze, which facilitated a partial restoration of the microbiome observed in vaginally delivered infants after the first month of life[4]. However, most recent evidence shows higher levels of species from the *Bacteroides* genus primarily drive differences between vaginal and C-section infants[5]. This bacterium originates from the maternal gut and is transferred during delivery via the faecal-oral route[6], raising important questions about the utility of vaginal seeding vs. maternal faecal seeding. Importantly, such procedures are not without risk and the short- and long-term consequences are not yet understood.

Within a few days, the initial colonisation is expanded as the infant is exposed to new microbes and the different ecosystems across the body become more distinctive[7]. The gut becomes increasingly anaerobic over the first weeks of life, resulting in loss of aerobic bacteria, the establishment of facultative anaerobes, and the new colonisation by anaerobic bacteria. In infants who receive mothers breast milk, which is rich in human milk oligosaccharides (HMOs) and other components that provide growth substrates for bacteria, a bloom in *Bifidobacterium* is notable[5,8]. *Bifidobacterium* have a distinctive ability to utilise HMOs and species from within this genus dominate the gut microbiome in breast-fed infants. This receipt of breastmilk is the single most important factor that influences the early life microbiome[5]. When an infant no longer receives breastmilk, the gut microbiome undergoes a rapid turnover, where the *Bifidobacterium* are lost and replaced with species from within the Firmicutes phylum[5]. Such species are typically found in adults and from around month 30 of life the gut microbiome of an individual will remain stable and individualised in the absence of deliberate manipulation, such as pro- or anti-biotics.



HOW DOES THE MICROBIOME IMPACT IMMUNITY?

In any complex ecosystem, the function of an individual species is typically wide spread and numerous. The trees of the Amazon convert carbon dioxide to oxygen as well as providing shelter, shade and nutrients to countless animals and birds. Similarly, the gut microbiome has essential roles in digestion, production of vitamins, promoting integrity of the epithelial cells that line the gut, and development and training of the immune system. Around ¾ of all human immune cells are located in the gut epithelium, making it the largest immune organ and primary site of immune development in early life. Innate and adaptive immunity of the neonatal intestine, including its role in short- and long-term health and disease is the focus of much investigation. The most important events in education of host immunity likely occur during the initial weeks and months of life, during which time the microbiome displays the highest variability within and between individuals, before reaching a more stable adult-like configuration at the age of ~3 years. Recent evidence has also highlighted the large variability of the human immune system within and between individuals during the first 3 months of life[9,10].

Innate immunity consists of the physical barriers and non-memory cells, including neutrophils and macrophages, that detect generic pathogen-associated molecular patterns. Mucus is produced by goblet cells residing in the epithelium and offers an important first line of defence to prevent the invasion of microbes into the submucosa. Like HMOs, mucin glycans also act as a growth substrate to specific bacteria including *Bifidobacterium*, facilitating closer host-microbial interaction with human epithelial cells[11]. Adaptive immunity is targeted and serves to respond and destroy specific pathogens, with education and memory from previous encounters. This adaptive branch includes B cells and T cells, which mainly reside in the intestine and develop from interaction with the gut microbiome[12,13]. Finally, secretory immunoglobulin A (SIgA) is abundant in human milk and is produced by an infant after the initial weeks of life. SIgA has important roles in shaping the host-microbial relationship and is highly effective at dampening T-cell activation and in binding enteric pathogens and their toxins[14,15].

WHAT ABOUT THE MICROBIOME IN PRETERM INFANTS?

Babies born significantly premature (<32 weeks gestation) have an unnatural start to life, with higher C-section rates, more antibiotics, reduced receipt of breastmilk, and housing in incubators limiting normal environmental exposure[16]. This directly influences the developing microbiome by reducing the normal diversification and increasing the number of pathobionts (i.e. potentially pathological bacteria that generally exists as a non-harming commensal). Indeed, the bacteria that exist within a given neonatal intensive care unit (NICU) seed the preterm infant, and the preterm infants also seeds the local NICU environment, explaining in part why the developing microbiome can differ between sites with seemingly comparable clinical practice[17].

Owing to the immaturity of a preterm infant, the risk of developing diseases including necrotising enterocolitis (NEC) and late onset sepsis (LOS) is greatly increased. The microbiome is also postulated to be involved in the pathogenesis of these diseases, both from a pathological and beneficial perspective[18]. Different studies, typically involving different NICUs, have failed to show a consistent bacterium pre-dating the onset of NEC. Nonetheless, some consistency has been observed at higher taxonomic levels, with higher levels of Proteobacteria (which includes *Enterobacter, Escherichia,* and *Klebsiella*) in NEC infants[19–25]. The overall bacterial profile has also been found to be different prior to disease in NEC, using both non-invasive stool and fixed tissue from the site of disease, highlighting an abnormal microbiome compared to matched controls[26]. Additionally, a single HMO, disialyllacto-N-tetraose, has been found to be lower in mothers' milk of infants who develop NEC, and administration of this HMO to rats prevented NEC from developing[27,28]. However, such findings are largely based on associations and do not inform if these differences are cause or effect. The role and mechanism of host-HMO-microbiome interaction in disease are actively being studied and it is tangible that the results of such research will inform tailored supplementation of infant diets alongside breast feeding or when breast milk is not available.

FOCUSING ON BIFIDOBACTERIUM

As described above, *Bifidobacterium* harbour unique properties that maximise their ability to colonise the infant gut, such as digestion of HMOs and mucin glycans. The synergistic relationship between *Bifidobacterium* and the human host during early life is notable and there is mounting evidence supporting a wide range of benefits from *Bifidobacterium* colonisation. For instance, *Bifidobacterium* species produce lactate and acetate which lower pH and inhibit growth of pathogens[29], reduce the transfer of antimicrobial resistance genes between pathogens[30], and improve gut barrier function and regulate gut mucosal immune responses[31]. In light of such important properties, *Bifidobacterium* has become the primary target for probiotics (i.e. the administration of viable bacteria to promote health), especially in early life. In preterm infants, many large RCTs have failed to show significant benefits of probiotics[32], although numerous systematic reviews and meta-analyses suggest probiotics are beneficial overall[33]. In term infants, probiotics are being studied in the context of atopic/allergic diseases, where the results relating to improved outcome are also inconsistent. Nonetheless, animal model and human cohort studies have demonstrated that *Bifidobacterium* can exert local (e.g., reduced penetration of antigens) or systemic (e.g., increased Treg production) influences[34].

It is notable that even within the context of an RCT, the probiotic strain can colonise placebo infants, likely confounding results. In addition, many elements of probiotic use are not understood, such as the exact bacterial strain(s) to use, the optimal dosage, the frequency of administration, what prebiotics to supplement, do different individuals require stratified or a personalised probiotic course based on their existing microbiome and/or demographic factors, etc. Thus, there remains a critical and urgent need to perform studies to determine many of these outstanding questions. This would allow RCTs to be designed that maximise the chances of the probiotic intervention showing significant results in improving health and reducing disease, potentially saving time and money in the long run.

WHAT NEXT – MOVING FROM BENCH TO BEDSIDE?

Much of our understanding of the microbiome in early life has come from association-based studies. Before microbiome associations are developed into preventative algorithms, biomarkers and novel therapeutics, a deeper understanding of host-microbiome interaction in early life is needed. Therefore, it is now essential that associations are further validated in large independent cohorts and followed up with targeted mechanistic experiments in the lab. One tool which offers great promise in studying host-microbe interaction in early life is a recently developed enteroid anaerobic co-culture system[35]. This vastly improves upon previous systems, which typically used immortalised cell lines and required experiments to be performed in an oxygenated environment, preventing growth of important anaerobic bacteria including *Bifidobacterium*. Furthermore, enteroids are derived from stem-cells from patient tissue[36], allowing the generation of a mini-intestine that is characteristic of the patient's life stage (i.e. infant different to adult), the region of the intestine the tissue was taken (i.e. small intestine different to large intestine), and are composed of all the major cell types of the intestinal epithelium (e.g. contain goblet cells which secrete mucus). With physiologically relevant oxygen conditions allowing the co-culture of enteroids in direct contact with the microbiome, such model systems have enormous potential to lead to paradigm shifting results.

CLOSING REMARK

In relatively recent times, the microbiome was largely viewed through the disease-causing lens, but recent research has changed this, with most bacteria now considered fundamental to human health. Nonetheless, a truly healthy microbiome has not been described and this likely varies from site-to-site, from person-to-person, and within an individual across the life course. Before the power of the microbiome can truly be harnessed, more research is needed, but early indicators are undoubtably positive and hugely exciting.

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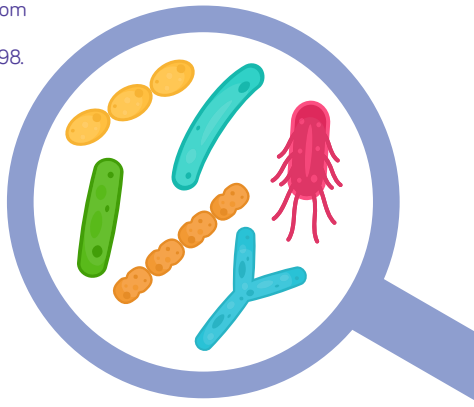

Long-chain polyunsaturated fatty acids (LCPUFAs) and the developing immune system

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IMMUNITY IN HEALTH AND DISEASE

The immune system protects individuals from pathogenic bacteria, viruses, fungi and parasites. To deal with this wide array of threats, the human immune system has evolved to include a myriad of cell types, communicating molecules and functional responses. It is obvious that a well-functioning immune system is key to providing effective defence against pathogenic organisms. Consequently, individuals with weakened immune systems are at a higher risk of becoming infected and of infections being more serious. The immune system also plays a role in assuring immunologic tolerance of non-threating exposures including commensal bacteria and food components. A breakdown in tolerance is linked to various diseases including inflammatory bowel diseases (loss of tolerance to commensal gut microbes) and food allergies (loss of tolerance to food components).

IMMUNE DEVELOPMENT IN HUMANS

All cells of the immune system develop in the bone marrow. Most immune cells also mature in the bone marrow, but T lymphocytes (T cells) mature in the thymus. Immune cells circulate in the blood stream and in the lymph and are found organised in discrete organs like the spleen and lymph nodes where they interact with one another. Mucosal barriers (e.g. the gastrointestinal tract, the respiratory tract, the genitourinary tract) also contain organised aggregations of immune cells. In humans it is estimated that 70% of immune cells are associated with the wall of the gastrointestinal (GI) tract, mainly in discrete structures such as lamina propria and Peyer's patches. The reason for such a large congregation of immune cells at mucosal barriers is that these are sites of high exposure to pathogens.

The human immune system begins to develop before birth with generation of a variety of immune cells and the population of the spleen and lymph nodes (**Fig 1**). Nevertheless, fetal immune cells are immature with limited functionality. Importantly therefore, the pregnant mother provides passive immunity to the fetus through the placental transfer of antibodies. Pregnancy is associated with immune changes in the mother with a dampening of T-helper 1 (Th1) type responses in favour of T-helper 2 (Th2) type responses; this is to assure maternal tolerance of the fetus. This Th2-skewing is also seen in the developing fetal immune system. Since Th1 type responses are involved in anti-bacterial and anti-viral immunity, pregnant women are at increased risk of these infections. After giving birth, the maternal immune system must reverse the pregnancy-associated Th2 skewing, while the newborn infant's immune system must develop its Th1 competence. The newborn infant has an immature immune system, and maternal transfer of antibodies and other protective molecules in breast milk is important in protection against infection. The newborn's immune system will develop over the course of months to a few years with acquisition of T cell and B lymphocyte (B cell) function and antibody production and the establishment of balances between Th1 and Th2 cells and between these effector T cells and regulatory T cells.

Breast milk-derived factors play important roles in this early life immune development (see next section) but exposure to antigens (from microbes and from foods) is also important, as is the acquisition of the infant gut microbiota. In turn, this is affected by the birthing process, by contact with maternal skin, by breast milk factors, and by environmental exposures. Ultimately, if an appropriate combination of immune maturation factors has been present, the infant develops an effective and balanced immune system that affords both protection against pathogens and tolerance of harmless environmental exposures. Conversely, impaired immune development leading to poor cellular responses or on-going immune imbalances (e.g. between the Th1 and Th2 systems) can result in enhanced infant susceptibility to infections or in development of immune-mediated diseases like food allergies.

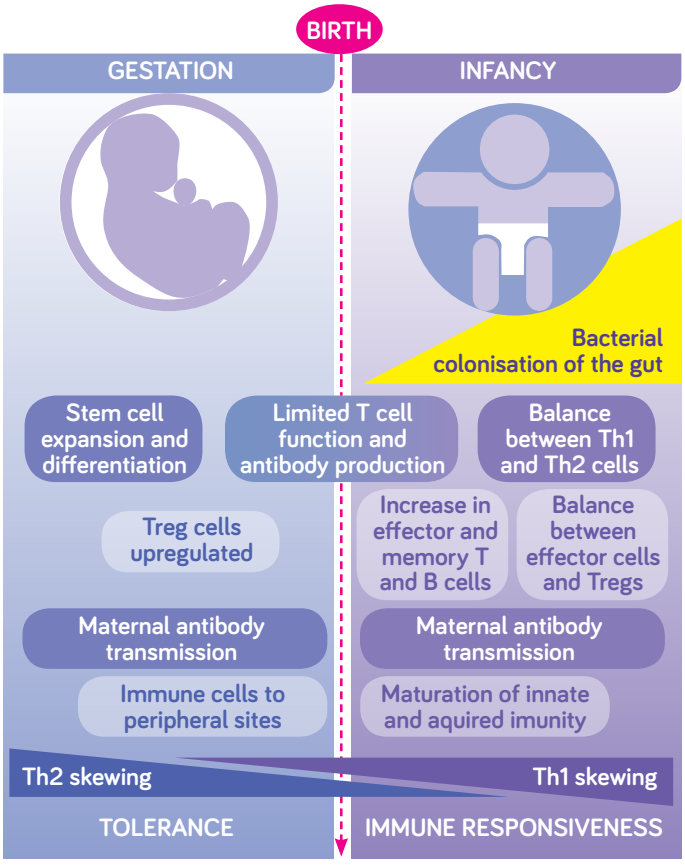

Fig 1. Overview of early life immune development

IMPORTANCE OF BREAST MILK FACTORS TO IMMUNE DEVELOPMENT

Human breast milk contains immune cells and numerous immune-active molecules and immune maturation factors[1,2]. These include immunoglobulins (Igs) like IgG, IgM and secretory IgA; anti-bacterial proteins like lactoferrin, lysozyme and complement C3; anti-viral mucins; many cytokines and growth factors; nucleotides and oligosaccharides. Some of these factors are also involved in promoting a healthy gut microbiota. The co-development of a healthy gut microbiota and a well-functioning immune system seems likely to be promoted through the dual action of breast milk derived factors. Human breast milk also contains long-chain polyunsaturated fatty acids (LCPUFAs) which have roles in the immune system. Breast feeding protects against childhood infections and may protect agains childhood allergies, and it may be that part of this protection is due to enhanced immune development in breast-fed infants.

LONG-CHAIN POLYUNSATURATED FATTY ACIDS (LCPUFAs)

In the context of human breast milk and infant development, LCPUFAs are considered to be the 20 and 22 carbon chain polyunsaturated fatty acids. These are members of the omega-6 (n-6) and omega-3 (n-3) fatty acid families. The major n-6 LCPUFA is arachidonic acid (AA) while the major n-3 LCPUFAs are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These LCPUFAs are synthesised from precursor essential fatty acids (linoleic acid and alpha-linolenic acid, respectively) using the pathway depicted in **Fig 2**. The essential fatty acids are consumed in the diet from seeds, nuts, vegetable oils and vegetable oil-based spreads. AA can be consumed from meat and eggs, while EPA and DHA are consumed from seafood, especially fatty fish, and from supplements. AA and DHA are the main LCPUFAs in human breast milk[3] and have important roles in infant visual and cognitive development[4].

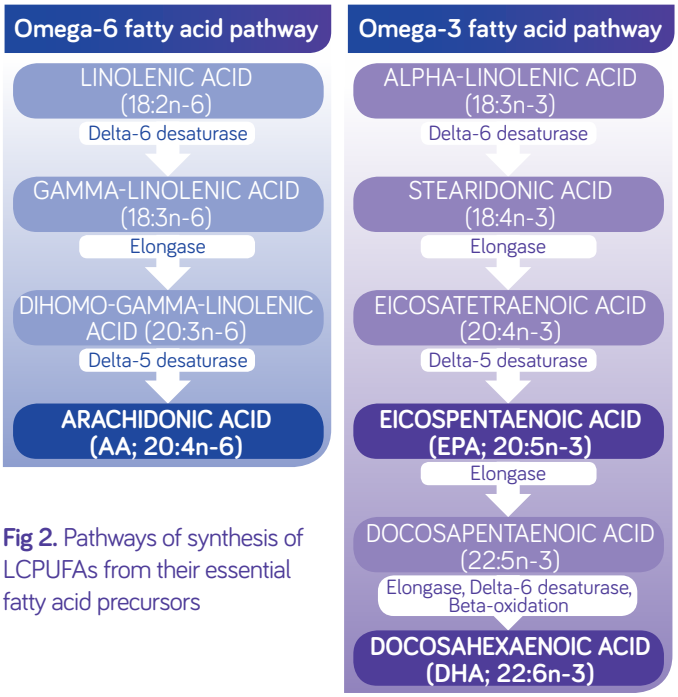

Fig 2. Pathways of synthesis of LCPUFAs from their essential fatty acid precursors

Immune cell membranes contain AA, EPA and DHA. Amongst the LCPUFAs, AA is usually the most abundant. DHA is usually the most abundant n-3 LCPUFA. Because of their highly unsaturated nature, LCPUFAs influence the physical nature of cell membranes (sometimes called membrane fluidity) and influence the function of membrane proteins, including their ability to move within membranes to form signalling platforms[5]. Hence, LCPUFAs modulate intracellular signalling within immune cells ultimately affecting transcription factor activation and gene expression[5]. Thus, LCUFAs have been reported to have roles in regulating the function of neutrophils, monocytes, macrophages, antigen-presenting cells, T-cells and B-cells[6]. Perhaps the best described function of LCPUFAs with regard to immune function, including the inflammatory component, is their role as substrates for the generation of bioactive lipid mediators (**Fig 3**). AA gives rise to prostaglandins, thromboxanes and leukotrienes that have roles in regulating immune cell function, including of antigen-presenting cells, T-cells and B-cells[7]. These mediators are also involved in inflammation and several AA-derived mediators including prostaglandin D2 and several leukotrienes are involved in the allergic response[7]. EPA is also a substrate for synthesis of prostaglandins, thromboxanes and leukotrienes, but these tend to be quite weak[8]. However, both EPA and DHA are substrates for a range of lipid mediators together termed "specialised pro-resolving mediators" (**Fig 3**)[9,10,11]. These include E- and D-series resolvins, protectins and maresins. SPMs are anti-inflammatory and inflammation resolving[9,10,11]. When the biological actions of the different lipid mediators formed from the n-6 LCPUFA AA and from the n-3 LCPUFAs EPA and DHA are considered it would seem that a balanced supply of precursors would be important in order to achieve "optimal" immune cell membrane contents of the various LCPUFAs, although what exactly constitutes "optimal" is currently unclear. It is known that increased intake of EPA and DHA (in adults) results in higher immune cell contents of those LCPUFAs and a lowered content of AA[8,12].

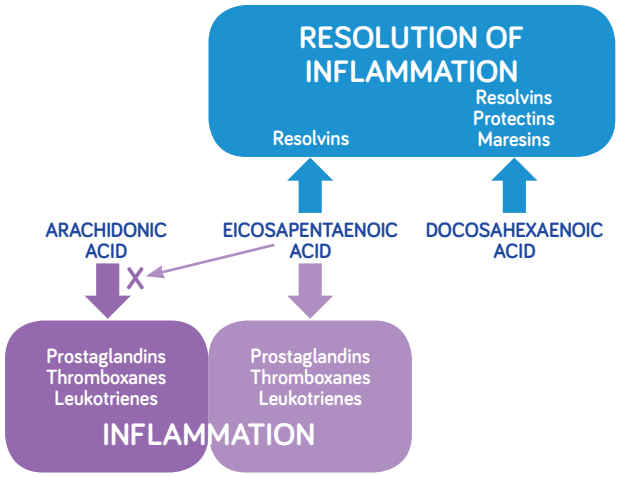

Fig 3. The role of LCPUFAs as substrates for lipid mediators involved in inflammation and its resolution

LCPUFAS AND EARLY LIFE IMMUNE DEVELOPMENT

A number of studies of increased intake of n-3 LCPUFAs in pregnancy or in pregnancy and lactation have reported reduced risk of allergic outcomes (e.g. skin prick test positivity, allergen specific IgE, food allergy, atopic dermatitis, wheeze/asthma) in infants and young children[13,14], suggesting that early exposure to n-3 LCPUFAs reprograms the immune response. A study with n-3 LCPUFAs in Thai schoolchildren reported less respiratory illness[15]. There are few trials of LCPUFA intervention in infants with immune system follow-up. Field *et al.*[16] compared human milk with standard formula or standard formula with added AA (0.49% of fatty acids) and DHA (0.35% of fatty acids) in 44 preterm infants. The duration of intervention was 42 days. An age-related increase in T-helper cells and in B-cells was seen in the human milk and the formula + LCPUFA groups

but not in the standard formula group. Likewise, in the human milk and formula + LCPUFA groups more T-cells were antigen mature at day 42 and fewer were antigen naïve. Production of the regulatory cytokine interleukin 10 was not different in the human milk and formula + LCPUFA groups but was low in the standard formula group. Conversly, production of the pro-inflammatory cytokine tumour necrosis factor was not different in the human milk and formula + LCPUFA groups but was lower than in the standard formula group. These findings suggest that adding AA and DHA to formula results in an immune profile that is consistent with that seen with human milk, suggesting that some of the immune effects of breast milk are due to its component LCPUFAs. This same group of researchers enrolled 30 term infants into a trial of formula compared with formula + AA (0.34% of fatty acids) and DHA (0.20% of fatty acids)[17]. The infants were aged 2 weeks and the duration of the trial was 4 weeks. There was also a breast-fed comparator group. Compared to the formula-fed infants, the infants fed formula + LCPUFAs had a blood immune cell distribution and a blood cytokine profile that did not differ from those of breast-fed infants. Danish researchers randomised 64 term infants to cows milk or infant formula each without or with added n-3 LCPUFAs (~570 mg EPA and 380 mg DHA daily) from age 9 to 12 months[18]. There was no difference between groups in plasma IgE, C-reactive protein, soluble interleukin 2 receptor or fecal IgA. However, n-3 LCPUFAs resulted in enhanced interferon-gamma production of whole blood cultures stimulated with *Lactobacillus paracasei*. Interferon-gamma is a Th1 type cytokine involved in anti-bacterial and anti-viral immunity. The authors concluded that their results indicated better immune maturation in infants given additional n-3 LCPUFAs. Taken together these three studies indicate that LCPUFAs result in an improved immune response in young infants, although it is not clear if both n-6 and n-3 LCPUFAs need to be present to achieve this effect. None of these studies investigated response to vaccination, infections or immune-mediated illnesses. Nor did they investigate the persistence of the immune effects reported beyond the end of the intervention period. Two trials in infants investigated illness outcomes in infants who received LCPUFAs in infant formula[19,20]. Birch et al.[19] followed up term infants who received standard formula ($n = 51$) or formula + LCPUFAs (AA 0.64-0.72% of fatty acids and DHA 0.32-0.36% of fatty acids) ($n = 38$) in two different studies. Infants had received the formulas from age < 6 days to 12 months and they were followed up to 3 years of age. Infants who had received LCPUFAs had much reduced risk of wheezing/asthma, wheezing/asthma plus atopic dermatitis, any allergy and upper respiratory tract infection. In another study[20], term infants received standard formula ($n = 248$) or formula + LCPUFAs (AA 0.64% of fatty acids and DHA 0.32% of fatty acids) from age ~ 1 month until one year. Infants who were receiving LCPUFAs were less likely to develop bronchitis or bronchiolitis at 5, 7 and 9 months. These two studies suggest that immune effects of LCPUFAs (e.g. promoting an enhanced balance between Th1 and Th2 responses) have benefit in protecting against allergic and respiratory disorders early in life.

SUMMARY AND CONCLUSIONS

The immune system is complex, involving many cell types and numerous chemical mediators and immune balances are vital to health. The immune system develops in early life and breast feeding promotes immune maturation and protects against infections and allergies. LCPUFAs are found in breast milk and influence immunity (including the inflammatory component) through multiple interacting mechanisms. Immune markers in preterm and term infants fed formula + LCPUFAs (AA+DHA) were similar to those in human milk fed infants, whereas those in infants fed formula without LCPUFAs were not. Infants who received formula + LCPUFAs show a lower risk of allergic disease and respiratory illness than infants who received standard formula. These findings suggest that LCPUFAs can play a role in immune development that is of clinical significance. The number of human studies in this area is limited and the separate effects of AA compared with n-3 LCPUFAs are not clear. The contribution of LCPUFAs to the immune benefits of breast milk is also unclear. Indeed, whether the effects of LCPUFAs delivered in breast milk, which contains many other immune active components, are the same as LCPUFAs delivered in formula, which lacks many of those components is not known.

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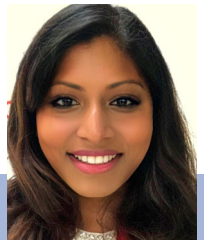
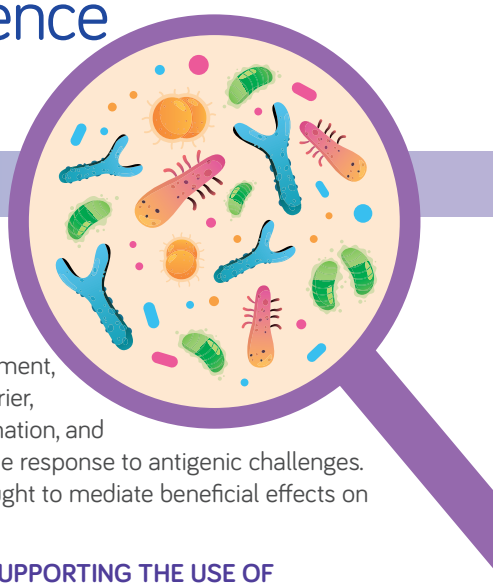
Or to read more about remote working practices for sustainability on our blog **Who Knew...The Doctor will now see you via video**



An update on the latest evidence on the use of probiotics

Bahee Van de Bor Specialist Paediatric Dietitian



One of the most significant discussions in the area of gut health today is probiotics. The past decade has seen a rapid growth in publications in this area encouraging its use in many conditions in children. However, given the wide number of probiotics available and limited data on dose and strain type used in the literature, it's not surprising that the health-care provider is often faced with uncertainties about whether or not to use probiotics and which one(s) to recommend[1].

WHAT ARE PROBIOTICS?

Probiotics are live microorganisms composed of non-pathogenic bacteria and fungi[2]. As they already occupy the human digestive system, they are considered to be generally safe to take and, when administered in adequate amounts, are thought to confer a health benefit on the host[3].

Probiotics are available either as single or multi-strain preparation in varying doses. To date, two hypoallergenic infant formulas containing probiotics are also available on prescription, for infants with diagnosed cow's milk allergy.

However, for children with co-morbidities that may modulate their immune function, health care professionals should assess the strength of evidence and risk to benefit ratio for each child before recommending probiotics.

WHAT ARE PREBIOTICS?

Prebiotics are dietary substances (mostly consisting of non-starch polysaccharides and oligosaccharides poorly digested by human enzymes) that nurture a selected group of microorganisms living in the gut. They favour the growth of beneficial bacteria over that of harmful ones.[4]

Prebiotics affect intestinal bacteria by increasing the numbers of beneficial anaerobic bacteria and decreasing the population of potentially pathogenic microorganisms.

HOW DO PROBIOTICS WORK (MECHANISMS OF ACTION)[4]

Probiotics affect the intestinal ecosystem by impacting mucosal immune mechanisms, by generating metabolic end products such as short-chain fatty acids, and by communicating with host cells through chemical signalling (**Fig.1**).

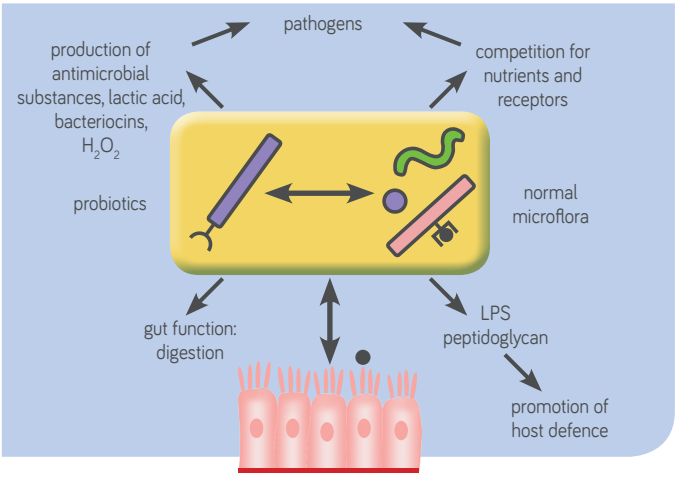


Fig 1. Mechanisms of probiotics

These mechanisms can lead to antagonism of potential pathogens, an improved intestinal environment, bolstering the intestinal barrier, down-regulation of inflammation, and up-regulation of the immune response to antigenic challenges. These phenomena are thought to mediate beneficial effects on the host.

WHAT'S THE EVIDENCE SUPPORTING THE USE OF PROBIOTICS?

Systematic reviews are available describing both the benefits and drawbacks when using probiotics in certain disease settings. It is also evident that the benefits of probiotics are strain specific, so it is essential that health care professionals know which strains to recommend and the quality of evidence supporting its use.

In contrast, adverse events associated with probiotics have been documented in the literature. Currently probiotics are not recommended as a new treatment for critically ill patients or if they are immunocompromised[5].

This article will focus on the few areas within paediatrics where the use of probiotics is supported with scientific evidence.

Disease and condition specific recommendations

ACUTE AND ANTIBIOTIC RELATED DIARRHOEA

Lactobacillus rhamnosus GG is one of the top probiotic strains shown to reduce the risk of infectious and antibiotic diarrhoea in healthy children. In a systematic review, combined data from 11 RCTs showed that *rhamnosus GG* was effective in reducing the duration of diarrhoea in European and non-European settings when used at a daily dose of $\geq 10^{10}$ colony-forming units (CFU)[5].

The ESPGHAN Working Group for Probiotics and Prebiotics[6] concluded that *rhamnosus GG* can be considered as an adjunct to rehydration therapy. Note, as of April 2020, *L. rhamnosus* has been officially reclassified to *Lacticaseibacillus rhamnosus* so the full strain name may also be referred to as *Lacticaseibacillus rhamnosus GG*®[7].

Similarly, a collection of RCTs in healthy children concluded that *Saccharomyces boulardii CNCM I745* has been effective in reducing the duration of diarrhoea by one day and also in the risk of developing diarrhoea in day 3.

Due to the lack of reliable evidence, other strains cannot be recommended in the management of diarrhoea.

Note that the AGA clinical guidelines[8], published in 2020, stated that "while there was evidence for probiotics in the prevention of *C. difficile*, the technical review found significant knowledge gaps in the use of probiotics in treatment of *C. difficile* and recommend this as an area for further study."

Table 1. Probiotics and their proposed clinical benefit in acute and antibiotic related diarrhoea

Strain	Proposed Clinical benefit	Dose
Lacticaseibacillus rhamnosus GG®	Infectious diarrhoea Treatment of gastroenteritis	≥ 10^{10} CFU/day (typically 5– 7 days)
Lacticaseibacillus rhamnosus GG®	Prevention of antibiotic-associated diarrhoea	1–2 × 10^{10} CFU (1 day)
Lacticaseibacillus rhamnosus GG®	Acute otitis media and upper respiratory infections in basically healthy children.	-
Saccharomyces boulardii	Prevention of antibiotic-associated diarrhoea	250–500 mg
Saccharomyces boulardii CNCM I745	Infectious diarrhoea Treatment of gastroenteritis	250–750 mg/day (typically 5– 7 days)
Lactobacillus reuteri DSM 17938	Infectious diarrhoea Treatment of gastroenteritis	10^{8} to 4 × 108 CFU (typically 5–7 days)
Lactobacillus casei DN-114 001 in fermented milk	Infections in children attending day-care centres	10^{10} CFU, once daily
Lactobacillus casei Shirota in fermented milk	Infections in children attending day-care centres	10^{10} CFU, once daily

NECROTISING ENTEROCOLITIS

Necrotising enterocolitis (NEC) remains a leading cause of mortality and morbidity in premature and very low birthweight infants. The aetiology of NEC is multifactorial, but the development of an abnormal gut microbiota is considered an important predisposing risk factor[9].

According to the World Gastroenterology Organisation Global Guidelines[4] there are no specific recommendations on which probiotic strains to use in preterm infants.

Despite this, a 2012 meta-analysis[10] updated in 2017 to include 25 RCTs and >7000 neonates, showed strong evidence for using multispecies probiotics to reduce NEC incidence (pooled OR = 0.36, 95% CI 0.24 to 0.53, p<0.00001), and associated mortality (OR = 0.58, 95% CI 0.43 to 0.79, p = 0.0006).

Yet, at present, most UK centres do not offer probiotics routinely due to heterogeneity in available studies.

Additionally, in 2020, Robertson *et al* also reported that probiotics administered daily to high-risk neonates in a UK-based neonatal centre reduced the rates of NEC by 50% (from 7.5% to 3.1%) using dual-species *Lactobacillus acidophilus* and *Bifidobacterium bifidum* combination probiotics. From April 2016, the strain *B. longum* subspecies *infantis* was included in the probiotic combination given.

The AGA Clinical Guidelines also recommend the following probiotic strains for low birth weight preterm infants less than 37 weeks. The recommendation is to use a combination of a variety of probiotic strains in the specific combinations below (**Table 2**).

Table 2. Suggested probiotic combinations in preterm infants

Preterm (<35 weeks) of low birth weight	Strain Combination
Combination 1	*Lactobacillus* spp and *Bifidobacterium* spp (L *rhamnosus ATCC 53103* and *B longum subsp infantis*; or *L casei* and *B breve*
Combination 2	*L rhamnosus, L acidophilus, L casei, B longum subsp infantis, B bifidum*, and *B longum subsp longum*
Combination 3	*L acidophilus* and *B longum subsp infantis*; or *L acidophilus* and *B bifidum*
Combination 4	*L rhamnosus ATCC 53103* and *B longum Reuter ATCC BAA-999*
Combination 5	*L acidophilus, B bifidum, B animalis subsp lactis*, and *B longum subsp longum)*
Combination 6	*B animalis subsp lactis (including DSM 15954)*, or *L reuteri (DSM 17938 or ATCC 55730)*
Combination 7	*L rhamnosus (ATCC 53103* or *ATC A07FA* or *LCR 35*)

In contrast, a brand-new systematic review[11] concluded that most strains were unfortunately only studied once or a few times. For this reason, the authors were unable to recommend precise probiotic strains or doses without further large adequately powered RCTs in premature infants. They added 'the number of reports in the most preterm neonates was very limited. Furthermore, it was not possible to determine optimal probiotic dosages, time of initiation, and duration of treatment course.'

Nevertheless, the authors commented that both *L. rhamnosus GG* and *B. lactis Bb-12/B94* appeared to be effective in reducing NEC. In addition, *B. longum BB536* showed a clear trend towards a similar effect. However, both the combination of *L. rhamnosus GG* with *B. longum BB536* and the combination of *B. lactis Bb-12* with *B. longum BB536* showed no measurable effect. The authors attributed this to a possible antagonistic effect of *B. longum BB536* together with the other two strains, or the relatively poor evidence base on which the study's network meta-analysis was built. In addition, the authors also commented that only *L. rhamnosus GG* simultaneously administered with *B. longum BB536* was able to reduce full enteral feeding significantly (based on one study with 94 infants studied)[12], whereas *L. rhamnosus GG* alone did not.

MANAGEMENT OF OTHER GASTROINTESTINAL DISORDERS

In conditions such as ulcerative colitis, Crohn's disease and irritable bowel syndrome (IBS), no recommendations for probiotics were made by AGA apart from in the context of clinical studies[8].

Although RCTs have been completed in children with IBS with benefits reported, sample sizes were small, IBS sub-types not identified and strain combinations used differed across studies. The main beneficial outcome reported was in abdominal pain scores when *rhamnosus GG* or the 8-combination strains in *VSL#3* was used[1].

In pouchitis, the AGA recommend the use of the 8-strain combination of *L. paracasei subsp paracasei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii subsp bulgaricus, B. longum subsp longum, B. breve, B. longum subsp infantis,* and *S. salivarius subsp thermophilus* over no or other probiotics[8].

ATOPIC DERMATITIS (AD)

Atopic dermatitis, is the most common form of eczema. It is also one of the most common chronic inflammatory skin disorders, affecting approximately 15% to 20% of children worldwide[13].

In the last two decades, the use of probiotics for the prevention of allergies in children has been extensively investigated in many randomised controlled trials.

Despite the early findings from a meta-analysis concluding that the evidence was more convincing in the probiotics' efficacy in prevention than in the treatment of paediatric atopic dermatitis[14], since then, both the European Academy of Allergy and Clinical Immunology and the American Academy of Paediatrics have stated that there is still no evidence to support the use of probiotics for the prevention of food allergy and anaphylaxis[15,16].

Despite prior evidence and expert recommendations, the more recent systematic review in 2015 by Zuccotti *et al*[17] refute these findings. It concluded that probiotics were effective in preventing infantile eczema when taken during pregnancy and when given orally to infants under 3 months of age.

Table 3. Systematic review findings on use of probiotics in Atopic Dermatitis (AD)

Systematic Review	Findings
Lee *et al*[14]	*Rhamnosus GG* had a positive effect on the prevention of AD.
Pelucci *et al*[18]	*Rhamnosus GG* during pregnancy and early infancy, showed a 20% statistically significant reduction in AD incidence.
Dang *et al*[19]	14 randomized, double-blind, placebo-controlled trials showed 31% reduction in the incidence of eczema.
Mansfield *et al*[20]	26% (18%–33) reduction in AD when taken from pregnancy until 6 months post. 52% (45%–69%) when the probiotic supplement included *Lactobacillus paracasei.*
Li *et al*[21]	*B. longum* (OR 0.39; 95% CI 0.18–0.83) or *B. breve* (OR 0.73; 95% CI 0.62–0.86) but *L. rhamnosus* with *L. paracasei* seemed most effective in AD prevention. Administration of probiotics for >12 months after birth was not effective in preventing AD compared with controls (OR 1.10; 95% CI 0.80–1.51).

The research to date has also focused on the prevention of atopic dermatitis using probiotics, please see **Table 3**. Therefore, an exciting development in the area of atopy was discovered in 2017 when a Finnish study[22] showed that a combination of probiotics, which included *rhamnosus GG* and *Bifidobacterium breve Bb99*, resulted in a 26% reduction in eczema at 2 years. This was maintained to 10 years, when given from 36 weeks' gestation and thereafter directly to babies with 0.8g galacto-oligosaccharides (prebiotics) for 6 months.

Similarly, more recently, Wickens *et al*[23] showed that *rhamnosus HN001* can protect against the development of eczema and atopic sensitization until at least age 11 years. This extends their previous findings of protection from age 6 years to 11 years, please see **Table 4**. *Rhamnosus HN001* was given at 6×10^9 colony-forming units from 35-week gestation to 6 months' post-partum in mothers while breastfeeding and from birth to age 2 years in infants.

Table 4. Probiotics and their proposed clinical benefit in eczema

Strain	Proposed Clinical benefit	Dose
Lacticaseibacillus rhamnosus GG®	Prevention of childhood eczema, hay fever	5×10^9 CFU, twice daily
Lactobacillus rhamnosus HN001	Prevention of childhood eczema and atopic sensitization	6×10^9 CFU
L. rhamnosus and *L. paracasei*	Prevention of childhood eczema	5×10^9 CFU
B. longum	Prevention of childhood eczema	
B. breve	Prevention of childhood eczema	

In conclusion, the overall evidence for the use of probiotics in the prevention of AD is positive with certain strains showing additional efficacy over others.

COW'S MILK PROTEIN ALLERGY (CMA)

Cow's milk allergy is one of the most commonly reported childhood food allergies, with increasing incidence, persistence and severity in many countries across the world[24].

There is growing interest in the potential role of the gut microbiota in earlier acquisition of cow's milk protein tolerance. Studies suggest that the intestinal microbiota may modulate immunologic and inflammatory systemic responses and so influence the development of sensitisation and allergy[25].

Several birth cohort studies have also shown altered gut microbiota, or dysbiosis, in allergic infants compared to healthy infants, as well as specifically in CMA[26], with a suggestion that the gut microbiota of infants with allergic conditions typically have low levels of *Bifidobacteria* and *Lactobacilli* compared with healthy infants[27].

So far, a prospective study of 260 children with cow's milk protein allergy showed that an extensively hydrolysed casein formula (EHCF) accelerated tolerance acquisition compared with other types of formula (hydrolysed rice formula; soy formula; and amino acid-based formula) and this effect was even greater with *Lactobacilli GG*[28]. However, note that children requiring amino acid-based formula typically have severe allergy and will therefore unsurprisingly take longer to acquire cow's milk tolerance.

Furthermore, a recent study that randomly allocated 220 children (median age 5.0 months) with suspected IgE-mediated CMA to either EHCF or EHCF + *LGG* showed reduced incidence of other allergic manifestations, including eczema and asthma, and hastened the development of oral tolerance in the probiotic-supplemented formula. There were also no differences in growth between the study groups[29].

A second strain called *Bifidobacteriumn breve M-16 V* has been examined in a several studies due to its natural presence in breastmilk. A trial of 90 infants under seven months of age compared infants who received extensively hydrolysed formula containing synbiotics (*Bifidobacteriumn breve M-16 V* enriched extensively hydrolysed formula with short chain galacto-oligosaccharides and long chain fructo-oligosaccharides) or the same formula without prebiotics for 12 weeks[30]. Although there was no difference in severity of atopic dermatitis between the groups, in the subgroup of infants with IgE-associated AD (n = 48), (SCORing Atopic Dermatitis) SCORAD score improvement was significantly greater in the synbiotic than in the placebo group at week 12[30]. Fewer children in the synbiotic group also reported episodes of dry stools (OR: 0.20, 95% CI 0.08–0.50, P = 0.001), constipation (P = 0.01) and diaper dermatitis (OR: 0.24, 95% CI: 0.08–0.68, P = 0.008). The study also showed that synbiotics favourably and significantly modulate the composition and metabolic activity of the intestinal microbiota of these children. This potentially explains the beneficial effects on constipation and diaper dermatitis prevalence observed. The synbiotic mixture was also well tolerated without adverse events.

Other benefits of amino acid (AA) formula inclusive of synbiotics was reported in the study of Fox *et al*[31]. Within 8 weeks of babies starting AA with prebiotics and *Bifidobacterium breve*, the synbiotic group had higher levels of bifidobacteria and lower *Eubacterium rectale/Clostridium coccoides* in their stools at percentages close to levels seen in age-matched healthy breastfed babies.[32] The synbiotic group also benefited from significantly reduced use of antibiotics and dermatologicals, as well as significantly fewer ear infections compared to the control group.

CONCLUSION

Current evidence demonstrates that probiotics and synbiotics are safe for children, including for preterm and term infants. Nevertheless, healthcare professionals should review the strength of evidence for individual or combinations of probiotic strains for the desired health outcomes in the paediatric population. Future larger and higher powered RCTs are still required to strengthen current findings, particularly on the precise dose and strain, or combination of strains, required to manage the various childhood health conditions described in this article.

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Up2 Date ...

An extensively hydrolysed synbiotic-containing formula improves gastrointestinal outcomes in infants with non-IgE cow's milk protein allergy, already well-established on extensively hydrolysed formula.

K Atwal, *et al.* *Poster Presentation. European Academy of Allergy and Clinical Immunology Food Allergy and Anaphylaxis Meeting 2020.*

BACKGROUND

Modulating the dysbiosis commonly seen in infants with cow's milk protein allergy (CMPA) may offer promising long-term health benefits. Infants with CMPA often present with vomiting, abdominal discomfort, constipation and/or diarrhoea amongst other manifestations of their allergy. Evidence indicates that pre- and probiotics (synbiotics) help normalise gut microbiota and improve gastrointestinal symptoms in infants with CMPA. This study evaluated gastrointestinal outcomes after commencing a new synbiotic extensively hydrolysed formula (SeHF) containing prebiotics (galacto-oligosaccharides (GOS)/ fructo-oligosaccharides (FOS)) and probiotics (B.breve M-16V) for 4 weeks in infants with CMPA.

METHOD

In this prospective, single-arm study, 25 infants (mean age 29.9±10.2 weeks, range 13.9-53.4) with non-IgE mediated CMPA conducted a 3-day baseline on existing eHF (mean intake 663±219ml/d) followed by a 4 week intervention with SeHF ((Aptamil Pepti Syneo, Nutricia), mean intake 614±223ml/d). Gastrointestinal symptom severity, stool frequency and consistency (using the Bristol Stool Chart) were assessed at baseline and at the end of study. Dietitians were also asked if they were satisfied with perceived tolerance of SeHF after intervention at the end of study. All data were analysed using SPSS (v24, IBM corp.) where paired samples t-tests were used for continuous data and non-parametric tests (Wilcoxon signed rank & Friedman tests) were used for ordinal data.

RESULTS

Significant reductions in constipation (p=0.046), abdominal pain (p=0.008), flatulence (p=0.017) & burping (p=0.05) were observed at the end of the study after SeHF use. An improvement in stool consistency was observed after study with SeHF (baseline type 6 Bristol Stool Chart score to type 5 at end of study, NS). There were no differences in stool frequency between baseline (mean 2.2±1.2/d) and end of study (mean 1.8±1.2/d, NS). 92% of dietitians agreed the SeHF was well tolerated by patients at the end of the study.

CONCLUSION

This study demonstrates that an extensively hydrolysed synbiotic-containing formula further improves gastrointestinal outcomes in infants with non-IgE mediated CMPA who are already established on an eHF. This supports existing evidence for the benefits of the addition of GOS, FOS & B. breve M-16V into hypoallergenic infant formulae, being safe and improving health outcomes.

Nutrition and the Immune System: A Complicated Tango

Venter, C., *et al.* *Nutrients,* 2020;**12**(3),818.

BACKGROUND

The interactions between the immune system and nutrition are varied and complex. The immune system impacts metabolism, and nutritional status influences the immune system, adding more complexity via this bi-directional relationship. This review aimed to investigate the current literature exploring this relationship between nutrition and the immune system.

EXISTING EVIDENCE

A mixture of human intervention, observational and very few randomised control trials are available, which investigate diet and immune system. Diet's role in noncommunicable disease has been investigated in many diseases with clearly defined immunopathologic processes, providing insight into the impacts of certain dietary components on the immune system. A European Academy of Allergy and Clinical Immunology (EAACI) position paper on allergic disease and diet exists suggests diet diversity may be associated with reduced allergic outcomes in childhood. This is thought to be due to the role diet diversity plays on the immune system and microbiome. The position paper discusses overall diet diversity and individual nutrients e.g. long-chain polyunsaturated fatty acids (LCPUFAs). Multiple studies investigating the role of fibre intake in disease modulation have been completed, showing the use of fibres in disease prevention.

FUTURE

The current evidence is limited and needs further investigation to learn more about diet, immune system and microbiome interactions, which play a role in immune-mediated pathologies. Studies need to focus on the interactions of multiple nutrients, as current evidence focusses on individual nutrients which doesn't factor in the interactions of these between each other. Therefore, the future of nutrition research would benefit greatly from randomised control trials investigating food patterns and whole diets.

CONCLUSION

Current evidence on single nutrients e.g. LCPUFAs and fibre and their effect on the immune system exist and can be used to make disease recommendations with confidence. However, to have a more broad understanding of the effect of diet on the immune system, multi-nutrient studies investigating overall food patterns need to be completed.

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