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REVIEW

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Impact of dietary fibers in infant formulas on gut microbiota and the intestinal immune barrier

Chunli Kong, * Marijke M. Faas, Paul de Vos and Renate Akkerman

Human milk (HM) is the gold standard for the nutrition of infants. An important component of HM is human milk oligosaccharides (hMOs), which play an important role in gut microbiota colonization and gut immune barrier establishment, and thereby contribute to the maturation of the immune system in early life. Guiding these processes is important as disturbances have life-long health effects and can lead to the development of allergic diseases. Unfortunately, not all infants can be exclusively fed with HM. These infants are routinely fed with infant formulas that contain hMO analogs and other non-digestible carbohydrates (NDCs) to mimic the effects of hMOs. Currently, the hMO analogs 2'-fucosyllactose (2'-FL), galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), and pectins are added to infant formulas; however, these NDCs cannot mimic all hMO functions and therefore new NDCs and NDC mixtures need to become available for specific groups of neonates like preterm and disease-prone neonates. In this review, we discuss human data on the beneficial effects of infant formula supplements such as the specific hMO analog 2'-FL and NDCs as well as their mechanism of effects like stimulation of microbiota development, maturation of different parts of the gut immune barrier and anti-pathogenic effects. Insights into the structure-specific mechanisms by which hMOs and NDCs exert their beneficial functions might contribute to the development of new tailored NDCs and NDC mixtures. We also describe the needs for new *in vitro* systems that can be used for research on hMOs and NDCs. The current data suggest that "tailored infant formulas" for infants of different ages and healthy statuses are needed to ensure a healthy development of the microbiota and the gut immune system of infants.

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1. Introduction

Human milk (HM) is the gold standard for infant nutrition.¹ It contributes to a healthy development of infants after birth. The composition of HM is highly variable and is believed to be evolutionary optimized to meet the needs of infants at different ages and stages of development.² HM therefore varies in composition during lactation and provides infants with all essential nutrients they need in their first months of life.³ HM is composed of various amounts of proteins, lipids and digestible and indigestible carbohydrates.⁴ Indigestible carbohydrates are predominantly human milk oligosaccharides (hMOs), which are the third most abundant solid component in HM after lactose and lipids.⁵ hMOs are important bioactive components of HM and have recently gained a considerable amount of attention due to the prebiotic effects they exert and their ability to guide the development of a stable microbiota

community and a healthy mucosal immune system in infants.^{6,7}

The colonization of infants' gastrointestinal tract (GIT) by microbiota is a critical and very vulnerable period in life and is influenced by various genetic and environmental factors.^{8,9} The development of a stable microbiota composition contributes to the development of an effective gut barrier and a balanced mucosal immune system. Any disturbance in the microbiota colonization, for example, by the use of antibiotics or diet changes may not only lead to immune-related diseases such as allergies but also to inflammatory bowel disease (IBD).^{10–12} This illustrates the importance and intertwined relationship between the microbiome and the host immune system. As hMOs are resistant to digestion in the upper GIT, they can reach the large intestine and stimulate the growth of key members of the microbiota such as *Bifidobacteria* and *Lactobacillus*.^{13–15}

In addition to the microbiota stimulating effects, hMOs also directly influence the development of the infants' mucosal immune system and gut barrier maturation.^{16,17} The gut barrier, consisting of resident microbiota, a firm mucus layer, the epithelium, and the underlying immune system together provide protection against various pathogens and toxins in the

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lumen of the gut.¹⁸ Infants are born with a relatively immature immune system, which is characterized by a loose epithelial barrier and a weak response to pathogenic stimuli.¹⁹ hMOs are known to exert direct immune modulatory effects on different cell types in the infants' GIT and thereby contribute to the maturation of the gastrointestinal immune system.^{20,21} Furthermore, hMOs can improve the barrier function by modulating mucus secretion and interaction with the epithelial lining of the gut.^{22,23} In this way, hMOs ensure a healthy and efficient development of the infants' gut immune system.²

hMOs are unique for HM and not found in the same compositions or complexity as milk of other species.²⁴ However, in specific cases, infants cannot be fed with HM, for example, when milk production is not sufficient to feed an infant, or when the mother has a disease that can possibly be transmitted through HM. Even though it was recently demonstrated that the currently emerging SARS-CoV-2 (COVID-19) is not transmitted through HM,²⁵ there are studies showing that in case of other viral infections like *M. tuberculosis*, *T. pallidum*, and *B. burgdorferi*, breastfeeding is not advisable.^{26,27} In these cases, and for many other valid reasons, infant formulas are the first option for the nutrition of newborn infants. Infant formulas, which are often cow milk derived, are nowadays often supplemented with hMO analogs or other non-digestible carbohydrates (NDCs) to substitute for some hMO functions.²⁸ Galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) are already broadly added to infant formulas, either individually or combined in a typical ratio of 9 : 1.^{29,30} Pectins are also of interest for supplementation in infant formulas.³¹ The production of synthetic hMOs is still challenging and very expensive; however, the hMO analog 2'-fucosyllactose (2'-FL) can be produced in sufficient quantities and therefore there are already infant formulas containing 2'-FL on the market. Addition of these NDCs to infant formulas has been proven effective for the prevention of allergic diseases and the development of a stable and breastfed-like microbiota composition.^{7,32} However, there are also other NDCs and NDC combinations available that are of potential interest as supplements for infant formula. Insight into the mechanisms by which NDCs exert their beneficial effects on microbiota and immunity might contribute to the development of new and more tailored mixtures of NDCs for specific groups of infants like preterm or disease-prone infants.

In this review, we review and discuss the current insights into the development of microbiota compositions in differently delivered neonates, the composition of the gastrointestinal immune barrier and subsequently how hMOs and currently applied NDCs can support maturation and differentiation of the gut microbiota and immune barrier. We will give an update on currently known hMO functions and the effects of the hMO analog 2'-FL and various NDCs on the regulation and modification of gut microbiota compositions, as well as their effects on the gut epithelium and gut immune barrier regulation. This knowledge might contribute to the development of new NDC interventions in infants to support their early life development.

2. The colonization of the infants' gastrointestinal tract by gut microbiota

The human GIT is colonized by a large diversity of microorganisms, collectively called the gut microbiota, which is mainly dominated by the gut bacteria, but also includes protozoans, viruses, and fungi.³³ The first 2–3 years of life are considered to be a critical window for the colonization of the GIT.^{34,35} It is widely believed that this colonization starts right after birth.³⁶ However, there are also studies available suggesting that colonization already starts *in utero*.³⁷ Most studies investigating the colonization period focus on colonization by bacterial species, which is therefore the main focus of this review. There are many factors identified that influence the bacterial colonization of the GIT.³⁸ The mode of delivery is one of those factors that highly impacts the colonization by the initial bacterial species of the gut microbiota.³⁹

Vaginally delivered infants are first colonized by bacteria from the mothers' vaginal, gastrointestinal, and skin microbiome. This is illustrated by a high abundance and diversity of bacteria from the genera *Bifidobacterium*, *Lactobacilli*, and *Bacteroides*, which belong to the phyla Actinobacteria and Bacteroidetes.^{40,41} In contrast, infants delivered through a caesarean section (c-section) are first colonized by the bacteria from the skin and oral microbiota, as well as by species from the surrounding environment.¹⁰ During the first 3 months of life, different bacteria of the phylum Firmicutes, such as *Clostridiaceae*, *Veillonella*, and *Klebsiella*, are abundantly present in the GIT of infants delivered through c-section. In addition, these infants are found to have a low abundance and diversity of Actinobacteria and Bacteroidetes.⁴¹ Notably, many *Bifidobacterium* spp. and *Bacteroides* spp. that are commonly found in vaginally delivered infants are not, or only in a very low abundance, present in c-section delivered infants.⁴²

The diversity in microbiota compositions between vaginally born infants and infants born *via* c-section gradually disappears after 4 to 12 months as a consequence of nutritional intervention.⁴⁰ In a recent longitudinal study, in which children were followed from 5 weeks of age until 6–11 years of age, fecal microbiota analysis illustrated that diet is the most important determining factor for the microbiota composition from 13 weeks onwards.⁴³ In this study, babies that were fed with mother milk developed a different gut microbiota composition compared to babies fed with infant formulas.⁴³ Infants born *via* vaginal delivery and breastfed afterwards tend to have a lower incidence of immunological disorders and a healthier microbiota composition with a high abundance of the beneficial bacteria *Bifidobacterium* spp. and *Lactobacillus* spp., as compared to c-section or infant formula fed infants.^{39,44,45} In a Swedish cohort study, babies fed with mother milk were found to have a high percentage of *Bifidobacterium* and *Lactobacillus* until 12 months of age, while for infant formula fed babies, species belonging to *Clostridia* were more prevalent.⁴⁶ This difference in the early life has large effects on the adult disease, *i.e.* low levels of *Bifidobacterium* and high abundance

of *Staphylococcus aureus* in early life are associated with a high risk of overweight by the age seven.⁴⁷ This illustrates the demand for improving infant formulas to achieve a similar gut microbiota to breastfed babies in early life.

The bacteria present in the infant's GIT play many important beneficial roles in infants. First of all, they play a role in the digestion of food components that are not absorbed in the stomach and in the small intestine like NDCs.⁴⁸ This is realized through special enzymes including glycoside hydrolases, glycosyltransferases, polysaccharide lyases, and carbohydrate esterases secreted by the gut bacteria, which can catalyze specific molecular linkages in the NDCs.⁴⁹ This process results in the formation of short-chain fatty acids (SCFAs) like acetate, propionate, and butyrate that can serve as immune regulatory molecules or as nutrients and an energy source for other beneficial bacteria.⁵⁰ SCFAs also have beneficial effects on intestinal epithelial cells, for example by stimulating their regeneration.⁵¹ In addition, commensal bacteria can synthesize a range of other molecules including vitamin K and vitamin B, which are also involved in host immune cell development.⁵²

As the microbiota compositions in newborn infants are rapidly changing over time, the ability of the gut microbiota to ferment and digest hMOs and NDCs is dependent on the infant's age and the bacterial species present in the GIT.^{43,50} As the complexity of microbiota compositions usually increases upon ageing, the ability of the microbiota to ferment and digest larger and more complex hMOs and NDCs also increases over time.⁴³ This was recently illustrated by a study of Logtenberg *et al.*, which showed that both fermentation capacity by infant fecal inoculum and SCFA production increased between 2 and 8 weeks of age in an *in vitro* batch fermentation of fructan-type NDCs.⁵³ For this reason, also the infant's age should be taken into account for the formulation of new NDC mixtures.

3. Gut immune barrier

The gut immune barrier is formed by the commensal microbiota, the gut mucus layer, the epithelial cell layer, and the resident and recruited immune cells in the lamina propria (Fig. 1). This barrier not only mediates food digestion and nutrient absorption, but it also forms a physical and immune barrier that prevents luminal pathogenic microorganisms, antigens, and detrimental luminal substances from entering the body and provides tolerance to commensal microbiota.¹⁸ In healthy adults almost 70% of the body's immune cells reside in the GIT, making it the largest immunologically active organ of the body.⁵⁴ hMOs and NDCs can impact the gut immune barrier at different levels, but before discussing their impact in the next sections, here the composition of the gut immune barrier will be explained in more detail.

The organization of the gut immune barrier differs between the small and large intestine (Fig. 1). In the small intestine, the immune barrier is highly organized with the presence of the majority of the immune cells in the lamina propria of the

GIT, the epithelial cell layer, and a thin mucus layer and a low abundance of bacteria in the lumen, which allows the direct crosstalk of the food antigens and microbiota with the epithelium and the underlying immune system. The epithelial cells in the small intestine form many finger-like structures, known as the villi, which largely extend the surface area for nutrient absorption and immune sampling.⁵⁵ The so-called crypts, containing intestinal stem cells and Paneth cells, are located at the base of the villi. Stem cells continuously generate new epithelial cells to replenish the cells lining the villi. Paneth cells secrete defensins in response to the invasion of pathogens ensuring a healthy balance in the gut microbiota population.⁵⁶

The immune components of the gut-associated lymphoid tissue (GALT) in the small intestine are organized into specialized globule-like structures called Peyer's patches (PPs) and mesenteric lymph nodes (MLNs) as well as the lamina propria.⁵⁷ The PPs in the small intestine are small lymphoid tissues that harbor many immune cells. They are covered by specialized epithelial cells, the microfold cells (M-cells), that sample antigens from the lumen and present them to the immune cells residing in the PPs. Dendritic cells present under the epithelial lining are also able to sample the luminal content by protruding their dendrites into the lumen.⁵⁸ Because of their antigen presenting function, PPs are considered to be important regulators of immune responses. B-cells are located in the follicles of the PPs and can secrete IgA, which can bind and neutralize pathogens and food antigens. The antigen presenting cells (APCs) in the PPs, like the dendritic cells, can migrate to the MLNs where they can also activate and instruct naïve lymphocytes.⁵⁸ Depending on the antigens presented to the naïve T-cells in PPs, they skew into different subsets, for example into T-regulatory (Treg) cells, which provide tolerance against food antigens and microbiota, or T-helper (Th) cells, that can be induced in response to pathogenic antigens.^{18,59}

The organization of the large intestine is different in several aspects. The epithelial layer of the large intestine has no villi, only crypts and is covered with a thick and firm mucus layer.⁶⁰ As a consequence, there is less direct contact between the luminal content and the mucosal immune system.⁶¹ The mucus layer of the large intestine is composed of two layers: the relatively loose outer mucus layer that harbors the commensal bacteria and the germ-free inner mucus layer. The outer mucus layer is usually made up of the secreted mucins, of which mucin-2 (MUC2) is the major component which is a highly glycosylated mucin produced by the goblet cells that are integrated in the epithelial lining.⁶² Mucins contain similar molecular structures to hMOs and can serve as a substrate for bacterial fermentation and thereby stimulate the growth and colonization of specific bacterial strains.⁶³ In addition, the mucus layer provides protection against intrusion of pathogenic bacteria.

The inner germ-free mucus layer is tightly attached to the intestinal epithelium and is mainly dominated by transmembrane mucins produced by intestinal epithelial cells with

Fig. 1 Schematic representation of the gut immune barrier. The gut immune barrier is organized with the commensal microbiota, mucus layer, the epithelium, and the immune cells in the lamina propria. The large intestine is organized with a higher abundance of commensal microbiota, and a thicker mucus layer compared with the small intestine. There are more immune cells present in the lamina propria of the small intestine due to the presence of Peyer's patches which harbor dendritic cells, B cells, T cells, and macrophages. The finger-like structures known as villi, and crypts in the small intestine extend the surface area for nutrient absorption and antigen sampling. There are only crypts in the large intestine with substantial goblet cells for mucus production.

various glycoproteins.⁶⁴ These glycoproteins form the backbone of the various glycans that build up a glycocalyx layer on the apical side of the brush border covering the intestinal epithelium.⁶⁵ A fully developed and intact glycocalyx layer is of significant importance for the mediation of the interaction between the gut microbes and the host immune system. The intestinal genes responsible for mucin production are already expressed before birth, but the development of a mature and protective mucus layer starts after birth and is of high importance for the establishment of a healthy immune system. Techniques such as glycomics are being explored to unravel the glycan reservoir of the glycocalyx layer to better understand the glycocalyx profiles, functions, and responses to the gut microbiota for giving dietary advice or improved medical treatment.^{66,67}

The intestinal epithelial cells are structurally assembled by intercellular tight junctions (TJ), adherens junctions, and desmosomes, of which TJ proteins are located at the most apical site of the epithelial cells and act as regulators of epithelial barrier integrity.^{68,69} They are also known as targets of immune regulation,^{70,71} as disruption of the TJ interactions will decrease barrier integrity and allow antigens, microbes, antibiotics, and toxins to enter the lamina propria and subsequently induce serious immune responses and gut inflammatory disorders.^{70,71} Many studies have demonstrated that microorganisms and dietary components influence the regulation of the TJ protein expression.^{72,73} Pattern recognition receptors (PRRs), which are widely expressed by the intestinal epithelial cells and intestinal immune cells, play an important role in the regulation of barrier integrity and TJ expression.^{74,75}

Toll-like receptors (TLRs) are the most well-studied PRRs, among which TLR2 and TLR4 are the best studied due to their interaction with gut bacteria and dietary components and their ability to mediate epithelial barrier integrity.^{74,75}

The gut immune barrier in newborn infants is relatively immature and has to develop into the structures described above. A characteristic of an infant is having a lower barrier function which is containing a looser epithelial barrier structure but also by an immature immune response and a microbiome with low diversity in species.¹⁹ Maturation processes of microbiota, barrier and immunity are considered to be under tight control by mother milk components such as hMOs and should therefore also be supported by hMO analogs or NDCs that are added to infant formulas. In the next section, after describing the diversity of hMOs in mother milk, we will review the current insight into how hMO and NDCs in infant formulas contribute to the development of different parts of the gut immune barrier and how it prevents pathogenic infections.

4. Human milk oligosaccharides (hMOs)

hMOs comprise a large family of over 200 different oligosaccharides that are composed of 5 main sugar building blocks: D-glucose (Glc), D-galactose (Gal), N-acetylglucosamine (GlcNAc), L-fucose (Fuc) and sialic acid (NeuAc) (Fig. 2). All hMOs are built up based on a backbone of lactose (Gal β 1-4Glc) as the reducing end, which can be further

Fig. 2 Structures of dietary fibers discussed in this review. (a) Main building blocks of human milk oligosaccharides (hMOs), galacto-oligosaccharides (GOS), and fructo-oligosaccharides (FOS); (b) disaccharide core structure of hMOs; (c) diverse hMO structures can be built by adding lacto-*N*-biose (LNB) or *N*-acetylglucosamine (LacNAc) to the core lactose structure, which can be decorated with fucose or sialic acid; (d) fucosylated and sialylated trisaccharides; (e) lactose can be elongated by galactose to form GOS ($n = 1-7$); (f) adding a certain number of fructose to glucose can make FOS ($n < 10$) or inulin ($n = \text{up to } 200$); (g) pectins include homogalacturonan (HG) and rhamnogalacturonan-I (RGI), HG is a line composed of galacturonic acid backbone, which can be methylated and interrupted with rhamnose. Rhamnose can be further branched with arabinose or galactose to form the more complex pectin structure of RGI.

elongated with two disaccharides lacto-*N*-biose (LNB) and *N*-acetylglucosamine (LacNAc) *via* either the $\beta 1-3$ or the $\beta 1-6$ linkage.⁷⁶ The core structure can also be decorated by Fuc (*via* $\alpha 1-2$, $\alpha 1-3$, or $\alpha 1-4$ linkage) or NeuAc (*via* $\alpha 2-3$ or $\alpha 2-6$ linkage), which forms the fucosylated (FL) or sialylated (SL) hMOs.⁷⁷ hMO profiles are determined by the enzymes expressed in the lactocytes of the mother.⁷⁸ For fucosylated hMOs, $\alpha 1-2$ linked Fuc is added through $\alpha 1-2$ -fucosyltransferase (FUT2), whereas Fuc linked through an $\alpha 1-3$ or $\alpha 1-4$ linkage is added through $\alpha 1-3/4$ -fucosyltransferase (FUT3). The FUT2 enzyme is encoded by the Secretor (*Se*) gene and the FUT3 enzyme is encoded by the Lewis (*Le*) gene. With the possibility of either a positive (+) or a negative (−) expression of the *Se* and *Le* genes, secretion patterns can be divided into four groups: *Se+Le+*, *Se−Le+*, *Se+Le−*, and *Se−Le−*.⁷⁸

The amounts and compositions of hMOs in mother milk change over time, from around 20 to 25 g L^{−1} in the colostrum

to 5 to 20 g L^{−1} in mature milk.⁷⁷ There are studies showing that the hMO profiles in mother milk are influenced by environmental factors and by the feeding practice of a mother. For example, a study by Davis *et al.*, in which hMO profiles of 33 Gambian mothers were analyzed, showed that the amount of hMOs in mother milk increased during the dry season, which could be related to the availability of food.⁷⁹ A Canadian study also showed that other environmental factors including sunlight, climate and allergen exposure influence hMO synthesis.⁸⁰ In the following sections, we describe the beneficial effects of hMOs on the different layers of the GIT in infants (Fig. 3).

4.1 Effects of hMOs on gut microbiota compositions

As mentioned before, hMOs are resistant to digestion in the upper GIT and can reach the large intestine intact. There they can modulate the gut microbiota composition and metabolism of infants in a structure-dependent manner.⁸¹ In general, the

Fig. 3 Schematic representation illustrating the effects of dietary fibers on the gut immune barrier in infants. (a) Dietary fiber can stimulate the growth and colonization of commensal bacteria, and (b) modulate the formation of metabolites, such as SCFAs which can interact with the gut immune system indirectly; (c) dietary fibers can reduce the pathogenic infections by serving as decoy receptors or (d) by increasing the mucus production to strengthen the barrier; (e) dietary fibers can increase the glycocalyx layer to provide more binding sites for commensal bacteria; furthermore, (f) dietary fibers can directly interfere with the antigen sampling by microfold cells (M cells) connecting to the Peyer's patches, (g) dendritic cells, and specialized Toll-like receptors (TLRs) around the absorptive cells (h) to regulate the immune responses; (i) dietary fibers can also regulate the gut permeability.

consumption of hMOs by breastfed infants is associated with a high abundance of many different beneficial *Bifidobacteria* as well as certain *Lactobacillus* species.^{13,82} These bacteria express dedicated glycoside hydrolases (GHs), transporters and other molecules that contribute to the degradation of hMOs. However, the ability to utilize specific hMOs highly differs between species.

For example, Asakuma *et al.* showed that *Bifidobacterium longum* subsp. *infantis* could grow well when cultured in a medium with pooled hMOs isolated from HM as the only carbon source. This was illustrated by a rapid decrease of 2'-FL, 3-FL, LDFT, LNnT, LNT, LNFP I, LNFP II, LNFP III, LNDFH I, and LNDFH II during the experiment, whereas *Bifidobacterium longum* subsp. *longum* and *Bifidobacterium breve* were only able to degrade LNT.⁸³ Thongaram *et al.* tested the ability of the selected *Bifidobacteria* and *Lactobacilli* to degrade individual hMO structures. They found that *Bifidobacterium longum* subsp. *infantis* ATCC 15697 and *Bifidobacterium infantis* M-63 could ferment 3'-SL, 6'-SL, 2'-FL and 3-FL, whereas *B. infantis* strains and *Bifidobacterium breve* ATCC 15700 were only able to ferment LNnT. Among *Lactobacilli*, *Lactobacillus acidophilus* NCFM could most efficiently degrade LNnT.⁸⁴ In a study by Perdijk *et al.*, the hMO sialyllactose (SL) stimulated the growth of *Bifidobacteria* strains, in particular *B. prevotella* and *B. thetaiotaomicron*, but not *Lactobacilli* during an *in vitro* batch fermentation using pooled faecal samples of 1–6-month-old, exclusively breastfed infants.⁸⁵

The difference in the ability to utilize different hMO structures is dependent on the sets of enzymes bacteria are equipped with. Some bacteria are able to internalize hMOs and subsequently deconstruct them using intracellular GHs. Examples of such bacteria are *B. infantis* species. These species were shown to abundantly express enzymes including

α -sialidase, α -fucosidase, β -galactosidases, and β -N-acetylhexosaminidases to degrade a wide spectrum of hMOs including α -sialidase, α -fucosidase, β -galactosidases, and β -N-acetylhexosaminidases.^{50,86} This can result in the formation of monosaccharides that can be released into the cytoplasm of the bacteria as well as in the formation of SCFAs as end products. There are also strains that utilize hMOs using extracellular enzymes. For example, in a study by Nishiyama *et al.*, it was found that *B. bifidum* species could selectively utilize fucosylated or sialylated hMOs using extracellular enzymes, including lacto-N-biosidase (LNBse) and galacto-N-biose (GNB), which are responsible for catalyzing the phosphorolysis of Gal β 1–3GalNAc and Gal β 1–3GlcNAc structures in these hMOs.^{86,87} The degradation of hMOs by this strain results in the formation of monosaccharides or disaccharides that can stimulate the growth of other species that cannot degrade hMOs themselves through cross-feeding.^{50,88}

Bacterial cross-feeding between hMO utilizing species and other species can contribute to a more diverse microbiota composition. There are various studies available showing the effect of hMO utilization by *Bifidobacteria* strains on the growth of other species. For example, Salli *et al.* found that 2'-FL promoted the growth of *Bifidobacteria* and increased the production of SCFAs and lactic acid in a human study.⁷ Lactic acid can be further utilized as a carbon source by secondary strains like *Eubacterium hallii*, *Faecalibacterium prausnitzii*, and *Clostridium butyricum* to produce butyrate.⁸⁵ In another recent study by Lawson *et al.*, *Bifidobacterium* strains isolated from breastfed infants were analyzed for their cross-feeding abilities. It was found that *B. longum* LH206 could partially degrade 2'-FL into fucose and lactose, and the conditioned media of *B. longum* LH206 could stimulate the growth of *B. longum* LH659 indicated by an increased acetate production. In the same study, *B. pseudocatenulatum* LH13 degraded 2'-FL into

acetate, ethanol, formate, and 1,2-propanediol, which could be further utilized by the non-degrader *B. longum* LH12.⁵⁰

4.2 hMOs and prevention of pathogen adhesion

hMOs are structurally similar to intestinal mucosal glycans, and can therefore serve as decoy receptors that bind pathogenic bacteria in the gut lumen and prevent them from adhering to the intestinal epithelial cells. There are various studies available demonstrating the decoy effects of hMOs. For example, fucosylated oligosaccharides were shown to effectively inhibit the adhesion of *Campylobacter jejuni*,^{89,90} *Listeria monocytogenes*,⁹¹ and enteropathogenic *Escherichia coli* (EPEC)⁹² to the gut epithelium. On the other hand, the sialylated oligosaccharide 3'-SL was shown to inhibit the adhesion of *Helicobacter pylori* in a rhesus monkey model.⁹³

Coppa *et al.* compared the effects of single hMOs with pooled hMO fractions on the inhibition of the adhesion of enterotoxigenic *Escherichia coli* (ETEC), *Vibrio cholerae*, and *Salmonella fytis* to epithelial Caco-2 cells.⁹⁴ Among the single hMOs only 3-FL, but not 2'-FL, 3'-SL, or 6'-SL, showed an inhibitory effect on the adhesion of ETEC and *S. fytis*, while the pooled hMO fractions showed a wider and also stronger inhibitory effect. This suggests that pathogens adhere to the epithelium of the host through multiple glycan structures, and specific oligosaccharide structures decoy the groups of pathogens that adhere through the same structures. However, this study did not provide mechanistic insight into the interference of hMOs and the binding of pathogens to specific receptors. A study by Ruiz-Palacios *et al.* further investigated the decoy abilities of hMOs. They found that α 1-2 fucosylated moieties of hMOs inhibited the adhesion of *C. jejuni* to intestinal mucosa and demonstrated that binding to intestinal H-2 antigen is essential for *C. jejuni* infection, which could be specifically inhibited by fucosal α 1-2 linked oligosaccharides.⁹⁰ More recently, Behrouz demonstrated that the hMO LNnT could prevent the adherence of *Staphylococcus pneumoniae* to chinchilla tracheal epithelial cells by blocking the Galb1-4GlcNacb1-3Gal receptor.⁹⁵

4.3 Effects of hMOs on the epithelial cell barrier

Nowadays, there are some studies available demonstrating the direct effect of hMOs on the different cells of the intestinal epithelial barrier. For example, Cheng *et al.* tested the effects of various individual hMOs including 2'-FL, 3-FL, and LNT2 on the gut mucus barrier function using a LS174 T goblet cell line in the presence of different stressors. They showed that 3-FL and LNT2 could improve *MUC2* gene expression during challenges with both IL-13 and TNF- α . 3-FL and LNT2 also stimulated *MUC2* secretion at the protein level.²² There are also studies showing the effects of hMOs on the epithelial glycocalyx layer. Angeloni *et al.* showed that hMOs influence the expression of different glycocalyx molecules that might have an effect on the ability of certain pathogens to bind to epithelial cells.⁹⁶ In another study by Kong *et al.*, the effects of 2'-FL and 3-FL on the average thickness and area of the intestinal epithelial glycocalyx components, hyaluronic acid (HA) and heparan sulfate (HS), in

Caco-2 cells were quantified using immunofluorescence staining. It was found that 3-FL, but not 2'-FL, could enhance albumin absorption and increase HA and HS, stimulating the development of the glycocalyx layer.⁹⁷ In addition to their mucus and glycocalyx modulating properties, hMOs can also stimulate epithelial cell maturation. Holscher *et al.* found that individual or combinations of 2'-FL, 3-FL, and 6'-SL reduced cell proliferation, and increased differentiation of both intestinal epithelial HT-29 and Caco-2Bbe cells, which were associated with the maturation of the intestinal epithelial cells.²³

4.4 Effects of hMOs on infection prevention and mucosal immune responses

Besides modulating the mucus protein and glycan expression, hMOs also exert immune regulating effects, for example *via* Toll-like receptors (TLRs).²¹ Cheng and colleagues tested the stimulatory effects of the individual hMOs 2'-FL, 3-FL, 6'-SL, LNT2, and LNnT on multiple TLRs, including TLR2, TLR3, TLR4, TLR5, TLR7, TLR8, and TLR9. They demonstrated that the activation and inhibition of specific TLRs are highly dependent on the hMO type, *e.g.* 3-FL activated TLR2 and inhibited TLR5, TLR7, and TLR8, while LNT2 activated all TLRs and led to a subsequent increase of IL-10 and TNF- α secretion in THP-1 macrophages.²¹ It was hypothesized that the unique *N*-acetylglucosamine end of this hMO was responsible for the observed effects.

Studying the TLR activation and inhibitory properties of hMOs is a relevant topic, as many infant diseases can be linked to specific TLR interactions. For example, TLR4 activation is involved in the pathogenesis of necrotizing enterocolitis (NEC), a neonatal disease with a high mortality. Upon TLR4 activation, necroptosis is activated by the intestinal epithelium, leading to the development of NEC.⁹⁸ hMO supplementation is beneficial for infants suffering from NEC, as it increases the epithelial cell differentiation and it accelerates the crypt cell turnover to protect the intestinal epithelial cell injury from NEC.⁹⁹ Importantly, hMOs also activate epidermal growth factor receptors and reduce the TLR4 expression and inhibit the downstream NF- κ B signaling, resulting in a lower expression of the inflammatory cytokines IL-6 and IL-8.⁹⁹ More specifically, Sodhi *et al.* showed that the supplementation of 2'-FL and 6'-SL, but not of lactose, to infant formulas prevents NEC in both mice and piglet models, and attenuates NEC-associated inflammation in human ileum samples, which is realized through the inhibition of TLR4.¹⁰⁰ Above all, hMOs have been listed by the FDA as nutritional supplements for gut disease treatment, *i.e.* irritable bowel syndrome. These functions have been more extensively reviewed by Cheng *et al.*²

5. Non-digestible carbohydrates (NDCs) in infant formulas to substitute hMO functions

Infants who cannot be fed with HM are routinely fed with infant formulas, which are often cow milk derived. As these

infant formulas do not contain hMOs they are supplemented with NDCs that partially resemble hMO effects.⁹⁹ Nowadays, there are more infant formulas coming to the market containing different NDCs. Until January 2020, there are 17 isolated or synthetic NDCs listed by the FDA (Food and Drug Administration), among which GOS, FOS, inulin, and polydextrose have been marketed in Europe and are under extensive clinical research in the United States.¹⁰¹ To date, GOS and FOS are commonly used in infant formulas as well as the hMO analog 2'-FL.^{102–104} An increasing amount of research is dedicated to the selection of other NDCs and NDC mixtures that can be used in infant formulas to support the development of similar gut microbiota composition and gut immune responses found in breastfed infants.²⁸ For example, it can be hypothesized that the mixtures of neutral NDCs like inulin-type fructans and acidic NDCs like pectin-derived oligosaccharides can resemble natural hMO mixtures more closely and therefore cover a wider range of beneficial effects.^{105,106} An important finding of this research is that the NDC type, structure, and dose influence the impact of NDCs on the gut microbiota and gut immune barrier functions.^{22,97,107} To optimize NDC formulations for infant formulas, insight into the mechanisms by which NDCs exert their beneficial effects is crucial. Therefore, we will review the current knowledge on the hMO analog 2'-FL as well as various NDCs that are available for the supplementation of infant formulas in the following sections, with a specific focus on the hMO effects they resemble (Fig. 3).

5.1 2'-Fucosyllactose (2'-FL)

Nowadays, it is possible to produce some small hMO molecules in sufficient quantities that allow application in infant formulas.¹⁰² 2'-FL is one of the commercially available hMO analogs that is not isolated from HM but is produced as a fermentation product of genetically engineered microorganisms which include the strains of *E. coli* and yeast.^{108,109} 2'-FL is composed of a lactose molecule decorated with one α 1–2 linked fucose unit (Fig. 2d). 2'-FL reaches the large intestine and can therefore exert direct and indirect effects by stimulating gut microbiota fermentation processes in the large intestine. In the past few years, 2'-FL has been added to infant formulas as an hMO analog and has been tested in several clinical studies to determine the optimal dosage that can be regarded as safe and which will be well tolerated in infants (Table 1).^{110–112} In contrast to hMOs in mother milk, 2'-FL is an individual molecule, not a mixture of various hMO structures. As a consequence, the dosage of 2'-FL differs from the total amount of hMOs found in human milk.^{110,111,113} In recent years, a growing number of studies have become available on the effect of supplementation of infant formulas with individual 2'-FL, which will be described in the next sections.

5.1.1 Effects of 2'-FL on microbiota compositions.

Nowadays, there are various studies available showing the effect of supplementation of infant formulas with 2'-FL on the gut microbiota compositions of infants. As described before, *Bifidobacteria* can selectively utilize hMO mixtures and are a dominant species in the intestine of breastfed infants. The

maternal fucosyltransferase 2 status has been proven to directly affect the gut bifidobacterial communities in the gut of breastfed infants.¹¹⁴ Children who are fed with breast milk of non-secretor mothers, who do not express α 1–2-fucosyltransferase responsible for making α 1–2 fucosylated hMOs, are shown to be delayed in the establishment of a bifidobacterial-laden microbiota, which is probably related to a difficulty in acquiring the species of *Bifidobacteria* that are able to consume the specific hMOs present in the mother milk. This illustrates the direct link between α 1–2 fucosylated hMOs like 2'-FL and the colonization by *Bifidobacteria*.¹¹⁴ Supplementation of infant formulas with 2'-FL might therefore also increase the abundance of *Bifidobacteria* in the infant's intestine. This is corroborated by an *in vitro* study by Van den Abbeele *et al.* who demonstrated that 2'-FL increased the relative abundance of *Bifidobacterium adolescentis* and butyrate-producing bacteria in a validated gut model using the microbiota of 6-month-old formula-fed infants.¹¹⁵ There are no clinical studies available yet that confirm the direct link between 2'-FL supplementation and an increase of *Bifidobacteria in vivo*.

5.1.2 2'-FL and prevention of pathogen adhesion. In an *in vitro* study by Weichert *et al.*, biotechnologically synthesized 2'-FL could inhibit the adhesion of *Campylobacter jejuni*, enteropathogenic *E. coli* (EPEC), *S. ftyris*, and *Pseudomonas aeruginosa* to the intestinal human cell line Caco-2 by 26%, 18%, 12% and 17%, respectively.¹¹⁶ 2'-FL could also inhibit the adherence of *Pseudomonas aeruginosa* to the human respiratory epithelial cell line A549 by 24%.¹¹⁶ Another study showed that 2'-FL could also significantly reduce the adhesion of *E. coli* to Caco-2 cells, while it did not have an effect on the adherence of *S. ftyris*.¹¹⁷ Yu *et al.* found that 2'-FL could attenuate *C. jejuni* invasion by 80% and decreased the release of mucosal pro-inflammatory signals IL-8 by 60–70%, IL-1 β by 80–90% and MIP-2 by 50% in an *in vitro* model using HEP-2 and HT-29 cells. Additionally, in a mouse model, 2'-FL also attenuated *C. jejuni* invasion by 80% and reduced weight loss by 5%. Histologic features of intestinal inflammation were also reduced by 50–70%.¹¹⁸

5.1.3 Effects of 2'-FL on epithelial cell responses. Next to its effect on microbiota and pathogen adhesion, there are also studies showing the direct effects of 2'-FL on epithelial cells. Kong *et al.* showed that 2'-FL, as well as 3-FL, significantly increased the thickness and coverage area of absorbed albumin in the glycocalyx layer on Caco-2 cells and thus possibly contributes to the development of the glycocalyx layer.⁹⁷ Zehra *et al.* found that 2'-FL modulated human epithelial cell responses related to allergic diseases by the selective inhibition of CCL20 release in response to the antigen–antibody complex, TNF- α or PGE2.¹⁷ The effect was PPAR γ dependent. This might be an important finding as 2'-FL can be used as a therapeutic in food allergy.¹⁷

5.1.4 Effects of 2'-FL on the prevention of infection and mucosal immune responses. Various *in vitro* studies show the immune modulating effects of 2'-FL. Cheng *et al.* showed that 2'-FL, among other hMO analogs, could specifically inhibit the immune receptor TLR5 and 7 and thus serve as an antagonist

Table 1 Effects of infant formula-derived dietary fibers on the infant health and gut microbiota in clinical trials

Dietary fibers	Dosing and experiment design	Main findings	Adverse effects	Ref.
2'-FL	Infant formula with only galacto-oligosaccharides (GOS) or GOS with 2'-fucosyllactose (2'-FL) of 0.2 g L ⁻¹ or 1 g L ⁻¹ , human milk served as a reference	All formula groups showed similar growth of the infants, and 2'-FL absorption was similar to the human milk group	Not observed	110
2'-FL	Infant formula with only GOS or GOS with 2'-FL of 0.2 g L ⁻¹ or 1.0 g L ⁻¹ , breastfeeding as a reference	2'-FL supplementation showed lower inflammatory cytokines, which is similar to the breastfeeding group	Not observed	111
2'-FL	Cow's milk-based infant formula with or without 2'-FL of 1.0 g L ⁻¹	2'-FL in infant formulas is safe, well-tolerated, supports growth, and is associated with lower parent-reported morbidity, bronchitis in particular	Not observed	112
GOS	Infant formula supplemented with GOS of 4.4 g L ⁻¹ , formula without GOS served as a control	Formula supplemented with GOS significantly increased <i>Bifidobacterium</i> counts, lowered stool pH, and lowered butyric acid concentration	Not observed	32
GOS	Infant formula supplemented with GOS (OM55N) of 3 g L ⁻¹ , formula without GOS served as a control	Formula supplemented with GOS significantly increased the abundance of <i>Bifidobacteria</i>	Not observed	123
GOS	Iron-containing micronutrient powders with 5 mg iron or 5 mg iron with 7.5 g GOS daily, powder without iron or GOS served as a control	Formula supplemented with iron or iron with GOS significantly decreased anaemia of infants. The addition of GOS relieved the adverse effects of iron, <i>i.e.</i> lower abundance of <i>Bifidobacterium</i> and <i>Lactobacillus</i>	Not observed	124
FOS	Infant formula supplemented with or without 1.5 g L ⁻¹ or 3.0 g L ⁻¹ fructo-oligosaccharides (FOS), breastfeeding served as a reference	Formula supplemented with FOS of 1.5 g L ⁻¹ resulted in more <i>Bifidobacteria</i> counts after 7 days compared to breastfeeding and the 3.0 g L ⁻¹ FOS group	Formula supplemented with FOS of 1.5 g L ⁻¹ increased <i>Clostridium</i> counts after 7 days	136
FOS	Infant formula supplemented with or without 2.0 g L ⁻¹ or 3.0 g L ⁻¹ FOS, breastfeeding served as a reference	Formula feeding group resulted in a similar abundance of <i>Bacteroides</i> , <i>Bifidobacterium</i> , and <i>Lactobacillus</i> to breastfeeding	Formula resulted in a higher abundance of <i>C. difficile</i> and <i>E. coli</i>	137
FOS	Infant formula supplemented with 4.0 g L ⁻¹ FOS, 4.0 g L ⁻¹ maltodextrins served as a control	Formula supplemented with FOS of 4 g L ⁻¹ resulted in higher <i>Bifidobacteria</i> counts after 2 months of age and higher IgA at 4 months of age	Not observed	141
Inulin	Infant formula supplemented with or without 2.0 g L ⁻¹ or 4.0 g L ⁻¹ inulin	Formula with inulin of 4 g L ⁻¹ resulted in more <i>Bifidobacteria</i> and <i>Lactobacilli</i> and lower pH	Not observed	135
scGOS/lcFOS/pectin	Infant formula with only 2 g L ⁻¹ acidic oligosaccharides pectin or a combination of 6 g L ⁻¹ of neutral scGOS/lcFOS mixture and 2 g L ⁻¹ pectin, maltodextrin served as a control	The combination of scGOS/lcFOS/pectin resulted in a higher abundance of <i>Bifidobacteria</i> and <i>Lactobacilli</i> , than only pectin and control	Not observed	160

for these receptors and prevent too strong immune activation.²¹ He *et al.* found that 2'-FL can also directly inhibit LPS-mediated inflammation during the invasion of ETEC in T84 and H4 intestinal epithelial cells by attenuating IL-8 production through decreasing CD14 induction.²⁰ There are also various *in vitro* studies on the effect of 2'-FL on the prevention of virus infection. Duska-McEwen *et al.* showed that 2'-FL could enhance innate immunity to airway infections by decreasing respiratory syncytial virus (RSV) viral load in airway epithelial cells.¹¹⁹ They also showed that 2'-FL could attenuate RSV-induced inflammatory cytokines IL-8 and TNF- α production in airway epithelial cells, and in RSV-infected peripheral blood mononuclear cells (PBMCs).¹¹⁹ 2'-FL, as well as 3'-SL and 6'-SL, could also reduce the infectivity of human rotaviruses G1P and G2P in African green monkey kidney epithelial cells (MA104 cells). As breastfed children are directly protected against human rotaviruses, this indicates that the supplementation of infant formulas with 2'-FL may confer these benefits

to formula-fed infants.¹²⁰ Koromyslova *et al.* found that 2'-FL can block the binding of noroviruses to histo-blood group antigens, preventing the viruses from infecting the cells.¹²¹ There are also *in vivo* studies showing the beneficial effects of the hMO analog 2'-FL in infant formulas. For example, in a study where healthy term infants were fed with formulas supplemented with 2'-FL, infants had similar levels of plasma inflammatory cytokines to breastfed infants which were 29–83% lower compared to those of children fed with the control formula.¹¹¹

5.2 Galacto-oligosaccharides (GOS)

Galacto-oligosaccharides (GOS) are a mixture of NDCs that have already been widely applied to infant formulas.¹⁰³ They are composed of galactose units with one glucose unit at the reducing end, and the degree of polymerization (DP) of GOS typically ranges between 2 and 10 (Fig. 2e).¹²² GOS are enzymatically synthesized using galactosyl transfer reactions,

which link the galactose units *via* β 1–3, β 1–4, or β 1–6 linkage, depending on the source of the enzymes.^{101,103} Like hMOs, GOS are resistant to digestion in the upper GIT and can therefore reach the large intestine, where they influence microbiota compositions and exert direct effects on the intestinal epithelium and immune reactions.¹⁰³

5.2.1 Effects of GOS on gut microbiota. The beneficial effect of GOS on gut microbiota in infants has been shown widely in studies that tested GOS as a supplement in infant formulas (Table 1). Matsuki *et al.* studied the effect of infant formulas supplemented with GOS at a concentration of 3 g L⁻¹ on microbiota composition in 35 healthy term infants of 0–2 months. After 2 weeks of feeding, fecal samples were collected and examined. GOS supplementation stimulated the abundance of indigenous *Bifidobacteria* in the infants' gut and reduced the α -diversity of the gut microbiota. Other fecal characteristics related to the gut microbiota metabolism such as SCFA production were not influenced by GOS.¹²³ In another study, Sierra *et al.* studied the impact of a higher GOS concentration of 4.4 g L⁻¹ in a study with 365 healthy term infants of 0–2 months.³² GOS were administered until 12 months of age and the effects on the gut microbiota composition and metabolism were studied at 4 months of age. GOS-containing infant formulas increased the *Bifidobacterium* counts, but a lower butyrate concentration was found in the fecal samples. In addition, it was found that GOS could also attenuate the adverse effects from the environment on the gut microbiota development of the infants. In a study among Kenyan infants aged 6.5–9.5 months, GOS could attenuate the adverse side effects on the microbiota caused by the use of iron-containing infant formulas to treat anaemia. Supplementation of these infant formulas with GOS resulted in a higher abundance of *Bifidobacterium* and *Lactobacillus* and a lower abundance of virulent and toxic genes of the pathogens.¹²⁴ Taken together, these studies show that GOS, like hMOs, can specifically stimulate beneficial strains in infants.

5.2.2 Effects of GOS on pathogen adhesion. Many studies have shown that GOS can also act as decoy receptors that block the adhesion of pathogenic bacteria to the intestinal epithelium. In a study by Shoaf *et al.*, the effect of purified GOS on the inhibition of the adherence of EPEC was investigated using an *in vitro* assay with epithelial HEP-2 and Caco-2 cells. The adherence was reduced by 65% and 70%, respectively, when compared to non-treated cells. Furthermore, it was proved that the inhibitory effects of GOS were mediated *via* the adherence factor BfpA, a bundle-forming pilus protein of EPEC.¹²⁵ Another study that investigated the protective effect of GOS on the adhesion of ETEC in neonate piglets demonstrated the binding ability of porcine albumin-conjugated GOS to various ETEC strains. ETEC K88 was found to have the greatest affinity, and the adhesion to the intestinal mucins was partially inhibited by GOS.¹²⁶ The inhibition effect of GOS on the adhesion of other pathogens including EPEC, *Salmonella*, and *C. rodentium* to the epithelial cells was also extensively reported.^{125,127,128}

5.2.3 Effects of GOS on gut epithelial cell responses. GOS can stimulate the gut barrier function by directly interacting

with the gut epithelial cells. Akbari *et al.* evaluated the effects of Vivinal GOS syrup and purified GOS fractions with different DPs on the barrier integrity of epithelial cells during a challenge with the fungal toxin deoxynivalenol (DON). Pre-incubation of the epithelial Caco-2 cells with GOS for 24 hours could prevent the disruption of the barrier integrity induced by DON. Vivinal GOS showed the most pronounced protective effect with accelerated reassembling of the tight junction and the production of Interleukin-8 (IL-8).¹²⁹ In another study, exposure of Caco-2 cells to GOS of DP2–7 (1.4% w/v) also showed the ability to improve the integrity of the tight junction *via* increasing the *trans*-epithelial electrical resistance (TER).⁷³ More recently, the transcriptome of epithelial Caco-2 cells was fully characterized after stimulation with GOS and the hMO SL, indicating a similarity in function between GOS and SL.⁸⁵ In another study, Cheng *et al.* showed that GOS could stimulate the gene expression of *MUC2* and *TFF3* in epithelial goblet cells in a dose- and time-dependent manner.²² In this study, medium containing GOS could also significantly stimulate *MUC2* at the protein level.²²

5.2.4 Effects of GOS on gut mucosal immune responses. The gut immune system can be modified by GOS as well, as illustrated by various studies. Bermudez-Brito *et al.* used an *in vitro* system to test the effects of GOS on T-cell populations. In this study, GOS selectively increased the Treg cytokine IL-10 and Th1 cytokine IFN- γ , while it decreased the secretion of the Th1 cytokine TNF- α , indicating that GOS modulates the gut immune responses and thereby contributes to a tolerogenic milieu.¹³⁰ Recently, the effect of GOS on mucosal immune homeostasis was evaluated in a suckling piglet model.¹³¹ In this study, newborn piglets were fed with GOS (1 g per kg body weight) for one week. The animals fed with GOS had a higher production of SCFAs, which subsequently reduced IL-8 and increased IL-10 measured in the large intestinal mucosa of piglets. In addition, the expression of the tight junction proteins ZO-1 and Claudin-1 was increased through suppressing the phosphorylation of NF- κ B and activating the AMPK signaling pathway.¹³¹

5.3 Fructo-oligosaccharides (FOS) and inulin

Fructo-oligosaccharides (FOS) and inulin are composed of linear chains of fructose units that are terminated by a single glucose unit with variations in glycosidic linkages and different DPs.¹⁰¹ FOS and inulin are categorized according to their chain length. Short-chain FOS (scFOS) are composed of 3 to 5 residues per chain, while long-chain FOS (lcFOS) have 6 to 10 residues. FOS can be enzymatically obtained from natural inulin. Inulin usually refers to fructans with a higher chain length, usually containing up to 200 fructose residues (Fig. 2f),^{101,132} and can be derived from different plant-based sources like chicory, dahlia, or Jerusalem artichoke.^{133,134}

5.3.1 Effects of FOS and inulin on gut microbiota. Supplementation of infant formulas with FOS and inulin has been widely shown to improve gut microbiota compositions in infants by exerting an enhancing effect on the abundance of *Bifidobacteria* species.^{104,135} Even though there are also studies reporting the minimal adverse effects of FOS supplementation on the gut microbiota by increasing the counts of *C. difficile*, *E. coli*, and *Clostridium* (Table 1),^{136,137} a higher abundance of *Bifidobacteria* in infants fed with FOS and inulin supplemented formulas will result in a closer resemblance of the microbiota compositions found in breastfed infants. This contributes to a lower incidence of fever, diarrhea, and infections during the antibiotic treatment.^{138–140} Over the last few years, there have been many studies showing the beneficial effects of FOS and inulin on the regulation of the gut microbiota composition, as listed in Table 1.^{135,141} For example, in a randomized controlled double-blind study with 164 healthy formula fed infants by Oswari *et al.*,¹³⁵ it was found that the supplementation of infant formulas with 4 g inulin per L increased the numbers of *Bifidobacteria* and *Lactobacilli* in the fecal microbiota as compared to the infant formula supplemented with 2 g inulin per L or without inulin. Even though FOS and inulin are well degraded by *Bifidobacteria*, their degradation by *Lactobacilli* is only limited to a few strains of *Lactobacillus casei* and *Lactobacillus paracasei* subsp.^{142,143} The inulin-degrading strain *Lactobacillus paracasei* I321 isolated from infant fecal microbiota was shown to resist the competitive environment of the fecal microflora *in vitro* and showed an anti-salmonella effect through the secretion of antibacterial agents and competitive adhesion mechanisms.¹⁴⁴ Notably, an increased intake of inulin is associated with a higher production of butyrate, which is believed to prevent colon cancer and inflammation.¹⁴⁵ However, Nielson showed that *Bifidobacteria* can proficiently degrade inulin, but did not contribute to the butyrogenic effect.⁸⁸

5.3.2 Effects of FOS and inulin on pathogen adhesion. FOS and inulin induce a strong inhibitory effect on the adhesion of pathogens. For inulin, this inhibitory effect seems to be dependent on the degradation of inulin by commensal bacteria rather than by the direct effects of inulin on the pathogens. This was illustrated by a study of Van Den Abbeele *et al.*, in which an *in vitro* assay was developed to evaluate the effects of inulin in PBS and inulin in intestinal fluid, which contained abundant commensal bacteria, on the adhesion of mucin-adhered *Clostridia* to the mucin agar. The adhesion of *Clostridia* was significantly inhibited in intestinal fluid; however, this inhibitory effect of inulin was not detected in PBS.¹⁴⁶ In a more recent study, Piotrowski *et al.* tested the effect of inulin on the adhesion of 12 *Clostridium difficile* strains to HT-29, mucus-secreting HT-29 MXT and CCD 841 human epithelial cell lines. The direct effects of inulin on the adhesion of *C. difficile* were not observed.¹⁴⁷ A study by Kanjan *et al.* showed that inulin was able to promote the competitive adhesion of *Lactobacillus paracasei* I321 isolated from infant fecal microbiota to mucin when co-cultured with *Salmonella typhimurium* and thereby reduced the adhesion of

S. typhimurium.¹⁴⁴ In another study, Shoaf *et al.* showed that FOS and inulin directly inhibited the adhesion of EPEC to Caco-2 cells by 32% and 42%, respectively.¹²⁵

5.3.3 Effects of FOS and inulin on gut epithelial cell responses. Inulin has been shown to exert direct effects on gut epithelial cells and can contribute to the development of a stronger defense line against pathogen-induced disruptions of the epithelial integrity. In an *in vitro* study by Wu *et al.*,¹⁴⁸ pre-incubation of inulin was found to protect the barrier integrity of Caco-2Bbe1 cells in a challenge with enterohemorrhagic *Escherichia coli* (EHEC), illustrated by a rescue of transepithelial electrical resistance (TER) levels. Pre-incubation of the cells with inulin alone also increased TER levels. The alteration of barrier integrity was further examined by measuring the expression levels of tight junction proteins and mRNA. It was found that inulin promoted the epithelial barrier function through a directly increased tight junction protein expression of occludin.¹⁴⁸ In another study, a mixture of inulin, containing molecules ranging from DP3 to 10, showed beneficial effects on the glycocalyx layer of Caco-2 cells.⁹⁷ It increased the amount of heparan sulfate in the glycocalyx layer. In the same study, a mixture of long-chain inulin and DP30–60 could specifically increase the amount of hyaluronic acid.⁹⁷

5.3.4 Effects of FOS and inulin on gut mucosal immune responses. Inulin can directly influence mucosal immune responses in a chain length and structure-dependent way. Vogt *et al.* found that short-chain inulin could induce an anti-inflammatory balanced IL-10/IL12 ratio in human peripheral blood mononuclear cells, *i.e.* more IL-10 compared with IL-12, while the long-chain length inulin induced a pro-inflammatory cytokine profile, *i.e.* more IL-12 compared with IL-10.¹⁴⁹ In a mouse model, long-chain inulin induced an anti-inflammatory cytokine production profile, promoted T-cell responses by increased regulatory T-cells, and enhanced tight junction proteins and anti-microbial peptides as well as the SCFA production.¹³⁴ Long-chain inulin (DP10–60) also supported the immune system and provided protection against pathogenic antigens induced by hepatitis-B vaccination through increasing Th1-cells, and stimulating a Th1-type cytokine profile and stronger TLR2 activation than the short-chain inulin (DP2–25).¹⁴⁹ These findings demonstrated that inulin can support different immune responses in a structure-specific way.

5.4 Pectins

Pectins are complex hetero-polysaccharides composed of an α 1–4 linked galacturonic acid backbone, which can be interrupted by the L-rhamnose unit with the side chains of neutral sugars including arabinose and galactose.¹⁵⁰ The carboxyl group of galacturonic acid on the galacturonic acid backbone can be esterified with methyl groups (Fig. 2g).¹⁵⁰ The percentages of the carboxyl groups of galacturonic acid that are methyl-esterified determine the degree of methyl esterification (DM).⁴⁹ According to differences in DM, pectins are classified as low DM pectins (25–50%) and high DM pectins (50–80%).¹⁵¹ Pectin is a common polymer in the cell wall of

plants, including vegetables and fruits, such as potatoes, lemon, and hawthorn.^{152,153} A major characteristic of pectin is its gel-forming ability, for which it is widely applied in the food and pharmaceutical industries.¹⁵² Pectin-derived acidic oligosaccharides are of great interest for the supplementation of infant formulas to mimic the sialic acid found in HM to promote the beneficial gut microbiota.^{31,153}

5.4.1 Effects of pectin on the gut microbiota. Pectin has the potential to shape the gut microbiota to obtain a balanced gut microbiota.^{154,155} Pectins can be degraded by various Gram-negative *Bacteroides* species, and are often less degraded by the Gram-positive bacteria like Firmicutes bacterial species.^{156,157} However, *Faecalibacterium prausnitzii* and *Eubacterium eligens* belonging to the Firmicutes species have recently also been identified as pectin degraders.¹⁵⁸ The growth of the species was tested on sugar beet pectin DP4 and DP5. *E. eligens* DSM3376 showed a rapid growth on both DP4 and DP5, *F. prausnitzii* A2-165 could utilize DP4, but not DP5, while *F. prausnitzii* S3L/3 grew better on DP5 than on DP4, and *F. prausnitzii* L2-6 showed a slow growth on both DP4 and DP5.¹⁵⁸ In a study by Bianchi *et al.*, citrus pectin was shown to enhance *Bifidobacterium longum* BB-46.¹⁵⁹ In another study, it was found that the acidic hydrolysate from pectin used for the supplementation of infant formulas at a concentration of 2 g L⁻¹ did not affect the *Bifidobacteria* and *Lactobacilli* counts. However, when combined with neutral GOS/FOS (9 : 1, 6 g L⁻¹), it resulted in a higher abundance of *Bifidobacteria* and *Lactobacilli* (Table 1).¹⁶⁰

Pectin can also promote bacterial cross-feeding resulting in increased abundances of non-degrading bacteria. This was illustrated in a study by Bui *et al.*, where lactate production resulting from pectin degradation by *Bacteroides thetaiotaomicron* stimulated the growth of *Anaerostipes rhamnosivorans* resulting in a higher butyrate production.¹⁶¹ Bang *et al.* also reported that the production of SCFAs after the fermentation of pectin increased bacterial strains of *Lachnospira*, *Dorea*, *Clostridium*, and *Sutterella*, with a subsequent increase in butyrate levels.¹⁶²

5.4.2 Pectin and prevention of pathogen adhesion. As illustrated by a number of studies, pectin oligosaccharides can prevent pathogen adhesion to the host and serve as decoy receptors to pathogens. Rhoades *et al.* evaluated the ability of pectin to inhibit the adhesion of six strains of pathogenic EPEC in an *in vitro* study using the human gut epithelial HT-29 cell line. The culture medium with different concentrations of pectin was tested on the EPEC viable counts to HT-29. It was found that the medium containing pectin at a concentration of 2.5 mg mL⁻¹ significantly decreased the adhesion of pathogenic bacteria by 70% when compared to the non-pectin-treated group.¹⁶³ The anti-adhesive effect of five different pectins, which varied in the extraction method, molecular weight, and degree of esterification, was also studied on the adhesion of EHEC O157:H7 to HT-29 cells.¹⁶⁴ Pectin concentrations used in this study ranged from 0.001 mg mL⁻¹ to 5 mg mL⁻¹ for all five pectin mixtures tested. Although all pectin samples showed anti-adhesive

effects, it was found that higher concentrations and lower molecular weights showed the strongest anti-adhesive effects, up to 100% when compared to the control group without pectin.¹⁶⁴ The above studies were performed by including pectins and the pathogens to the epithelial cells at the same time. In another study by Thöle *et al.*, it was found that the pre-incubation of *H. pylori* with pectin-like okra extracts decreased their ability to adhere to human gastric cells. Among the extract fractions by ammonium sulfate precipitation with 30%, 60%, and 90% saturation levels, the 60% and 90% saturation fractions significantly decreased *H. pylori* adhesion. The pectin fractions were found to exert their decoy effects through binding to the bacteria cell membrane proteins BabA, SabA, and HpA.¹⁶⁵

5.4.3 Effects of pectin on gut epithelial cell responses. Pectin has been demonstrated to induce morphological changes of the small intestine *in vivo*, and the mechanism has been extensively explored *in vitro*. In a study conducted by Nishida *et al.*, pectin from *Prunus domestica* L. was incubated with the human intestinal epithelial Caco-2 cell line for 24 h. Although the total amount of the glycocalyx component HS was not affected, the disaccharide compositions of HS were markedly altered through the binding of pectin with fibronectin and $\alpha 5 \beta 1$ integrin at the Caco-2 cells.¹⁶⁶ The structural change of HS was further found to promote the secretion of Wnt3a, a protein that strongly binds to HS in Caco-2 cells, which can stimulate the proliferation of the rat IEC6 cell, a model of intestinal crypt cells.¹⁶⁷ In a more recent study, Kong *et al.* evaluated pectins from lemon with different DMs and their ability to influence the glycocalyx compositions of Caco-2 cells. Consistently, neither the amount of HS nor HA was changed, but pectin significantly increased the absorbed albumin of Caco-2 cells.⁹⁷ The direct regulatory effect was also observed in an *in vitro* study with the HT-29 cell line. Both high DM and low DM pectins stimulated the mucin secretion of HT-29.⁹⁷

5.4.4 Effects of pectin on gut mucosal immune responses. Pectin supplementation to infant formulas stimulates a Th1-type and regulatory T-cell immune response and attenuates the Th2-type immune response.^{106,168,169} Vos *et al.* demonstrated that this effect is achieved through selectively promoting cecal *Lactobacilli* growth in a murine vaccination model. Pectin also exerts immune modulating effects that are independent of the gut microbiota.¹⁷⁰ For example, Sahasrabudhe *et al.* found that pectins with different DMs (DM7, DM22, DM45, DM60, and DM75) could directly bind and block TLR2 and thereby inhibit the pro-inflammatory TLR2-TLR1 pathway.¹⁷¹ In this study, the low DM pectins showed the most pronounced inhibitory effect. The effect of low DM pectins (DM7) on the mucosal barrier function was also tested in an *in vivo* mouse model. It was shown that low DM pectins up-regulated the gene expression of tight junction proteins (ZO-1 and occludin), the anti-microbial peptide β defensin-1, and increased SCFA production.¹⁷² These results are in line with the observation that low DM pectins are better digested than high DM pectins.¹⁷³

6. *In vitro* systems to evaluate the effects of dietary fibers

The development of more effective hMOs and NDC mixtures for the supplementation of infant formulas to mimic beneficial hMO functions has been hampered due to the lack of efficient *in vitro* test models. *In vitro* models are needed because testing in infants is ethically not approved and animal studies are very labor-intensive. In addition, the results from currently applied *in vitro* models are not always easy to translate to humans *in vivo*.^{174,175} Even for the animal studies, many factors can cause the ineffective translation to human trails, *i.e.* specific animal models are needed for humans of different age, sex, health status, and dose translation. Especially in some animal models, translation of the used dosage to clinical trials is not tolerated or even toxic in humans.¹⁷⁶ Considering these issues, it is of essential importance to develop new *in vitro* systems that resemble the human gut conditions more closely. A few potential new *in vitro* systems will be discussed.

Research on the development of intestinal organoids from stem cells, progenitor cells, or tissue explants has increased rapidly over the past few years.^{72,99,177} These organoids might be of interest for studying new NDCs and NDC mixtures as they closely resemble the *in vivo* GIT. Intestinal organoids are epithelial 3D spheroids that are cultured on extracellular matrixes and represent either intestinal villi or crypts containing a mixture of complex intestinal epithelial cell populations mimicking the intestinal physiology and pathology (Fig. 4a).^{178,179} In the case of NDC research, these organoids might be used to study NDC effects on host–microbiota interactions, medicine and antibiotic interferences, effects on tissue repair, and regeneration of the intestine.^{180–182} The culturing techniques still need to be extensively optimized to improve organoid viability and quality control, as currently there are high batch-to-batch variations.¹⁸³ Techniques that can be employed to measure NDC effects include immunohistochemistry, which is commonly applied to visualize and localize structures such as the villi, crypts, and certain proteins. In addition, the culturing medium can be collected to quantify the cytokine secretion, and the gene expression of different cell types in the organoids can also be measured using PCR techniques.^{180,184} However, due to the heterogeneity in size and shape, and generally the small size of the 3D organoids, analysis of the barrier integrity is very complicated.¹⁸⁵ The closed configuration of the 3D organoid also makes it technically difficult to reach the apical lumen side.¹⁸⁶ With the application of the transwell inserts, epithelial cells, for example grown as 3D organoids and later dissociated into the monolayers, make it possible to do barrier integrity analysis.¹⁸⁷

Another approach is the use of gut epithelial cells grown in transwells (Fig. 4b). The transwell system is built up around a porous transwell on which epithelial cells are grown. Insertion of these transwells into normal cell culture plates results in the creation of two separate culturing chambers, apically and

Fig. 4 Schematic overview of intestinal *in vitro* systems. (a) Intestinal organoids cultured in the Petri dish; (b) transwell system that is separated by a porous membrane on which the gut epithelial cells can grow, with the upper chamber to mimic the gut lumen where the gut microbiota and the dietary fibers can be cultured and the lower chamber to mimic the lamina propria where the endothelial cells and immune cells are present; (c1) the gut-on-a-chip system, which is established by introducing the shear force to the static transwell system on a polydimethylsiloxane chip with two separate chambers. The side vacuum chambers exert the cyclic stretching force across the chip to generate the intestinal peristalsis; (c2) vertical view of (c1) with the direction of flow; (d1) the gut-on-a-chip system with a gradual decreasing shear force by a gradual increasing cell culture area; (d2) vertical view of (d1) with the direction of flow; (e1) the ibidi system with a constant and continuous shear force; (e2) vertical view of (e1) with the direction of flow.

on the basolateral side of the epithelial cell layer. By culturing immune cells in the basolateral compartment of the transwell system, it can be used to mimic the communication between intestinal epithelial cells and the immune cells in the lamina propria.¹³⁰ The dietary fiber interventions in these interactions can also be investigated using this *in vitro* transwell co-culture system.^{188,189} Experiments using the transwell culture system are most often conducted under static conditions. However, food intake, gut microbiota, and the intestinal epithelium are continuously exposed to shear force generated by the intestinal peristalsis.¹⁹⁰ Shear force has been shown to affect gut microbiota colonization,^{191,192} gut microbiota interaction with the host,¹⁹³ and intestinal epithelium characteristics including the gut mucus layer and tight junctions.^{194,195} A wide range of studies have shown the effect of shear forces on the intestinal

epithelial cell behavior and the bacterial colonization, showing the need to mimic shear force in *in vitro* models to more closely mimic the *in vivo* situation.^{196,197}

Recently, a more advanced transwell system named microfluidic device was constructed to develop a gut-on-a-chip system involving the key elements of the *in vivo* environment, in which the gut lumen shear force effects were introduced to the normal transwell system, allowing the interactions of the gut microbiota in the gut lumen, the intestinal epithelial cells, and the immune cells or endothelial cells in the lamina propria to be investigated *in vitro*.¹⁹⁰ It is established based on the polydimethylsiloxane (PDMS) chip with two separate chambers, resulting in the upper gut lumen flow chamber and the lower capillary flow chamber.¹⁹⁰ The epithelial cells are seeded on the median porous membrane, and the immune cells or endothelial cells can be introduced in the lower chamber. The vacuum chambers on the two sides of the main transwell chambers make a physiological cyclic stretching force across the membrane to generate intestinal peristalsis (Fig. 4c1 and c2).¹⁹⁸ Peristaltic shear force was recently proposed to stimulate the differentiation of the human-induced pluripotent stem cells to induce the polarized 3D villi structure forming the *in vivo* characteristic of intestinal epithelium with different cell types, *i.e.* absorptive enterocytes, goblet cells, and Paneth cells. This gut-on-a-chip system gives research opportunities to involve as many genetic risk factors as associated with the Celiac disease by establishing a Celiac disease-on-a-chip model to find the treatment of the patients.¹⁸⁶ The gut-on-a-chip device has been used to study the interaction of the gut commensal bacteria or gut pathogens with the host including the colonization to the gut epithelium and the impact on gut barrier function.^{178,190} As the shear forces vary in different parts of the intestine, another gut-on-a-chip model was made with a gradually increasing culturing area, which resulted in a gradually decreasing shear force ranging between 0.002 and 0.03 dyn cm⁻² from one side of the device to the other (Fig. 4d1 and d2). With an increase of the shear force in this model, the organization of the cytoskeleton and the formation of microvilli were found to increase, and the mucus production and the tight junction of the epithelial cells also increased but not at the highest shear force, *i.e.* 0.026–0.03 dyn cm⁻².¹⁹⁷

However, to date there is only one experimental set-up available that investigated the effects of dietary fibers on the intestinal epithelial cells and one gut commensal bacterium in the presence of peristaltic shear force. This study used a so-called ibidi system in which a constant and continuous shear force can be exerted on the gut epithelium (Fig. 4e1 and e2). In this study, the effects of three individual hMOs, *i.e.* 2'-FL, 3-FL, and LNT2, were investigated, combined with intestinal peristaltic shear force on the crosstalk of the intestinal epithelial Caco-2 cells and *L. plantarum* WCFS1. It was found that 3-FL and LNT2 supported the crosstalk between *L. plantarum* WCFS1 and Caco-2 cells, and shear force showed increasing effects on the modulating effects of hMOs.¹⁹⁹ Including shear force as a factor in the current *in vitro* models would increase their trans-

lation to *in vivo* GIT and would make translation of the results generated using these models to the *in vivo* situation easier.

7. Concluding remarks

As outlined in this review, the beneficial functions of hMOs for the development of the gut microbiota and the gut immune system in early life have been extensively studied in both *in vitro* and *in vivo* studies.^{85,95} Despite these many identified health benefits, most of the hMOs are currently not applied in infant formulas, except for 2'-FL and LNnT,¹⁰² because the synthesis of the specific oligosaccharide structures is still challenging both technically and financially. Currently, it is difficult to generate more complex hMOs for basic research studies and clinical trials. On the other hand, research focuses on the functions of the individual hMO and NDC structures or only on the mixtures of well-defined composition is still scarce. Also, the number of studies exploring new NDC mixtures is still very limited and very focused on GOS-FOS.^{2,28}

In recent years, emerging research has demonstrated that the supplementation of NDCs to infant formulas can partially mimic hMO functions.^{123,139} Nowadays, especially the direct immune regulatory effects of NDCs are more recognized in addition to their conventional prebiotic effects.^{129,200} However, not all NDCs have the same regulatory effects on the host immune responses. Immune active NDCs often either exert Th1 or Th2 immune regulatory properties, depending on their molecular structures.^{130,201} Specific structures and specific Th1 or Th2 regulating NDCs might be needed as preterm infants are experiencing high proinflammatory Th1-prone immune response, and allergy-prone infants develop too strong anti-inflammatory Th2 immune responses.²⁰² Tailoring specific infant formulas for these groups might benefit the development and long-term health in these babies.

Another important factor that might change the type and composition of NDCs applied in infant formulas is the novel insights into the needs of the gut; in early life some hMOs and also some NDCs seem to be poorly digested by the microbiota of newborn infants,^{53,203} which suggests that in the early phases of life other more simple NDCs and hMOs might be needed to support a healthy development of the gut microbiota.⁴³ This is less important at increasing age as gut microbiota tend to develop and express more enzymes that facilitate the fermentation of hMOs and NDCs.⁷ The importance of the use of tailored NDCs for infants of different age classes was illustrated by a study of Logtenberg *et al.*, in which microbiota of 8-week-old infants were shown to have an increased fermentation capacity of fructan-type NDCs compared to infants of 2 weeks old.⁵³ Thus, understanding how and which hMO and NDC molecules can support microbiota development, modulate infant immune responses and gut barrier function, as well as investigating how hMO and NDCs can be utilized by the gut microbiota of infants of specific ages and health status is necessary for creating effective health supporting infant formula.

In addition, the most optimal dosage of hMO analogs and NDCs in infant formulas needs to be considered carefully. For example, it still remains to be determined whether 2'-FL in the applied dosage can achieve the same benefits as GOS and FOS which is used in higher concentrations. It is known that the amount of hMOs in mother milk decreases between 2 and 12 weeks of lactation, whereas the amount of NDCs added to infant formulas usually remains stable. Insight into specific molecular hMO structures that increase and decrease over time might provide insight into the needs of infants of specific ages as well. A recent study by Borewicz *et al.* showed that even though the total amount of hMOs was decreasing during the first weeks of lactation, the amount of specific hMOs, *i.e.* 3-FL and LNFP III, increased in this period.²⁰⁴ This might indicate a specific role of these molecules in infants of specific ages as well as the importance of proper dosage of NDCs for infants. Dosage of NDCs in infant formulas is of high importance and dosages used in *in vitro* studies are often extremely high when compared to the amount of hMOs in mother milk and might therefore not always be directly translatable to the *in vivo* situation. Appropriate clinical trials to determine the dosages for specific groups of infants with different health statuses are therefore indispensable.

Our expectation is that formulas will be created for specific infant target groups. By reviewing current insight into the health benefits of hMOs and NDCs we hope to contribute to the design of "tailored infant formula" for infants of different age groups and different health statuses, to ensure a healthy development of the gut microbiota and immune system of infants.

Conflicts of interest

The authors declare no conflicts of interest.

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References

- 1 G. W. Tannock, B. Lawley, K. Munro, S. G. Pathmanathan, S. J. Zhou, M. Makrides, *et al.*, Comparison of the compositions of the stool microbiotas of infants fed goat milk formula, cow milk-based formula, or breast milk, *Appl. Environ. Microbiol.*, 2013, **79**(9), 3040–3048.
- 2 L. Cheng, R. Akkerman, C. Kong, M. T. C. Walvoort and P. de Vos, More than sugar in the milk: human milk oligosaccharides as essential bioactive molecules in breast milk and current insight in beneficial effects, *Crit. Rev. Food Sci. Nutr.*, 2020, 1–17.
- 3 O. Ballard and A. L. Morrow, Human Milk Composition. Nutrients and Bioactive Factors, *Pediatr. Clin. North Am.*, 2013, **60**(1), 49–74.
- 4 N. J. Andreas, B. Kampmann and K. Mehring Le-Doare, Human breast milk: A review on its composition and bioactivity, *Early Hum. Dev.*, 2015, **91**(11), 629–635.
- 5 B. Casado, M. Affolter and M. Kussmann, OMICS-rooted studies of milk proteins, oligosaccharides and lipids, *J. Proteomics*, 2009, **73**(2), 196–208.
- 6 Y. He, S. Liu and N. D. Leone S, Human colostrum oligosaccharides modulate major immunologic pathways of immature human intestine, *Physiol. Behav.*, 2014, **7**(6), 1326–1339.
- 7 K. Salli, H. Anglenius, J. Hirvonen, A. A. Hibberd, I. Ahonen, M. T. Saarinen, *et al.*, The effect of 2'-fucosyllactose on simulated infant gut microbiome and metabolites; a pilot study in comparison to GOS and lactose, *Sci. Rep.*, 2019, **9**(1), 1–15.
- 8 A. Amedei and F. Boem, I've gut a feeling: Microbiota impacting the conceptual and experimental perspectives of personalized medicine, *Int. J. Mol. Sci.*, 2018, **19**(12), 3756.
- 9 P. D. Houghteling, W. Allan Walker and M. D. Mucosal, Why is initial bacterial colonization of the intestine important to the infant's and child's health?, *J. Pediatr. Gastroenterol. Nutr.*, 2008, **6**(9), 2166–2171.
- 10 A. Eck, N. B. M. M. Rutten, M. M. J. Singendonk, G. T. Rijkers, P. H. M. Savelkoul, C. B. Meijssen, *et al.*, Neonatal microbiota development and the effect of early life antibiotics are determined by two distinct settler types, *PLoS One*, 2020, **15**(2), 1–15.
- 11 S. M. Huse, L. Dethlefsen, J. A. Huber, D. M. Welch, D. A. Relman and M. L. Sogin, Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing, *PLoS Genet.*, 2008, **6**(11), e280.
- 12 G. K. Fragiadakis, S. A. Smits, E. D. Sonnenburg, W. Van Treuren, G. Reid, R. Knight, *et al.*, Links between environment, diet, and the hunter-gatherer microbiome, *Gut Microbes*, 2019, **10**(2), 216–227.
- 13 M. B. Engfer, B. Stahl, B. Finke, G. Sawatzki and H. Daniel, Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract, *Am. J. Clin. Nutr.*, 2000, **71**(6), 1589–1596.
- 14 S. Karav, A. Le Parc, J. M. Leite Nobrega de Moura Bell, S. A. Frese, N. Kirmiz, D. E. Block, *et al.*, Oligosaccharides released from milk glycoproteins are selective growth substrates for infant-associated bifidobacteria, *Appl. Environ. Microbiol.*, 2016, **82**(12), 3622–3630.
- 15 J. E. Becerra, J. M. Coll-Marqués, J. Rodríguez-Díaz, V. Monedero and M. J. Yebra, Preparative scale purification of fucosyl-N-acetylglucosamine disaccharides and their evaluation as potential prebiotics and antiadhesins, *Appl. Microbiol. Biotechnol.*, 2015, **99**(17), 7165–7176.
- 16 C. Wang, M. Zhang, H. Guo, J. Yan, L. Chen, W. Teng, *et al.*, Human Milk Oligosaccharides Activate Epidermal Growth Factor Receptor and Protect Against Hypoxia-

- Induced Injuries in the Mouse Intestinal Epithelium and Caco2 Cells, *J. Nutr.*, 2020, **150**(4), 756–762.
- 17 S. Zehra, I. Khambati, M. Vierhout, M. F. Mian, R. Buck and P. Forsythe, Human Milk Oligosaccharides Attenuate Antigen–Antibody Complex Induced Chemokine Release from Human Intestinal Epithelial Cell Lines, *J. Food Sci.*, 2018, **83**(2), 499–508.
 - 18 S. Figueroa-Lozano and P. de Vos, Relationship Between Oligosaccharides and Glycoconjugates Content in Human Milk and the Development of the Gut Barrier, *Compr. Rev. Food Sci. Food Saf.*, 2019, **18**(1), 121–139.
 - 19 J. C. Yu, H. Khodadadi, A. Malik, B. Davidson, S. L. Salles É da, J. Bhatia, *et al.*, Innate Immunity of Neonates and Infants, *Front. Immunol.*, 2018, **9**(July), 1759.
 - 20 Y. Y. He, S. B. Liu, D. E. Kling, S. Leone, N. T. Lawlor, Y. Huang, *et al.*, The human milk oligosaccharide 2'-fucosyllactose modulates CD14 expression in human enterocytes, thereby attenuating LPS-induced inflammation, *Gut*, 2016, **65**(1), 33–46.
 - 21 L. Cheng, M. B. G. Kiewiet, A. Groeneveld, A. Nauta and P. de Vos, Human milk oligosaccharides and its acid hydrolysate LNT2 show immunomodulatory effects via TLRs in a dose and structure-dependent way, *J. Funct. Foods*, 2019, **59**(May), 174–184.
 - 22 L. Cheng, C. Kong, M. T. C. Walvoort, M. M. Faas and P. de Vos, Human Milk Oligosaccharides Differently Modulate Goblet Cells Under Homeostatic, Proinflammatory Conditions and ER Stress, *Mol. Nutr. Food Res.*, 2019, **1900976**, e1900976.
 - 23 H. D. Holscher, L. Bode and K. A. Tappenden, Human Milk Oligosaccharides Influence Intestinal Epithelial Cell Maturation in Vitro, *J. Pediatr. Gastroenterol. Nutr.*, 2017, **64**(2), 296–301.
 - 24 C. Kunz, S. Rudloff, W. Baier, N. Klein and S. Strobel, Oligosaccharides in human milk: structural, functional, and metabolic aspects, *Annu. Rev. Nutr.*, 2000, **20**(1), 699–722.
 - 25 K. A. Lackey, R. M. Pace, J. E. Williams, L. Bode, S. M. Donovan, K. M. Järvinen, *et al.*, SARS-CoV-2 and human milk: What is the evidence?, *Matern. Child Nutr.*, 2020, 1–26.
 - 26 S. A. Rasmussen and D. J. Jamieson, Coronavirus disease 2019 (COVID-19) and pregnancy: Responding to a rapidly evolving situation, *Obstet. Gynecol.*, 2020, **135**(5), 999–1002.
 - 27 R. M. Lawrence and R. A. Lawrence, Breast milk and infection, *Clin. Perinatol.*, 2004, **31**(3), 501–528.
 - 28 R. Akkerman, M. M. Faas and P. de Vos, Non-digestible carbohydrates in infant formula as substitution for human milk oligosaccharide functions: Effects on microbiota and gut maturation, *Crit. Rev. Food Sci. Nutr.*, 2018, **59**(9), 1486–1497.
 - 29 H. D. C. R. Júnior, T. C. M. Ribeiro, A. P. De Mattos, M. Pontes, R. O. S. Sarni, M. L. S. Cruz, *et al.*, Normal growth of healthy infants born from HIV+ mothers fed a reduced protein infant formula containing the prebiotics galacto-oligosaccharides and fructo-oligosaccharides: a randomized controlled trial, *Clin. Med. Insights Pediatr.*, 2015, **9**, 37–47.
 - 30 F. Huet, M. Abrahamse-Berkeveld, S. Tims, U. Simeoni, G. Beley, C. Savagner, *et al.*, Partly Fermented Infant Formulae With Specific Oligosaccharides Support Adequate Infant Growth and Are Well-Tolerated, *J. Pediatr. Gastroenterol. Nutr.*, 2016, **63**(4), e43–e53.
 - 31 J. A. Garthoff, S. Heemskerk, R. A. Hempenius, B. A. R. Lina, C. A. M. Krul, J. H. Koeman, *et al.*, Safety evaluation of pectin-derived acidic oligosaccharides (pAOS): Genotoxicity and sub-chronic studies, *Regul. Toxicol. Pharmacol.*, 2010, **57**(1), 31–42.
 - 32 C. Sierra, M. J. Bernal, J. Blasco, R. Martínez, J. Dalmau, I. Ortuño, *et al.*, Prebiotic effect during the first year of life in healthy infants fed formula containing GOS as the only prebiotic: a multicentre, randomised, double-blind and placebo-controlled trial, *Eur. J. Nutr.*, 2014, **54**(1), 89–99.
 - 33 L. Van den Bossche, *Bile acids as novel therapeutic agents for inflammatory bowel disease*, PhD Thesis, 2017, (January).
 - 34 S. M. Jandhyala, R. Talukdar, C. Subramanyam, H. Vuyyuru, M. Sasikala and D. N. Reddy, Role of the normal gut microbiota, *World J. Gastroenterol.*, 2015, **21**(29), 8836–8847.
 - 35 A. O'Sullivan, M. Farver and J. T. Smilowitz, The Influence of early infant-feeding practices on the intestinal microbiome and body composition in infants, *Nutr. Metab. Insights*, 2015, **8**, 1–9.
 - 36 M. E. Perez-Muñoz, M. C. Arrieta, A. E. Ramer-Tait and J. Walter, A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: Implications for research on the pioneer infant microbiome, *Microbiome*, 2017, **5**(1), 1–19.
 - 37 L. W. J. van den Elsen, J. Garssen, R. Burcelin and V. Verhasselt, Shaping the Gut Microbiota by Breastfeeding: The Gateway to Allergy Prevention?, *Front. Pediatr.*, 2019, **7**(February), 1–10.
 - 38 C. Milani, S. Duranti, F. Bottacini, E. Casey, F. Turrone, J. Mahony, C. Belzer, S. D. Palacio, S. A. Montes, L. Mancabelli, G. A. Lugli, *et al.*, The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota, *Microbiol. Mol. Biol. Rev.*, 2017, **81**(4), 1–67.
 - 39 P. M. Munyaka, E. Khafipour and J. E. Ghia, External influence of early childhood establishment of gut microbiota and subsequent health implications, *Front. Pediatr.*, 2014, **2**(Oct), 1–9.
 - 40 F. Bäckhed, J. Roswall, Y. Peng, Q. Feng, H. Jia, P. Kovatcheva-Datchary, *et al.*, Dynamics and stabilization of the human gut microbiome during the first year of life, *Cell Host Microbe*, 2015, **17**(5), 690–703.
 - 41 E. Rutayisire, K. Huang, Y. Liu and F. Tao, The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: A systematic review, *BMC Gastroenterol.*, 2016, **16**(1), 1–12.

- 42 H. Makino, A. Kushiro, E. Ishikawa, H. Kubota, A. Gawad, T. Sakai, *et al.*, Mother-to-infant transmission of intestinal bifidobacterial strains has an impact on the early development of vaginally delivered infant's microbiota, *PLoS One*, 2013, **8**(11), e78331.
- 43 G. Galazzo, N. van Best, L. Bervoets, I. O. Dapaah, P. H. Savelkoul, M. W. Hornef, *et al.*, Development of the Microbiota and Associations With Birth Mode, Diet, and Atopic Disorders in a Longitudinal Analysis of Stool Samples, Collected From Infancy Through Early Childhood, *Gastroenterology*, 2020, (March), 1–13.
- 44 E. Rinninella, P. Raoul, M. Cintoni, F. Franceschi, G. A. D. Miggiano, A. Gasbarrini, *et al.*, What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases, *Microorganisms*, 2019, **7**(1), 14.
- 45 M. Tanaka and J. Nakayama, Development of the gut microbiota in infancy and its impact on health in later life, *Allergol. Int.*, 2017, **66**(4), 515–522.
- 46 X. He, M. Parenti, T. Grip, B. Lönnerdal, N. Timby, M. Domellöf, *et al.*, Fecal microbiome and metabolome of infants fed bovine MFGM supplemented formula or standard formula with breast-fed infants as reference: a randomized controlled trial, *Sci. Rep.*, 2019, **9**(1), 1–14.
- 47 M. Kalliomäki, M. C. Collado, S. Salminen and E. Isolauri, Early differences in fecal microbiota composition in children may predict overweight, *Am. J. Clin. Nutr.*, 2008, **87**(3), 534–538.
- 48 V. Singla and S. Chakkaravarthi, Applications of prebiotics in food industry: A review, *Food Sci. Technol. Int.*, 2017, **23**(8), 649–667.
- 49 X. Fu, Z. Liu, C. Zhu, H. Mou and Q. Kong, Nondigestible carbohydrates, butyrate, and butyrate-producing bacteria, *Crit. Rev. Food Sci. Nutr.*, 2019, **59**(S1), S130–S152.
- 50 M. A. E. Lawson, I. J. O'Neill, M. Kujawska, S. Gowrinadh Javvadi, A. Wijeyesekera, Z. Flegg, *et al.*, Breast milk-derived human milk oligosaccharides promote Bifidobacterium interactions within a single ecosystem, *ISME J.*, 2020, **14**(2), 635–648.
- 51 J. Langhorst, A. K. Koch, P. Voiss, G. J. Dobos and A. Rueffer, Distinct patterns of short-chain fatty acids during flare in patients with ulcerative colitis under treatment with mesalamine or a herbal combination of myrrh, chamomile flowers, and coffee charcoal: secondary analysis of a randomized controlled trial, *Eur. J. Gastroenterol. Hepatol.*, 2020, **32**(2), 175–180.
- 52 J. R. Brestoff and D. Artis, Commensal bacteria at the interface of host metabolism and the immune system, *Nat. Immunol.*, 2013, **14**(7), 676–684.
- 53 M. J. Logtenberg, R. Akkerman, R. An, G. D. A. Hermes, B. J. Haan, M. M. Faas, *et al.*, Fermentation of Chicory Fructo-oligosaccharides and Native Inulin by Infant Faecal Microbiota Attenuates Pro-inflammatory Responses in Immature Dendritic Cells in an Infant-age Dependent and Fructan-Specific Way, *Mol. Nutr. Food Res.*, 2020, **2000068**, 2000068.
- 54 G. Vighi, F. Marcucci, L. Sensi, G. Di Cara and F. Frati, Allergy and the gastrointestinal system, *Clin. Exp. Immunol.*, 2008, **153**(Suppl. 1), 3–6.
- 55 D. Ramanan and K. Cadwell, Intrinsic Defense Mechanisms of the Intestinal Epithelium, *Physiol. Behav.*, 2016, **19**(4), 434–441.
- 56 M. J. Koslowski, Z. Teltschik, J. Beisner, E. Schaeffeler, G. Wang, I. Kübler, *et al.*, Association of a functional variant in the wnt co-receptor LRP6 with early onset ileal Crohn's Disease, *PLoS Genet.*, 2012, **8**(2), e1002523.
- 57 P. Brandtzaeg, *Immunity in the gut: Mechanisms and functions*, in *Viral gastroenteritis: Molecular Epidemiology and Pathogenesis*, Academic Press., 2016, pp. 23–46.
- 58 H. Tezuka and T. Ohteki, Regulation of IgA production by intestinal dendritic cells and related cells, *Front. Immunol.*, 2019, **10**(Aug), 1–15.
- 59 O. I. Coleman and D. Haller, Bacterial signaling at the intestinal epithelial interface in inflammation and cancer, *Front. Immunol.*, 2018, **8**(Jan), 1–11.
- 60 J. M. Allaire, S. M. Crowley, H. T. Law, S. Y. Chang, H. J. Ko and B. A. Vallance, The Intestinal Epithelium: Central Coordinator of Mucosal Immunity, *Trends Immunol.*, 2018, **39**(9), 677–696.
- 61 A. Hausmann and W.-D. Hardt, The Interplay between Salmonella enterica Serovar Typhimurium and the Intestinal Mucosa during Oral Infection, *Microbiol. Spectrum*, 2019, **7**(2), 1–16.
- 62 S. Song, X. Li, C. Geng, Y. Li and C. Wang, Somatostatin stimulates colonic MUC2 expression through SSTR5-Notch-Hes1 signaling pathway, *Biochem. Biophys. Res. Commun.*, 2020, **521**(4), 1070–1076.
- 63 R. Y. Wu, B. Li, Y. Koike, P. Määttänen, H. Miyake, M. Cadete, *et al.*, Human Milk Oligosaccharides Increase Mucin Expression in Experimental Necrotizing Enterocolitis, *Mol. Nutr. Food Res.*, 2019, **63**(3), 1–11.
- 64 M. E. V. Johansson, J. M. Holmén Larsson and G. C. Hansson, The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**(Suppl. 1), 4659–4665.
- 65 H. Schneider, E. Berger, B. Dolan, B. Martinez-Abad, L. Arike, T. Pelaseyed, *et al.*, The human transmembrane mucin MUC17 responds to TNF α by increased presentation at the plasma membrane, *Biochem. J.*, 2019, **476**(16), 2281–2295.
- 66 T. Pelaseyed, J. H. Bergström, J. K. Gustafsson, A. Ermund, G. M. H. Birchenough, A. Schütte, *et al.*, The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system, *Immunol. Rev.*, 2014, **260**(1), 8–20.
- 67 G. Patsos and A. Corfield, Management of the human mucosal defensive barrier: Evidence for glycan legislation, *Biol. Chem.*, 2009, **390**(7), 581–590.
- 68 K. C. Johnson-Henry, K. A. Donato, G. Shen-Tu, M. Gordanpour and P. M. Sherman, Lactobacillus rhamnosus strain GG prevents enterohemorrhagic Escherichia

- coli O157:H7-induced changes in epithelial barrier function, *Infect. Immun.*, 2008, **76**(4), 1340–1348.
- 69 L. Liu, C. Wu, D. Chen, B. Yu, Z. Huang, Y. Luo, *et al.*, Selenium-Enriched Yeast Alleviates Oxidative Stress-Induced Intestinal Mucosa Disruption in Weaned Pigs, *Oxid. Med. Cell. Longevity*, 2020, **2020**, 1–11.
 - 70 S. C. Bischoff, G. Barbara, W. Buurman, T. Ockhuizen, J. D. Schulzke, M. Serino, *et al.*, Intestinal permeability - a new target for disease prevention and therapy, *BMC Gastroenterol.*, 2014, **14**(1), 1–25.
 - 71 K. Sugita and K. Kabashima, Tight junctions in the development of asthma, chronic rhinosinusitis, atopic dermatitis, eosinophilic esophagitis, and inflammatory bowel diseases, *J. Leukocyte Biol.*, 2020, **107**(5), 749–762.
 - 72 D. Dutta and H. Clevers, Organoid culture systems to study host–pathogen interactions, *Curr. Opin. Immunol.*, 2017, **48**, 15–22.
 - 73 G. M. Flaujac Lafontaine, N. M. Fish and I. F. Connerton, In vitro evaluation of the effects of commercial prebiotic GOS and FOS products on human colonic caco-2 cells, *Nutrients*, 2020, **12**(5), 1–24.
 - 74 M. B. G. Kiewiet, R. Dekkers, L. H. Ulfman, A. Groeneveld, P. De Vos and M. M. Faas, Immunomodulating protein aggregates in soy and whey hydrolysates and their resistance to digestion in an: In vitro infant gastrointestinal model: New insights in the mechanism of immunomodulatory hydrolysates, *Food Funct.*, 2018, **9**(1), 604–613.
 - 75 C. Xu, S. Yan, Y. Guo, L. Qiao, L. Ma, X. Dou, *et al.*, Lactobacillus casei ATCC 393 alleviates Enterotoxigenic Escherichia coli K88-induced intestinal barrier dysfunction via TLRs/mast cells pathway, *Life Sci.*, 2020, **244**, 117281.
 - 76 L. Bode, The functional biology of human milk oligosaccharides, *Early Hum. Dev.*, 2015, **91**(11), 619–622.
 - 77 L. Bode, Human milk oligosaccharides: Every baby needs a sugar mama, *Glycobiology*, 2012, **22**(9), 1147–1162.
 - 78 M. Elwakiel, J. A. Hageman, W. Wang, I. M. Szeto, J. B. Van Goudoever, K. A. Hettinga, *et al.*, Human Milk Oligosaccharides in Colostrum and Mature Milk of Chinese Mothers: Lewis Positive Secretor Subgroups, *J. Agric. Food Chem.*, 2018, **66**(27), 7036–7043.
 - 79 J. C. C. Davis, Z. T. Lewis, S. Krishnan, R. M. Bernstein, S. E. Moore, A. M. Prentice, *et al.*, Growth and Morbidity of Gambian Infants are Influenced by Maternal Milk Oligosaccharides and Infant Gut Microbiota, *Sci. Rep.*, 2017, **7**(August 2016), 1–16.
 - 80 M. B. Azad, B. Robertson, F. Atakora, A. B. Becker, P. Subbarao, T. J. Moraes, *et al.*, Human Milk Oligosaccharide Concentrations Are Associated with Multiple Fixed and Modifiable Maternal Characteristics, Environmental Factors, and Feeding Practices, *J. Nutr.*, 2018, **148**(11), 1733–1742.
 - 81 C. Quin, S. D. Vicaretti, N. A. Mohtarudin, A. M. Garner, D. M. Vollman, D. L. Gibson, *et al.*, Influence of sulfonated and diet-derived human milk oligosaccharides on the infant microbiome and immune markers, *J. Biol. Chem.*, 2020, **295**(12), 4035–4048.
 - 82 G. N. Bidart, J. Rodríguez-Díaz, V. Monedero and M. J. Yebra, A unique gene cluster for the utilization of the mucosal and human milk-associated glycans galacto-N-biose and lacto-N-biose in Lactobacillus casei, *Mol. Microbiol.*, 2014, **93**(3), 521–538.
 - 83 S. Asakuma, E. Hatakeyama, T. Urashima, E. Yoshida, T. Katayama, K. Yamamoto, *et al.*, Physiology of consumption of human milk oligosaccharides by infant gut-associated bifidobacteria, *J. Biol. Chem.*, 2011, **286**(40), 34583–34592.
 - 84 T. Thongaram, J. L. Hoeflinger, J. M. Chow and M. J. Miller, Human milk oligosaccharide consumption by probiotic and human-associated bifidobacteria and lactobacilli, *J. Dairy Sci.*, 2017, **100**(10), 7825–7833.
 - 85 O. Perdijk, B. P. Van, M. M. Fernandez-Gutierrez, B. E. Vanden, F. H. J. Schuren, S. Brugman, *et al.*, Sialyllactose and galactooligosaccharides promote epithelial barrier functioning and distinctly modulate microbiota composition and short chain fatty acid production in vitro, *Front. Immunol.*, 2019, **10**(Feb), 1–14.
 - 86 M. Sakanaka, A. Gotoh, K. Yoshida, T. Odamaki, H. Koguchi, J. Z. Xiao, *et al.*, Varied pathways of infant gut-associated Bifidobacterium to assimilate human milk oligosaccharides: Prevalence of the gene set and its correlation with bifidobacteria-rich microbiota formation, *Nutrients*, 2020, **12**(1), 1–21.
 - 87 K. Nishiyama, A. Nagai, K. Uribayashi, Y. Yamamoto, T. Mukai and N. Okada, Two extracellular sialidases from Bifidobacterium bifidum promote the degradation of sialyl-oligosaccharides and support the growth of Bifidobacterium breve, *Anaerobe*, 2018, **52**, 22–28.
 - 88 N. T. Baxter, A. W. Schmidt, A. Venkataraman, K. S. Kim, C. Waldron and T. M. Schmidt, Dynamics of Human Gut Microbiota and Short-Chain Fatty Acids in Response to Dietary Interventions with Three Fermentable Fibers, *mBio*, 2019, **10**(1), 1–13.
 - 89 A. Cravioto, A. Tello, H. Villafan, J. Ruiz, S. Del Vedovo and J. R. Neeser, Inhibition of localized adhesion of enteropathogenic Escherichia coli to HEP-2 cells by immunoglobulin and oligosaccharide fractions of human colostrum and breast milk, *J. Infect. Dis.*, 1991, **163**(6), 1247–1255.
 - 90 G. M. Ruiz-Palacios, L. E. Cervantes, P. Ramos, B. Chavez-Munguia and D. S. Newburg, Campylobacter jejuni binds intestinal H(O) antigen (Fucα1, 2Galβ1, 4GlcNAc), and fucosyloligosaccharides of human milk inhibit its binding and infection, *J. Biol. Chem.*, 2003, **278**(16), 14112–14120.
 - 91 P. Chen, T. Reiter, B. Huang, N. Kong and B. C. Weimer, Prebiotic oligosaccharides potentiate host protective responses against L. Monocytogenes infection, *Pathogens*, 2017, **6**(4), 1–25.
 - 92 S. Kerneis, S. S. Bilge, V. Fourel, G. Chauviere, M. H. Coconnier and A. L. Servin, Use of purified F1845 fimbrial adhesin to study localization and expression of receptors for diffusely adhering Escherichia coli during enterocytic differentiation of human colon carcinoma cell

- lines HT-29 and Caco-2 in culture, *Infect. Immun.*, 1991, **59**(11), 4013–4018.
- 93 J. V. Mysore, T. Wigginton, P. M. Simon, D. Zopf and D. A. Heman-Ackah LM, Treatment of Helicobacter Pylori Infection in Rhesus Monkeys Using a Novel Antiadhesion Compound, *Gastroenterology*, 1999, **117**(6), 1316–1325.
 - 94 G. V. Coppa, L. Zampini, T. Galeazzi, B. Facinelli, L. Ferrante, R. Capretti, *et al.*, Human milk oligosaccharides inhibit the adhesion to Caco-2 cells of diarrheal pathogens: Escherichia coli, Vibrio cholerae, and Salmonella typhi, *Pediatr. Res.*, 2006, **59**(3), 377–382.
 - 95 B. Farhadihosseinabadi, M. Salimi, B. Kazemi, A. Samadikuchaksaraei, H. Ghanbarian, M. Mozafari, *et al.*, Inducing type 2 immune response, induction of angiogenesis, and anti-bacterial and anti-inflammatory properties make Lacto-n-Neotetraose (LNnT) a therapeutic choice to accelerate the wound healing process, *Med. Hypotheses*, 2020, **134**, 109389.
 - 96 S. Angeloni, J. L. Ridet, N. Kusy, H. Gao, F. Crevoisier, S. Guinchard, *et al.*, Glycoprofiling with micro-arrays of glycoconjugates and lectins, *Glycobiology*, 2005, **15**(1), 31–41.
 - 97 C. Kong, M. Elderman, L. Cheng, B. J. de Haan, A. Nauta and P. de Vos, Modulation of Intestinal Epithelial Glycocalyx Development by Human Milk Oligosaccharides and Non-Digestible Carbohydrates, *Mol. Nutr. Food Res.*, 2019, **63**(17), e1900303.
 - 98 A. D. Werts, W. B. Fulton, M. R. Ladd, A. Saad-Eldin, Y. X. Chen, M. L. Kovler, *et al.*, A Novel Role for Necroptosis in the Pathogenesis of Necrotizing Enterocolitis, *CMGH*, 2020, **9**(3), 403–423.
 - 99 C. Wang, M. Zhang, H. Guo, J. Yan, F. Liu, J. Chen, *et al.*, Human Milk Oligosaccharides Protect against Necrotizing Enterocolitis by Inhibiting Intestinal Damage via Increasing the Proliferation of Crypt Cells, *Mol. Nutr. Food Res.*, 2019, **63**(18), 1–12.
 - 100 C. P. Sodhi, P. Wipf, Y. Yamaguchi, W. B. Fulton, M. Kovler, D. F. Niño, *et al.*, The human milk oligosaccharides 2'-fucosyllactose and 6'-sialyllactose protect against the development of necrotizing enterocolitis by inhibiting toll-like receptor 4 signaling, *Pediatr. Res.*, 2020, (February), 1–11.
 - 101 D. L. Ackerman, K. M. Craft and S. D. Townsend, Infant food applications of complex carbohydrates: Structure, synthesis, and function, *Carbohydr. Res.*, 2017, **437**, 16–27.
 - 102 Y. Vandenplas, B. Berger, V. P. Carnielli, J. Ksiazek, H. Lagström, M. S. Luna, *et al.*, Human milk oligosaccharides: 2'-fucosyllactose (2'-FL) and lacto-n-neotetraose (LNnT) in infant formula, *Nutrients*, 2018, **10**(9), 1161.
 - 103 E. Jantscher-Krenn, T. Lauwaet, L. A. Bliss, S. L. Reed, F. D. Gillin and L. Bode, Human milk oligosaccharides reduce Entamoeba histolytica attachment and cytotoxicity in vitro, *Br. J. Nutr.*, 2012, **108**(10), 1839–1846.
 - 104 M. Haarman and J. Knol, Quantitative Real-Time PCR Assays To Identify and Quantify Fecal, *Appl. Environ. Microbiol.*, 2005, **71**(5), 2318–2324.
 - 105 P. Piemontese, M. L. Gianni, C. P. Braegger, G. Chirico, C. Grüber, J. Riedler, *et al.*, Tolerance and safety evaluation in a large cohort of healthy infants fed an innovative prebiotic formula: A randomized controlled trial, *PLoS One*, 2011, **6**(11), e28010.
 - 106 J. Stam, M. van Stuijvenberg, J. Garssen, K. Knipping and P. J. J. Sauer, A mixture of three prebiotics does not affect vaccine specific antibody responses in healthy term infants in the first year of life, *Vaccine*, 2011, **29**(44), 7766–7772.
 - 107 C. Alexander, K. S. Swanson, G. C. Fahey and K. A. Garleb, Perspective: Physiologic Importance of Short-Chain Fatty Acids from Nondigestible Carbohydrate Fermentation, *Adv. Nutr.*, 2019, **10**(4), 576–589.
 - 108 G. A. Sprenger, F. Baumgärtner and C. Albermann, Production of human milk oligosaccharides by enzymatic and whole-cell microbial biotransformations, *J. Biotechnol.*, 2017, **258**(July), 79–91.
 - 109 F. Baumgärtner, L. Seitz, G. A. Sprenger and C. Albermann, Construction of Escherichia coli strains with chromosomally integrated expression cassettes for the synthesis of 2'-fucosyllactose, *Microb. Cell Fact.*, 2013, **12**(1), 1–13.
 - 110 B. J. Marriage, R. H. Buck, K. C. Goehring, J. S. Oliver and J. A. Williams, Infants Fed a Lower Calorie Formula with 2 ' FL Show Growth and 2 ' FL Uptake Like Breast-Fed Infants, *J. Pediatr. Gastroenterol. Nutr.*, 2015, **61**(6), 649–658.
 - 111 K. C. Goehring, B. J. Marriage, J. S. Oliver, J. A. Wilder, E. G. Barrett and R. H. Buck, Similar to Those Who Are Breastfed, Infants Fed a Formula Containing 2'-Fucosyllactose Have Lower Inflammatory Cytokines in a Randomized Controlled Trial, *J. Nutr.*, 2016, **146**(12), 2559–2566.
 - 112 G. Puccio, P. Alliet, C. Cajozzo, E. Janssens, G. Corsello, N. Sprenger, *et al.*, Effects of infant formula with human milk oligosaccharides on growth and morbidity: A randomized multicenter trial, *J. Pediatr. Gastroenterol. Nutr.*, 2017, **64**(4), 624–631.
 - 113 M. W. Larsson, V. Lind M, R. P. Laursen, C. Yonemitsu, A. Larnkjær, C. Mølgaard, *et al.*, Human Milk Oligosaccharide Composition Is Associated With Excessive Weight Gain During Exclusive Breastfeeding—An Explorative Study, *Front. Pediatr.*, 2019, **7**(July), 1–14.
 - 114 Z. T. Lewis, S. M. Totten, J. T. Smilowitz, M. Popovic, E. Parker, D. G. Lemay, *et al.*, Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants, *Microbiome*, 2015, **3**(1), 15–17.
 - 115 P. Van den Abbeele, C. Duysburgh, E. Vazquez, J. Chow, R. Buck and M. Marzorati, 2'-Fucosyllactose alters the composition and activity of gut microbiota from formula-fed infants receiving complementary feeding in a validated intestinal model, *J. Funct. Foods*, 2019, **61**(March), 103484.
 - 116 S. Weichert, S. Jennewein, E. Hübner, C. Weiss, J. Borkowski, J. Putze, *et al.*, Bioengineered 2'-fucosyllac-

- tose and 3-fucosyllactose inhibit the adhesion of *Pseudomonas aeruginosa* and enteric pathogens to human intestinal and respiratory cell lines, *Nutr. Res.*, 2013, **33**(10), 831–838.
- 117 B. Facinelli, E. Marini, G. Magi, L. Zampini, L. Santoro, C. Catassi, *et al.*, Breast milk oligosaccharides: effects of 2'-fucosyllactose and 6'-sialyllactose on the adhesion of *Escherichia coli* and *Salmonella typhimurium* to Caco-2 cells, *J. Matern.-Fetal Neonatal Med.*, 2019, **32**(17), 2950–2952.
 - 118 Z.-T. Yu, N. N. Nanthakumar and D. S. Newburg, The Human Milk Oligosaccharide 2'-Fucosyllactose Quenches *Campylobacter jejuni*-Induced Inflammation in Human Epithelial Cells HEP-2 and HT-29 and in Mouse Intestinal Mucosa, *J. Nutr.*, 2016, **146**(10), 1980–1990.
 - 119 G. Duska-McEwen, A. P. Senft, T. L. Ruetschilling, E. G. Barrett and R. H. Buck, Human Milk Oligosaccharides Enhance Innate Immunity to Respiratory Syncytial Virus and Influenza in Vitro, *Food Nutr. Sci.*, 2014, **05**(14), 1387–1398.
 - 120 D. R. Laucirica, V. Triantis, R. Schoemaker, M. K. Estes and S. Ramani, Milk Oligosaccharides Inhibit Human Rotavirus Infectivity in MA104 Cells, *J. Nutr.*, 2017, **147**(9), 1709–1714.
 - 121 A. Koromyslova, S. Tripathi, V. Morozov, H. Schroten and G. S. Hansman, Human norovirus inhibition by a human milk oligosaccharide, *Virology*, 2017, **508**(February), 81–89.
 - 122 G. T. Macfarlane, H. Steed and S. Macfarlane, Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics, *J. Appl. Microbiol.*, 2008, **104**(2), 305–344.
 - 123 T. Matsuki, S. Tajima, T. Hara, K. Yahagi, E. Ogawa and H. Kodama, Infant formula with galacto-oligosaccharides (OM55N) stimulates the growth of indigenous bifidobacteria in healthy term infants, *Benefic. Microbes*, 2016, **7**(4), 453–461.
 - 124 D. Paganini, M. A. Uyoga, C. I. Cercamondi, D. Moretti, E. Mwasi, C. Schwab, *et al.*, Consumption of galacto-oligosaccharides increases iron absorption from a micronutrient powder containing ferrous fumarate and sodium iron EDTA: A stable-isotope study in Kenyan infants, *Am. J. Clin. Nutr.*, 2017, **106**(4), 1020–1031.
 - 125 K. Shoaf, G. L. Mulvey, G. D. Armstrong and R. W. Hutkins, Prebiotic galactooligosaccharides reduce adherence of enteropathogenic *Escherichia coli* to tissue culture cells, *Infect. Immun.*, 2006, **74**(12), 6920–6928.
 - 126 H. M. Sarabia-Sainz, C. Armenta-Ruiz, J. A. I. Sarabia-Sainz, A. M. Guzmán-Partida, A. I. Ledesma-Osuna, L. Vázquez-Moreno, *et al.*, Adhesion of enterotoxigenic *Escherichia coli* strains to neoglycans synthesised with prebiotic galactooligosaccharides, *Food Chem.*, 2013, **141**(3), 2727–2734.
 - 127 L. E. J. Searle, W. A. Cooley, G. Jones, A. Nunez, B. Crudgington, U. Weyer, *et al.*, Purified galactooligosaccharide, derived from a mixture produced by the enzymic activity of *Bifidobacterium bifidum*, reduces *Salmonella enterica* serovar Typhimurium adhesion and invasion in vitro and in vivo, *J. Med. Microbiol.*, 2010, **59**(12), 1428–1439.
 - 128 H. Kittana, M. I. Quintero-Villegas, L. B. Bindels, J. C. Gomes-Neto, R. J. Schmaltz, R. R. Segura Munoz, *et al.*, Galactooligosaccharide supplementation provides protection against citrobacter rodentium-induced colitis without limiting pathogen burden, *Microbiology*, 2018, **164**(2), 154–162.
 - 129 P. Akbari, J. Fink-Gremmels, R. H. A. M. Willems, E. Difilippo, H. A. Schols, M. H. C. Schoterman, *et al.*, Characterizing microbiota-independent effects of oligosaccharides on intestinal epithelial cells: insight into the role of structure and size: Structure–activity relationships of non-digestible oligosaccharides, *Eur. J. Nutr.*, 2017, **56**(5), 1919–1930.
 - 130 M. Bermudez-Brito, N. M. Sahasrabudhe, C. Rösch, H. A. Schols, M. M. Faas and P. De Vos, The impact of dietary fibers on dendritic cell responses in vitro is dependent on the differential effects of the fibers on intestinal epithelial cells, *Mol. Nutr. Food Res.*, 2015, **59**(4), 698–710.
 - 131 J. Wang, S. Tian, H. Yu, J. Wang and W. Zhu, Response of Colonic Mucosa-Associated Microbiota Composition, Mucosal Immune Homeostasis, and Barrier Function to Early Life Galactooligosaccharides Intervention in Suckling Piglets, *J. Agric. Food Chem.*, 2019, **67**(2), 578–588.
 - 132 A. Firmansyah, N. Chongviriyaphan, D. H. S. Dillon, N. C. Khan, T. Morita, K. Tontisirin, *et al.*, Fructans in the first 1000 days of life and beyond, and for pregnancy, *Asia Pac. J. Clin. Nutr.*, 2016, **25**(4), 652–675.
 - 133 F. T. Moerman, M. B. Van Leeuwen and J. A. Delcour, Enrichment of higher molecular weight fractions in inulin, *J. Agric. Food Chem.*, 2004, **52**(12), 3780–3783.
 - 134 K. Chen, H. Chen, M. M. Faas, B. J. de Haan, J. Li, P. Xiao, *et al.*, Specific inulin-type fructan fibers protect against autoimmune diabetes by modulating gut immunity, barrier function, and microbiota homeostasis, *Mol. Nutr. Food Res.*, 2017, **61**(8), 1601006.
 - 135 H. Oswari, A. D. Widodo, F. Handayani, M. Juffrie, T. Sundjaya, J. Bindels, *et al.*, Dosage-related prebiotic effects of inulin in formula-fed infants, *Pediatr. Gastroenterol. Hepatol. Nutr.*, 2019, **22**(1), 63–71.
 - 136 A. R. Euler, D. K. Mitchell, R. Kline and L. K. Pickering, Prebiotic effect of fructo-oligosaccharide supplemented term infant formula at two concentrations compared with unsupplemented formula and human milk, *J. Pediatr. Gastroenterol. Nutr.*, 2005, **40**(2), 157–164.
 - 137 Q. Xia, T. Williams, D. Hustead, P. Price, M. Morrison and Z. Yu, Quantitative analysis of intestinal bacterial populations from term infants fed formula supplemented with fructo-oligosaccharides, *J. Pediatr. Gastroenterol. Nutr.*, 2012, **55**(3), 314–320.
 - 138 N. M. Koropatkin, E. A. Cameron and E. C. Martens, How glycan metabolism shapes the human gut microbiota, *Nat. Rev. Microbiol.*, 2012, **10**(5), 323–335.

- 139 S. Fehlbauer, K. Prudence, J. Kieboom, M. Heerikhuisen, T. van den Broek, F. H. J. Schuren, *et al.*, In vitro fermentation of selected prebiotics and their effects on the composition and activity of the adult gut microbiota, *Int. J. Mol. Sci.*, 2018, **19**(10), 3097.
- 140 S. Lohner, V. Jakobik, K. Mihályi, S. Soldi, S. Vasileiadis, S. Theis, *et al.*, Inulin-type fructan supplementation of 3- To 6-year-old children is associated with higher fecal Bifidobacterium concentrations and fewer febrile episodes requiring medical attention, *J. Nutr.*, 2018, **148**(8), 1300–1308.
- 141 D. Paineau, F. Respondek, V. Menet, R. Sauvage, F. Bornet and A. Wagner, Effects of short-chain fructooligosaccharides on faecal bifidobacteria and specific immune response in formula-fed term infants: A randomized, double-blind, placebo-controlled trial, *J. Nutr. Sci. Vitaminol.*, 2014, **60**(3), 167–175.
- 142 R. Takagi, Y. Tsujikawa, R. Nomoto and R. Osawa, Comparison of the Growth of *Lactobacillus delbrueckii*, *L. paracasei* and *L. plantarum* on Inulin in Co-culture Systems, *Biosci. Microbiota, Food Health*, 2014, **33**(4), 139–146.
- 143 O. Hernandez-Hernandez, A. Muthaiyan, F. J. Moreno, A. Montilla, M. L. Sanz and S. C. Ricke, Effect of prebiotic carbohydrates on the growth and tolerance of *Lactobacillus*, *Food Microbiol.*, 2012, **30**(2), 355–361.
- 144 P. Kanjan and T. Hongpattarakere, Prebiotic efficacy and mechanism of inulin combined with inulin-degrading *Lactobacillus paracasei* I321 in competition with *Salmonella*, *Carbohydr. Polym.*, 2017, **169**, 236–244.
- 145 J. Luettig, R. Rosenthal, C. Barmeyer and J. D. Schulzke, Claudin-2 as a mediator of leaky gut barrier during intestinal inflammation, *Tissue Barriers*, 2015, **3**(1), e977176.
- 146 P. V. D. Abbeele, C. Grootaert, S. Possemiers, W. Verstraete, K. Verbeken and T. Van De Wiele, In vitro model to study the modulation of the mucin-adhered bacterial community, *Appl. Microbiol. Biotechnol.*, 2009, **83**(2), 349–359.
- 147 M. Piotrowski, D. Wultańska, P. Obuch-Woszczatyński and H. Pituch, Fructooligosaccharides and mannose affect *Clostridium difficile* adhesion and biofilm formation in a concentration-dependent manner, *Eur. J. Clin. Microbiol. Infect. Dis.*, 2019, **38**(10), 1975–1984.
- 148 R. Y. Wu, M. Abdullah, P. Määttänen, A. V. C. Pilar, E. Scruten, K. C. Johnson-Henry, *et al.*, Protein kinase C σ signaling is required for dietary prebiotic-induced strengthening of intestinal epithelial barrier function, *Sci. Rep.*, 2017, **7**(September 2016), 1–10.
- 149 L. Vogt, U. Ramasamy, D. Meyer, G. Pullens, K. Venema, M. M. Faas, *et al.*, Immune Modulation by Different Types of β 2 $\rightarrow$ 1-Fructans Is Toll-Like Receptor Dependent, *PLoS One*, 2013, **8**(7), 1–12.
- 150 H. Cheng, Z. Zhang, J. Leng, D. Liu, M. Hao, X. Gao, *et al.*, The inhibitory effects and mechanisms of rhamnogalacturonan i pectin from potato on HT-29 colon cancer cell proliferation and cell cycle progression, *Int. J. Food Sci. Nutr.*, 2013, **64**(1), 36–43.
- 151 B. Knaup, M. Kempf, J. Fuchs, A. Valotis, K. Kahle, A. Oehme, *et al.*, Model experiments mimicking the human intestinal transit and metabolism of D-galacturonic acid and amidated pectin, *Mol. Nutr. Food Res.*, 2008, **52**(7), 840–848.
- 152 B. Yapo and K. Koffi, Extraction and Characterization of Highly Gelling Low Methoxy Pectin from Cashew Apple Pomace, *Foods*, 2013, **3**(1), 1–12.
- 153 R. Zhu, M. Hong, C. Zhuang, L. Zhang, C. Wang, J. Liu, *et al.*, Pectin oligosaccharides from hawthorn (*Crataegus pinnatifida* Bunge. Var. major) inhibit the formation of advanced glycation end products in infant formula milk powder, *Food Funct.*, 2019, **10**(12), 8081–8093.
- 154 N. Larsen, C. B. De Souza, L. Krych, T. B. Cahú, M. Wiese, W. Kot, *et al.*, Potential of pectins to beneficially modulate the gut microbiota depends on their structural properties, *Front. Microbiol.*, 2019, **10**(Feb), 1–13.
- 155 M. Beukema, M. M. Faas and P. V. de, The effects of different dietary fiber pectin structures on the gastrointestinal immune barrier: impact via gut microbiota and direct effects on immune cells, *Exp. Mol. Med.*, 2020, **52**, 1364–1376.
- 156 A. A. Salyers, S. E. H. West, J. R. Vercellotti and T. D. Wilkins, Fermentation of mucins and plant polysaccharides by anaerobic bacteria from the human colon, *Appl. Environ. Microbiol.*, 1977, **34**(5), 529–533.
- 157 P. O. Sheridan, J. C. Martin, T. D. Lawley, H. P. Browne, H. M. B. Harris, A. Bernalier-Donadille, *et al.*, Polysaccharide utilization loci and nutritional specialization in a dominant group of butyrate-producing human colonic firmicutes, *Microb. Genom.*, 2016, **2**(2), 1–16.
- 158 W. S. F. Chung, M. Meijerink, B. Zeuner, J. Holck, P. Louis, A. S. Meyer, *et al.*, Prebiotic potential of pectin and pectic oligosaccharides to promote anti-inflammatory commensal bacteria in the human colon, *FEMS Microbiol. Ecol.*, 2017, **93**(11), 1–9.
- 159 F. Bianchi, N. Larsen, T. de Mello Tieghi, M. A. T. Adorno, W. Kot, S. M. I. Saad, *et al.*, Modulation of gut microbiota from obese individuals by in vitro fermentation of citrus pectin in combination with *Bifidobacterium longum* BB-46, *Appl. Microbiol. Biotechnol.*, 2018, **102**(20), 8827–8840.
- 160 S. Fanaro, J. Jelinek, B. Stahl, G. Boehm and R. Kock VV, Acidic oligosaccharides from pectin hydrolysate as new component for infant formulae: Effect on intestinal flora, stool characteristics, and pH, *J. Pediatr. Gastroenterol. Nutr.*, 2005, **41**(2), 186–190.
- 161 T. P. N. Bui, H. A. Schols, M. Jonathan, A. J. M. Stams, W. M. de Vos and C. M. Plugge, Mutual Metabolic Interactions in Co-cultures of the Intestinal Anaerostipes rhamnosivorans With an Acetogen, Methanogen, or Pectin-Degrader Affecting Butyrate Production, *Front. Microbiol.*, 2019, **10**(November), 1–12.
- 162 S. J. Bang, G. Kim, M. Y. Lim, E. J. Song, D. H. Jung, J. S. Kum, *et al.*, The influence of in vitro pectin fermentation on the human fecal microbiome, *AMB Express*, 2018, **8**(1), 98.

- 163 J. Rhoades, K. Manderson, A. Wells, A. T. Hotchkiss, G. R. Gibson, K. Formentin, *et al.*, Oligosaccharide-mediated inhibition of the adhesion of pathogenic *Escherichia coli* strains to human gut epithelial cells in vitro, *J. Food Prot.*, 2008, **71**(11), 2272–2277.
- 164 R. Di, M. S. Vakkalanka, C. Onumpai, H. K. Chau, A. White, R. A. Rastall, *et al.*, Pectic oligosaccharide structure-function relationships: Prebiotics, inhibitors of *Escherichia coli* O157:H7 adhesion and reduction of Shiga toxin cytotoxicity in HT29 cells, *Food Chem.*, 2017, **227**, 245–254.
- 165 C. Thöle, S. Brandt, N. Ahmed and A. Hensel, Acetylated rhamnogalacturonans from immature fruits of *Abelmoschus Esculentus* inhibit the adhesion of *helicobacter pylori* to human gastric cells by interaction with outer membrane proteins, *Molecules*, 2015, **20**(9), 16770–16787.
- 166 M. Nishida, K. Murata, Y. Kanamaru and T. Yabe, Pectin of *Prunus domestica* L. alters sulfated structure of cell-surface heparan sulfate in differentiated Caco-2 cells through stimulation of heparan sulfate 6-O-endosulfatase-2, *Biosci., Biotechnol., Biochem.*, 2014, **78**(4), 635–643.
- 167 M. Nishida, K. Murata, K. Oshima, C. Itoh, K. Kitaguchi, Y. Kanamaru, *et al.*, Pectin from *Prunus domestica* L. induces proliferation of IEC-6 cells through the alteration of cell-surface heparan sulfate on differentiated Caco-2 cells in co-culture, *Glycoconjugate J.*, 2015, **32**(3–4), 153–159.
- 168 A. P. Vos, M. Haarman, J. W. H. VanGinkel, J. Knol, J. Garssen, B. Stahl, *et al.*, Dietary supplementation of neutral and acidic oligosaccharides enhances Th1-dependent vaccination responses in mice, *Pediatr. Allergy Immunol.*, 2007, **18**(4), 304–312.
- 169 J. P. Van Den Berg, E. A. M. Westerbeek, F. R. M. Van Der Klis, E. A. M. Sanders, G. A. M. Berbers and R. M. Van Elburg, Response on Pneumococcal Vaccine in Preterm Infants After Neutral and Acidic Oligosaccharides Supplementation, *Pediatr. Infect. Dis. J.*, 2015, **34**(9), 976–982.
- 170 A. P. Vos, J. Knol, B. Stahl, L. M'Rabet and J. Garssen, Specific prebiotic oligosaccharides modulate the early phase of a murine vaccination response, *Int. Immunopharmacol.*, 2010, **10**(5), 619–625.
- 171 N. M. Sahasrabudhe, M. Beukema, L. Tian, B. Troost, J. Scholte, E. Bruininx, *et al.*, Dietary fiber pectin directly blocks toll-like receptor 2–1 and prevents doxorubicin-induced ileitis, *Front. Immunol.*, 2018, **9**(Mar), 1–19.
- 172 Y. Sun, Y. He, F. Wang, H. Zhang, P. de Vos and J. Sun, Low-methoxyl lemon pectin attenuates inflammatory responses and improves intestinal barrier integrity in caerulein-induced experimental acute pancreatitis, *Mol. Nutr. Food Res.*, 2017, **61**(4), 1–9.
- 173 L. Tian, G. Bruggeman, M. van den Berg, K. Borewicz, A. J. W. Scheurink, E. Bruininx, *et al.*, Effects of pectin on fermentation characteristics, carbohydrate utilization, and microbial community composition in the gastrointestinal tract of weaning pigs, *Mol. Nutr. Food Res.*, 2017, **61**(1), 1–10.
- 174 J. Karczewski, F. J. Troost, I. Konings, J. Dekker, M. Kleerebezem, R.-J. M. Brummer, *et al.*, Regulation of human epithelial tight junction proteins by *Lactobacillus plantarum* in vivo and protective effects on the epithelial barrier, *Am. J. Physiol.: Gastrointest. Liver Physiol.*, 2010, **298**(6), G851–G859.
- 175 J. R. Singer, E. G. Blosser, C. L. Zindl, D. J. Silberger, S. Conlan, V. A. Laufer, *et al.*, Preventing dysbiosis of the neonatal mouse intestinal microbiome protects against late-onset sepsis, *Nat. Med.*, 2019, **25**(11), 1772–1782.
- 176 H. B. V. D. Worp, D. W. Howells, E. S. Sena, M. J. Porritt, S. Rewell, V. O'Collins, *et al.*, Can animal models of disease reliably inform human studies?, *PLoS Med.*, 2010, **7**(3), e1000245.
- 177 S. R. Finkbeiner and J. R. Spence, A gutsy task: Generating intestinal tissue from human pluripotent stem cells, *Dig. Dis Sci.*, 2013, **58**(5), 1176–1184.
- 178 A. Grassart, V. Malardé, S. Gobba, A. Sartori-Rupp, J. Kerns, K. Karalis, *et al.*, Bioengineered Human Organ-on-Chip Reveals Intestinal Microenvironment and Mechanical Forces Impacting Shigella Infection, *Cell Host Microbe*, 2019, **26**(3), 435–444.
- 179 S. J. A. Buczacki, H. I. Zecchini, A. M. Nicholson, R. Russell, L. Vermeulen, R. Kemp, *et al.*, Intestinal label-retaining cells are secretory precursors expressing lgr5, *Nature*, 2013, **495**(7439), 65–69.
- 180 P. Brooks, T. Zur Bruegge, E. C. Boyle, S. Kalies, S. N. Villarreal, A. Liese, *et al.*, CD14 and ALPK1 Affect Expression of Tight Junction Components and Proinflammatory Mediators upon Bacterial Stimulation in a Colonic 3D Organoid Model, *Stem Cells Int.*, 2020, **2020**, 4069354.
- 181 R. Okamoto, H. Shimizu, K. Suzuki, A. Kawamoto, J. Takahashi, M. Kawai, *et al.*, Organoid-based regenerative medicine for inflammatory bowel disease, *Regen. Ther.*, 2020, **13**, 1–6.
- 182 D. Qi, W. Shi, A. R. Black, M. A. Kuss, X. Pang, Y. He, *et al.*, Repair and regeneration of small intestine: A review of current engineering approaches, *Biomaterials*, 2020, **240**(December 2018), 119832.
- 183 R. Cruz-Acuña, M. Quirós, A. E. Farkas, P. H. Dedhia, S. Huang, D. Siuda, *et al.*, Synthetic hydrogels for human intestinal organoid generation and colonic wound repair, *Nat. Cell Biol.*, 2017, **19**(11), 1326–1335.
- 184 S. Yoshida, H. Miwa, T. Kawachi, S. Kume and K. Takahashi, Generation of intestinal organoids derived from human pluripotent stem cells for drug testing, *Sci. Rep.*, 2020, **10**(1), 5989.
- 185 J. Barrila, A. Crabbé, J. Yang, K. Franco, S. D. Nydam, R. J. Forsyth, *et al.*, Modeling host-pathogen interactions in the context of the microenvironment: Three-dimensional cell culture comes of age, *Infect. Immun.*, 2018, **86**(11), 1–28.
- 186 R. Moerkens, J. Mooiweer, S. Withoff and C. Wijmenga, Celiac disease-on-chip: Modeling a multifactorial disease in vitro, *United Eur. Gastroenterol. J.*, 2019, **7**(4), 467–476.

- 187 J. In, J. Foulke-Abel, N. C. Zachos, A. M. Hansen, J. B. Kaper, H. D. Bernstein, *et al.*, Enterohemorrhagic *Escherichia coli* Reduces Mucus and Intermicrovillar Bridges in Human Stem Cell-Derived Colonoids, *Cell. Mol. Gastroenterol. Hepatol.*, 2016, **2**(1), 48–62.
- 188 M. Rimoldi, M. Chieppa, V. Salucci, F. Avogadri, A. Sonzogni, G. M. Sampietro, *et al.*, Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells, *Nat. Immunol.*, 2005, **6**(5), 507–514.
- 189 A. F. P. Lépine, N. de Wit, E. Oosterink, H. Wichers, J. Mes and P. de Vos, *Lactobacillus acidophilus* attenuates *Salmonella*-induced stress of epithelial cells by modulating tight-junction genes and cytokine responses, *Front. Microbiol.*, 2018, **9**(Jul), 3–8.
- 190 H. J. Kim, D. Huh, G. Hamilton and D. E. Ingber, Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow, *Lab Chip*, 2012, **12**(12), 2165–2174.
- 191 T. D. Leser and L. Mølbak, Better living through microbial action: The benefits of the mammalian gastrointestinal microbiota on the host, *Environ. Microbiol.*, 2009, **11**(9), 2194–2206.
- 192 M. Eshрати, F. Amadei, S. Staffer, W. Stremmel and M. Tanaka, Shear-Enhanced Dynamic Adhesion of *Lactobacillus rhamnosus* GG on Intestinal Epithelia: Correlative Effect of Protein Expression and Interface Mechanics, *Langmuir*, 2019, **35**(2), 529–537.
- 193 M. Marzorati, B. Vanhoecke, T. De Ryck, M. Sadaghian Sadabad, I. Pinheiro, S. Possemiers, *et al.*, The HMI™ module: A new tool to study the Host-Microbiota Interaction in the human gastrointestinal tract in vitro, *BMC Microbiol.*, 2014, **14**, 133.
- 194 Y. S. Kim and S. B. Ho, Intestinal goblet cells and mucins in health and disease: Recent insights and progress, *Curr. Gastroenterol. Rep.*, 2010, **12**(5), 319–330.
- 195 D. Maggiorani, R. Dissard, M. Belloy, J. S. Saulnier-Blache, A. Casemayou, L. Ducasse, *et al.*, Shear stress-induced alteration of epithelial organization in human renal tubular cells, *PLoS One*, 2015, **10**(7), 1–21.
- 196 M. Soyer and G. Duménil, Introducing Shear Stress in the Study of Bacterial Adhesion, *J. Visualized Exp.*, 2011, (55), 2–6.
- 197 L. C. Delon, Z. Guo, A. Oszmiana, C. C. Chien, R. Gibson, C. Prestidge, *et al.*, A systematic investigation of the effect of the fluid shear stress on Caco-2 cells towards the optimization of epithelial organ-on-chip models, *Biomaterials*, 2019, **225**(December 2018), 119521.
- 198 H. J. Kim, H. Li, J. J. Collins and D. E. Ingber, Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**(1), E7–15.
- 199 C. Kong, L. Cheng, G. Krenning, J. Fledderus, B. J. de Haan, M. T. C. Walvoort, *et al.*, Human Milk Oligosaccharides Mediate the Crosstalk Between Intestinal Epithelial Caco-2 Cells and *Lactobacillus Plantarum* WCFS1 in an In Vitro Model with Intestinal Peristaltic Shear Force, *J. Nutr.*, 2020, **150**(8), 2077–2088.
- 200 L. M. Vogt, M. E. Elderman, T. Borghuis, B. J. de Haan, M. M. Faas and P. de Vos, Chain length-dependent effects of inulin-type fructan dietary fiber on human systemic immune responses against hepatitis-B, *Mol. Nutr. Food Res.*, 2017, **61**(10), 1–11.
- 201 P. G. Thomas, M. R. Carter, O. Atochina, A. A. Da'Dara, D. Piskorska, E. McGuire, *et al.*, Maturation of Dendritic Cell 2 Phenotype by a Helminth Glycan Uses a Toll-Like Receptor 4-Dependent Mechanism, *J. Immunol.*, 2003, **171**(11), 5837–5841.
- 202 A. Berger, Science commentary: Th1 and Th2 responses: What are they?, *Br. Med. J.*, 2000, **321**(7258), 424.
- 203 J. C. C. Davis, S. M. Totten, J. O. Huang, S. Nagshbandi, N. Kirmiz, D. A. Garrido, *et al.*, Identification of Oligosaccharides in Feces of Breast-fed Infants and Their Correlation with the Gut Microbial Community, *Mol. Cell. Proteomics*, 2016, **15**(9), 2987–3002.
- 204 K. Borewicz, F. Gu, E. Saccenti, C. Hechler, R. Beijers, C. de Weerth, *et al.*, The association between breastmilk oligosaccharides and faecal microbiota in healthy breastfed infants at two, six, and twelve weeks of age, *Sci. Rep.*, 2020, **10**(1), 1–12.