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Babies, Bugs, and Barriers: Dietary Modulation of Intestinal Barrier Function in Early Life

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Keywords

intestinal barrier, breastfeeding, early life nutrition, immunity, infection, inflammation

Abstract

The intestinal barrier is essential in early life to prevent infection, inflammation, and food allergies. It consists of microbiota, a mucus layer, an epithelial layer, and the immune system. Microbial metabolites, the mucus, antimicrobial peptides, and secretory immunoglobulin A (sIgA) protect the intestinal mucosa against infection. The complex interplay between these functionalities of the intestinal barrier is crucial in early life by supporting homeostasis, development of the intestinal immune system, and long-term gut health. Exclusive breastfeeding is highly recommended during the first 6 months. When breastfeeding is not possible, milk-based infant formulas are a safe alternative. Breast milk contains many bioactive components that help to establish the intestinal microbiota and influence the development of the intestinal epithelium and the immune system. Importantly, breastfeeding lowers the risk for intestinal and respiratory tract infections. Here we review all aspects of intestinal barrier function and the nutritional components that impact its functionality in early life, such as

micronutrients, bioactive milk proteins, milk lipids, and human milk oligosaccharides. These components are present in breast milk and can be added to milk-based infant formulas to support gut health and immunity.

Contents

1. INTRODUCTION

The gastrointestinal (GI) barrier plays an important role in protection against intestinal infection, inflammation, and the development of food allergy. At birth, the infant's immune system is not fully developed, but it has already been exposed to many external stimuli such as the (intestinal) microbiota, pathogens, and innocuous proteins such as food proteins and allergens. The intestinal barrier is instrumental in coping with these challenges by functioning not only as a physical barrier but also as an immunological barrier that plays a key role in immune system development in early life.

The GI tract of the human body is where food components are digested and nutrients and water are absorbed to meet the energy need of daily activities. Another important function of the intestine is to resist the invasion of pathogens and transport of harmful substances from the gut lumen, via a physical barrier and a chemical barrier formed by intestinal cell secretions. An intact intestinal barrier, which is essential for maintaining intestinal immune homeostasis and gut health, is supported by a mucus layer, the intestinal epithelium, and the lamina propria (LP) beneath the epithelial cells (32, 33, 120). In addition, the gut microbiota and their metabolites are important biological components that regulate intestinal barrier function.

In our opinion, intestinal barrier function (described in 33, 60) comprises four multifactorial barriers (**Figure 1**):

GI: gastrointestinal

IECs: intestinal epithelial cells

1. The biological barrier, which is made up of the intestinal microbiota, provides colonization resistance, a generic term for the mechanisms by which the microbiota limits the introduction of exogenous microorganisms and overgrowth of pathobionts.
2. The mechanical barrier consists of the mucus layer and the closed lining of intestinal epithelial cells (IECs). Permeability of the lateral space between adjacent epithelial cells (termed

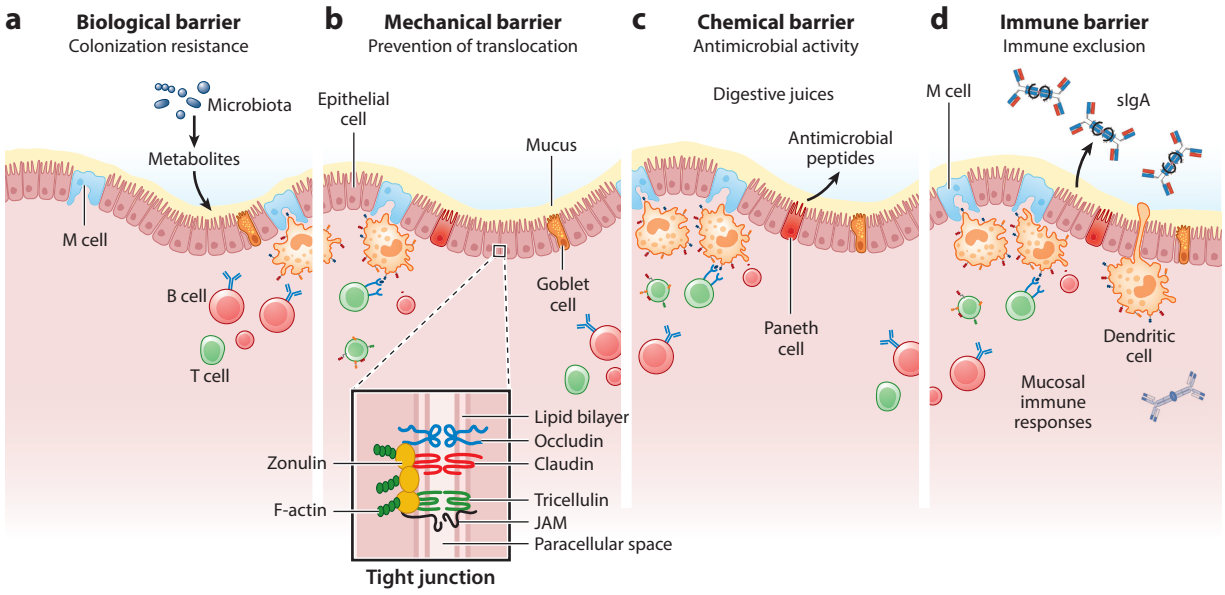

Figure 1

The multifaceted barrier functions of the intestinal epithelium, as defined in References 33 and 60. The biological barrier, the mechanical barrier, the chemical barrier, and the immune barrier together maintain intestinal barrier function. (*a*) The biological barrier consists of the microbiota and their metabolites. The microbiota can prevent the outgrowth of unwanted pathogens (colonization resistance) and produce metabolites that can strengthen the mechanical barrier. (*b*) The mechanical barrier consists of the mucus layer and the intestinal epithelial cells. The epithelial cells are joined together by TJs, as well as by the adherens junctions, desmosomes, and gap junctions (not indicated in this figure). The TJs consist of zonulins, occludins, claudins, F-actin, tricellulin, and JAMs. TJs are located at the top of the epithelial cell and are essential for preventing leakage of macromolecules across the epithelium. Panel inset adapted from Reference 261. (*c*) The chemical barrier is provided by intestinal digestive juices and antimicrobial peptides and proteins produced by enterocytes and Paneth cells. (*d*) The immune barrier consists of the cells of the immune system in the intestinal mucosa, the cytokines they produce, and sIgA that is produced by plasma cells and transported through enterocytes into the intestinal lumen. sIgA binds to bacteria, viruses, and toxins to prevent adhesion and pathogenesis (immune exclusion). Abbreviations: JAM, junctional adhesion molecule; sIgA, secretory IgA; TJ, tight junction.

the paracellular space) is controlled by apical tight junctions (TJs) consisting of a complex of cytoplasmic and membrane-associated proteins.

3. The chemical barrier refers to the secreted mucus and the physical barrier of the epithelium chemical barrier, consisting of digestive secretions, as well as antimicrobial peptides (AMPs), regenerating islet-derived protein 3 (Reg3) proteins, lysozyme, and other factors produced by epithelial cells and immune cells in the underlying mucosa (e.g., cytokines, inflammatory mediators). Additionally, membrane-associated mucins may sterically inhibit close interaction of the epithelial cell surface with bacteria and large molecules.
4. The immune barrier is composed of gut-associated lymphoid tissue (GALT), organized in Peyer's patches, lymphoid follicles, and diffuse immune cells in the LP. The key components are effector and regulatory T cells (Tregs), IgA-producing B (plasma) cells, innate lymphoid cells, resident macrophages, and dendritic cells.

TJs: tight junctions

AMPs: antimicrobial peptides

SCFAs: short-chain fatty acids

The intestinal microbial community is critical for the development and maturation of the intestinal epithelium and the immune system. Colonization resistance of pathogens by commensal bacteria occurs through competition for resources, killing of pathogens by produced bacteriocins, and inhibition of pathogen virulence or growth by short-chain fatty acids (SCFAs)

(68). In addition, microbial metabolites can impact the microbiota composition through indirect mechanisms involving the stimulation of innate and adaptive immune responses, as well as secretion of AMPs by intestinal epithelial and goblet cells (40, 186).

Barrier function is further regulated by the physical barrier of the epithelium and TJs. The epithelium regulates the paracellular permeability of micro- and macromolecules and actively transports some nutrients from the apical to the basolateral side. In addition to the epithelium, the barrier to pathogens, bacterial components, and intestinal food components is further strengthened by the secretion of highly glycosylated membrane-associated mucins, which form a physical barrier between the intestinal lumen and the epithelial cells (143). Secreted mucus is present as (*a*) a tight layer close to the epithelium that is almost devoid of bacteria and (*b*) a loose apical layer containing members of the gut microbiota that can degrade the carbohydrate structures that are present on glycosylated mucin. Less mucus is secreted in the small intestine than in the colon due to differences in the number of goblet cells. The importance of mucins in intestinal barrier function is clearly illustrated by the fact that *Muc2*-deficient mice spontaneously develop colitis.

In addition to barrier function, epithelial cells express intracellular and membrane-bound pattern recognition receptors (PRRs) of the innate immune system; PRRs bind to conserved molecular structures found on microorganisms. Signaling via these receptors upregulates expression of chemical intestinal defenses such as mucin 2, specific β-defensins, and the Reg3γ protein in humans (reviewed in 261). Additionally, PRR signaling induces expression of several cytokines and chemokines that interact with immune cells and with other somatic cells in the LP.

Sampling of the intestinal antigens by dendritic cells in the Peyer's patches or mucosa leads to antigen presentation, the induction of T and B cell responses, and the migration and homing of activated T and B cells to other mucosal sites. In the LP, mature plasma B cells produce dimeric sIgA, which is transported across the mucosal epithelium into the intestinal lumen via the polymeric IgA receptor. sIgA can neutralize bacterial toxins and prevent infection via immune exclusion, but its role in shaping the microbiota is not completely understood (175).

2. DEVELOPMENT OF THE HUMAN INTESTINAL BARRIER AFTER BIRTH

The adult intestinal barrier is well developed to protect against the infectious threats in the external environment. The situation is quite different in early life. In the neonatal intestine, immature gut mucosal immunity, underdeveloped gut epithelium architecture, and developing microbiota ecology open a window of opportunity for potential pathogens (262). Thus, neonates are more susceptible to infections than are adults. In 2013, 2.8 million neonates died globally, with almost half of these deaths caused by infections in the late neonatal periods (7–27 days after birth) (174).

There are only a few studies on postnatal development of the human intestine due to the ethical considerations. However, the maturation of the gut and the impact of maternal nutrition are of enormous interest from both a nutritional and a medical point of view in terms of caring for early preterm neonates (less than 28 weeks gestation).

Permeability of the small intestine is maintained by cell junctions between adjacent enterocytes, and the most apical TJ complexes play a key role in permeability (reviewed in 281). In some mammals, intestinal transfer of macromolecules such as Igs across the intestinal epithelium occurs in the first few days of life through epithelial endocytosis. However, in humans the intestine is thought to already be closed in utero, thus preventing transfer of large macromolecules after birth.

Intestinal permeability has been studied in healthy newborn infants by using the simple noninvasive dual-sugar test. This test is based on measuring the paracellular permeability of lactulose (L) (a large, minimally absorbed sugar) and a sugar-alcohol mannitol (M) (which is taken across

the epithelium by the transcellular pathway). The L:M ratio has been used as a marker in several studies on children under 5 years of age, although differences in the assay method and in data reporting limit interstudy comparisons (63). Catassi et al. (51) performed the dual-sugar test on full-term healthy babies 1, 7, and 30 days after birth. There was a significant decrease in the L:M ratio over time, suggesting permeability decreases over the first month of life. The decrease in permeability was faster in breastfed babies than in those fed formula milk.

NEC: necrotizing enterocolitis

In the human fetal intestine, *Muc2* expression can be detected as early as 9 weeks of gestation (45), but infants still have an underdeveloped intestinal mucus layer in the first few days after birth (240). Anatomically, the human intestinal epithelium reaches maturity with structured crypt-villus units during the gestational period (42). Moreover, Paneth cells are present in the neonatal intestine at birth (192) and contribute to the AMPs identified in the meconium and feces during the first weeks after birth (46). These AMPs presumably play a key role in protecting intestinal infections, especially given the immaturity of the adaptive immune system at birth.

Murine models show that, in the first few days of life, mRNA expression of *Muc2* and *Muc5ac* is low in the epithelium, resulting in a thinner mucus layer (241).

The formation of the gut immune system in humans begins at the embryonic stage, further develops in the fetal period, and attains maturity several years after birth. In the human fetus, Peyer's patches were found as early as 11 weeks of gestation. B and T cells without germinal centers could be clearly identified in the intestine as early as 12 to 16 weeks of gestation, continuously increasing in abundance throughout pregnancy (42). In the first 2 weeks after birth, Ig-producing plasma cells are present in relatively low numbers but increase from the first month of life and throughout the first years of life (134).

Although a number of B cells are present in the neonatal gut at birth, they are not functionally mature. Therefore, the amount of Ig in the infant mucosa is lower during the first 2 years of life than in young adults. It has been suggested that T cell–independent antibody responses are absent at birth and that only at the age of 3–5 years do they reach maturity (266). Moreover, previous studies showed that IgM, IgG, and IgA reach adult-equivalent levels after approximately 6 months, 5 years, and 15 years, respectively (156). A recent study demonstrated the age-related reference values of serum IgG, IgA, and IgM levels in healthy children. This study showed that the levels of IgG, IgA, and IgM reached maximum values at 16–18 years of age (21). Notably, IgG is the only Ig class that can be actively transferred from the mother to the fetus through placental transport to support neonatal passive immunity (38). Therefore, at birth the serum IgG concentration is greater than that of IgA and IgM but decreases over the 1–3 months of life. In conclusion, although GALT and several immune cells are present in the gut prior to and at birth (42), the adaptive immune system is far from being mature and needs to develop over time.

3. BENEFICIAL EFFECTS OF THE MICROBIOTA AND SPECIFIC BACTERIAL SPECIES ON INTESTINAL BARRIER FUNCTION

The development of the microbiota colonizing the skin and mucosal surfaces of the body after birth is influenced by environmental exposures, such as antibiotics use, mode of birth [vaginal delivery versus cesarean section (C-section)], and maternal factors such as genetics. The intestinal microbiota, in particular, undergoes large changes in the first 3 years of life and is shaped mainly by the availability of different nutrients in (breast)milk and food as well as by maternal IgA and IgG in breast milk; the microbiota at 2–3 years of age resembles that of the adult (201). Strongly altered microbiota composition and functionality are associated with many human disease states and are seen in neonatal necrotizing colitis (NEC) (reviewed recently in 236). An important protective function of the intestinal microbiota is colonization resistance to enteric pathogens, which can

damage the intestinal barrier and stimulate inflammatory responses. Such protection could be crucial in early life, when adaptive responses are still developing.

HMOs: human milk oligosaccharides

Natural birth promotes intestinal colonization by species of the vaginal microbiota (e.g., *Lactobacillus* species), whereas opportunist bacteria prevalent in hospital settings (e.g., *Enterococcus* and *Klebsiella*) are more abundant in the intestines of C-section babies in the early stages of life. However, these differences in the intestinal microbiota diminish over time and can be partially corrected by exposure of cesarean-born infants to vaginal microbiota after birth (67).

Studies have suggested that microbial colonization starts in the uterus and develops during and after birth (232). Recent research in mice has raised the possibility of microbe transfer to the fetus intestine in vivo, for example, via the amniotic fluid. The origin of bacteria that colonize the infant GI tract after birth is likely the mother's skin and vagina during natural birth. Low numbers of bacteria are also found in breast milk, likely due to contamination from the mother's skin. Several studies on the development of infant microbiota show that it is shaped by the introduction of formula, solid food, illness, and antibiotic treatment until it reaches a stable adult-like composition and diversity at 2–3 years of age (117).

Bacterial colonization of the intestinal tract influences many aspects of normal gut epithelial physiology such as crypt depth, proliferation, blood vessel density, and mucus thickness (203). The formation of the mucosal-associated lymphoid tissue (i.e., Peyer's patches, intestinal lymph follicles, and mesenteric lymph nodes) is initiated in utero via the development of intestinal lymphoid tissue inducer cells, which secrete CXCL13 to attract other immune cells (247). However, intestinal colonization by microbes is crucial for maturation of these tissues (203). The abovementioned responses to intestinal colonization are rather generic, but certain species or groups of bacteria have been found to have specific effects on intestinal immunity and epithelial functions.

Intestinal *Bifidobacterium* species frequently isolated from breastfed infants are *Bifidobacterium longum* subsp. *infantis*, *Bifidobacterium longum* subsp. *longum*, *Bifidobacterium bifidum*, and *Bifidobacterium breve* (reviewed in 146). Human milk oligosaccharides (HMOs) support the growth of *Bifidobacterium* species that are abundant in the intestine during the first months of life (146). HMOs are not digested in the small intestine and pass to the colon, where they are selectively fermented by specific species such as *Bifidobacterium*, which has all the genes required for efficient utilization of HMOs. The introduction of solid food at 4 to 6 months increases the diversity and complexity of the microbiota, consistent with the increased consumption of nondigestible dietary fibers, which are fermented largely in the colon. At weaning, increased abundance of *Bacteroides*, *Clostridium*, Ruminococcaceae, and Lachnospiraceae occurs with a decrease in *Bifidobacterium*, Enterobacteriaceae, and Streptococcaeae (11).

Administration of live infant-associated *Bifidobacterium* strains in the first stage of life may result in the prevention of NEC and reduce the risk of developing atopic disease (52, 59). Recently, different *Bifidobacterium* species and strains were tested for their capacity to alter the transepithelial resistance of human intestinal Caco-2 cell monolayers in a Transwell system. The probiotic *B. bifidum* strain BB1 increased transepithelial electrical resistance (TEER), which was dependent on Toll-like receptor (TLR)-2 and activation of the p38 kinase pathway, but not on NF-κB activation. Strain BB1 did not have a significant effect on the expression of TJ proteins ZO-1, claudin-1, claudin-2, or claudin-3 but increased expression of occludin in Caco-2 monolayers.

Many strains of *Bifidobacterium* produce an exopolysaccharide (EPS) that may be involved in adhesion, persistence, and signaling in the host via carbohydrate recognition receptors (50). EPS can also be used as a carbohydrate-fermentable source by some gut commensals, resulting in the release of bacterial metabolites that have positive benefits for the host. EPSs produced by *Lactobacillus* and *Bifidobacterium* species protect mice against infection from *Citrobacter rodentium*, which

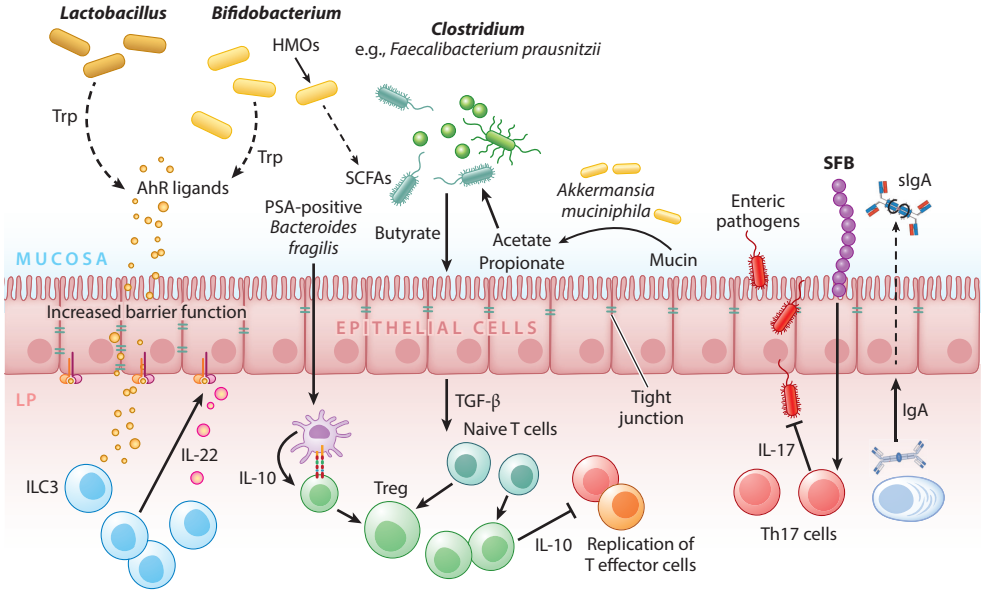

Figure 2

The proposed effects of potentially beneficial intestinal bacteria on intestinal barrier function. *Lactobacillus* and *Bifidobacterium* can metabolize tryptophan to produce AhR agonists that increase innate lymphocytes type 3 and IL-22 production in vivo. IL-22 interacts with its cognate receptor on the basal epithelium membrane to stimulate intestinal defenses and proliferation. IL-22 is also crucial for the maintenance of intraepithelial lymphocytes. HMOs support the growth of *Bifidobacterium*, and their fermentation leads to SCFA production. Members of the anaerobic Clostridia, including *Faecalibacterium prausnitzii* and spore-forming species of *Clostridium*, ferment fiber reaching the colon to generate SCFAs, including butyrate. Butyrate produced by Clostridia indirectly induces IL-10-producing Tregs from naive T cells in the LP. Tregs maintain an anti-inflammatory tone by suppressing T effector cell proliferation. PSA-expressing strains of *Bacteroides fragilis* induce IL-10 in dendritic cells, thus promoting Treg induction. *Akkermansia muciniphila* digests and ferments carbohydrates that decorate host mucin, thereby modulating the growth of other symbionts. SFB attach to the ileal epithelium, stimulating the differentiation and maturation of Th17 cells and the production of IgA. The enhanced innate response to SFB protects against enteric pathogens. Abbreviations: AhR, aryl hydrocarbon receptor; HMO, human milk oligosaccharide; LP, lamina propria; PSA, polysaccharide A; SCFA, short-chain fatty acid; SFB, segmented filamentous bacteria; sIgA, secretory IgA; TGF-β, transforming growth factor β; Treg, regulatory T cell.

has virulence mechanisms similar to those of enteropathogenic *Escherichia coli* in humans (reviewed in 50). Recently, the purified EPSs of *Bifidobacterium animalis* subsp. *lactis* were shown to specifically activate TLR signaling (49). Nevertheless, there remains an important gap in our understanding of the potential health effects of different strains and the precise mechanisms by which *Bifidobacterium* contributes to protection from NEC and to epithelial barrier function.

In addition to Bifidobacteria and Lactobacilli, several potentially beneficial bacteria, mostly conferring protective effects in colitis models via promoting immune regulation by Tregs, have been identified in the last decade (as reviewed in 103, 203) (**Figure 2**). For example, spore-forming species of *Clostridium* were shown to induce $CD4^+$ $FoxP3^+$ Tregs and anti-inflammatory IL-10 expression in the mouse colon through butyrate-dependent induction of transforming growth factor (TGF)-β1 in colonic epithelial cells (15). Proteolytically activated TGF-β1 was proposed to induce naive T cell differentiation into Tregs in the LP. SCFAs were later confirmed to regulate

the number and function of Tregs in the colon of mice in a GPCR43-dependent mechanism (219). As a follow-up to these studies, a mixture of 17 spore-forming human Clostridia strains that induced Tregs in mice was selected. Oral administration of these strains to young adult mice attenuated disease in models of colitis and allergic diarrhea (15, 219). These findings suggest that colonization of the infant gut by spore-forming species of anaerobic Clostridia will also be an important step in the establishment of T cell–regulatory mechanisms in the colon.

AhR: aryl hydrocarbon receptor

Faecalibacterium prausnitzii is a common member of the human microbiota and a strict anaerobe beneficial to intestinal health and epithelial barrier function. It is abundant but extremely oxygen sensitive and is a core butyrate-producing species found in human fecal microbiota. Several studies showed that administration of *F. prausnitzii* or EPSs derived from strain HTF-F can attenuate colitis in mouse models of inflammatory bowel disease (157, 202, 259). *Akkermansia muciniphila*, another common member of the adult and intestinal microbiota (64), colonizes the loose mucus layer in the colon, which it can use as a sole carbon source for growth. Fermentation of host mucin generates predominantly acetate and propionate. *A. muciniphila* can be detected in infants even in the first month of life but increases in abundance over the first 2–3 years of life. Colonization of infants by *A. muciniphila* is supported by its ability to metabolize HMOs (122).

Segmented filamentous bacteria (SFB) are another example of bacteria that have potent effects on intestinal immunity and barrier function. SFB attach to epithelial cells and trigger the postnatal maturation of the intestinal immune system in mice (14, 109). Through proximity to epithelial cells, SFB stimulate innate immunity and an abundance of mucosa-associated Th17 cells in mice (109). Consequently, mice colonized with SFB are less susceptible to infection with enteropathogens and produce higher amounts of IgA than do mice lacking SFB. The downside of SFB colonization is that it increases inflammation in colitis and other inflammatory diseases such as autoimmune arthritis.

In mammals, SFB have been detected in early life by qPCR or histology but decline in abundance throughout life. PCR and metagenomic DNA sequencing have been used to detect SFB in the liminal fluids of some children in both US and Chinese populations (55). The presence of SFB is correlated with total IgA production in the terminal ileum, suggesting an immunity effect similar to that observed in mice. However, the very low amounts of SFB in the human microbiota have prevented the identification of SFB genome sequences via metagenomics sequencing (129).

The microbiota ferment indigestible food components and excrete metabolites, some of which are used as an energy source by the body. Some bacteria primarily ferment dietary fibers into SCFAs, whereas others ferment proteins, which results in the formation of metabolites, such as ammonia, amines, phenols, sulfides, and nitric oxide, that are considered harmful for gut health (88). In contrast, the metabolism of tryptophan by specific species in the intestine can produce ligands of the aryl hydrocarbon receptor (AhR), which have a role in regulating intestinal barrier function and intestinal immune cells, as well as intestinal homeostasis (165). The gut microbiota is also essential for gut barrier function and intestinal homeostasis (68).

Germ-free animal models indicate that the lack of gut microbiota causes severe defects in the function of the immune system, with reduced levels of Igs, plasma cells, and circulating B and T cells (213). The gut microbiota may provide signals to specific lymphocyte subsets, promoting their development (class switching) and further influencing Ig production (155). Gut microbiota is also considered to contribute to the regulation of the intestinal mucus layer. A thinner inner mucus layer was observed in the colon of germ-free mice relative to conventionally raised mice (112).

These results indicate the important role that many types of bacteria play in the maintenance of a healthy microbiota, in the establishment of the epithelial barrier, and in the maturation of the intestinal immune system.

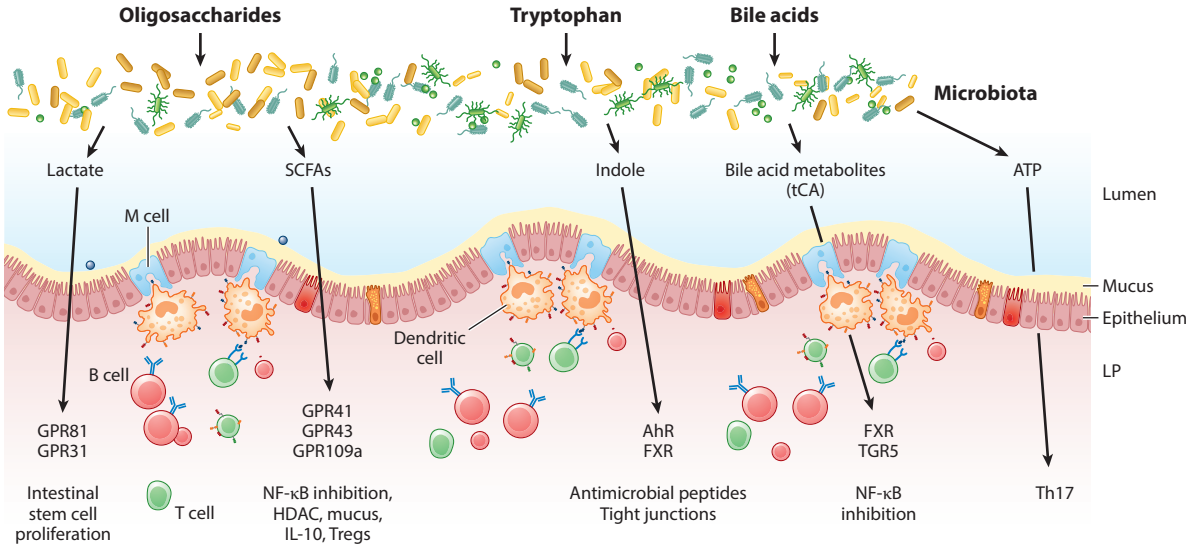

Figure 3

Receptors and effects of microbial metabolites on intestinal barrier function. The fermentation of oligosaccharides results in the production of SCFAs, which interact with GPRs on epithelial and immune cells in the LP. Tryptophan is metabolized by specific bacterial species to produce agonists, inducing nuclear translocation of AhR or PXR and altering mucosal gene expression in immune and epithelial cells. Bile acid metabolites interact with FXR and TGR5 to regulate cell metabolism. Abbreviations: AhR, aryl hydrocarbon receptor; FXR, farnesoid X receptor; GPR, G protein–coupled receptor; HDAC, histone deacetylase; LP, lamina propria; PXR, pregnane X receptor; SCFA, short-chain fatty acid; Treg, regulatory T cell.

4. MICROBIAL METABOLITES

4.1. Short-Chain Fatty Acids

Metabolites produced by the microbiome play a key role in gut health and immunity (100, 114, 116) (**Figure 3**). SCFAs, fatty acids with short aliphatic tails consisting of one to six carbon atoms, are produced when dietary fiber or nondigestible poly- and oligosaccharides are fermented in the colon. SCFAs are well known to enhance barrier function in epithelial cells (recently reviewed by 177). SCFAs can bind to G protein–coupled receptor 41 (GPR41), GPR43, and GPR109a. These receptors have different selectivity for SCFAs; GPR109A binds only butyrate (217, 237), GPR43 and GPR41 bind to all SCFAs, GPR41 is most selective for propionate and butyrate, and GPR43 is most selective for acetate and propionate (41, 135). SCFAs not only signal through binding cognate GPRs expressed in enteroendocrine and immune cells in the body but also induce epigenetic changes in the genome through effects on the activity of histone acetylase and histone deacetylase enzymes (99).

Several studies have shown that butyrate increases expression of AMPs and Reg3 proteins via a GPR43-dependent mechanism (279) and enhances the intestinal barrier by facilitating TJ formation in Caco-2 cell monolayers (179). SCFAs also enhance the intestinal epithelial barrier by increasing the expression of MUC2 and specific membrane-associated mucins in colon cancer cell lines. In vivo, butyrate increases crypt depth, villi length, and mucosa thickness in weaning piglets fed with supplemented Na butyrate (123). The piglets treated with Na butyrate also had an increased number of goblet cells in the colon (152), suggesting an enhanced development of jejunal and ileal mucosa in formula-fed piglets.

GPR: G protein–coupled receptor

Addition of acetate to the drinking water of mice after weaning can protect against allergic airway disease (238). Protection from allergic airway disease is also observed in the offspring when acetate is given to the mothers only during pregnancy, suggesting that acetate causes epigenetic imprinting of the fetus in utero. More recent studies have also shown that the onset of autoimmune type 1 diabetes can also be prevented in NOD (nonobese diabetic) mice in early life through specialized diets to deliver acetate and butyrate to the colon (153).

4.2. Tryptophan Metabolites as Aryl Hydrocarbon Receptor Ligands

Host and bacterial metabolites of tryptophan are ligands of the AhR, a ligand-activated regulator that is translocated to the nucleus to activate gene expression. The AhR is best known for its role in regulating expression of cell enzymes that metabolize halogenated aromatic hydrocarbons, including toxic compounds such as dioxins, but also regulates the immune system. In 2011, Li et al. (141) showed that AhR is a crucial regulator in maintaining intraepithelial lymphocytes in the intestine. Genetic deletion of AhR or the lack of exogenous AhR ligands from diet and microbiota alters the composition of the microbiota, leading to heightened immune activation and increased vulnerability to epithelial damage and infection by enteropathogens (141, 194). The link between AhR ligands and the gut barrier is thought to be mainly due to the role of AhR signaling in the expansion of innate lymphocytes type 3 and IL-22 production in vivo. IL-22 has a homeostatic role in the intestine signaling via receptors on the basal side of the epithelium to increase expression of mucins, AMPs, Reg3 proteins, fucosyl transferase 2 (FUT2), and TJ proteins and increases IEC proliferation (222, 223, 227). Furthermore, IL-22 promotes α1,2-fucosyl linkages on epithelial glycoproteins at the interface for host–microbe interaction by increasing FUT2 expression. Thus, there may be a critical role for an interaction between microbes and IL-22 (91).

AhR activation induced $Foxp3^+$ Treg cell differentiation in vivo and in vitro (81), and $AhR^{-/-}$ mice have decreased numbers of Tregs (73, 195). However, another study showed no effect of constitutive AhR activation on $Foxp3^+$ Treg cell differentiation (80). Type 1 Treg (Tr1) cells are $Foxp3^-$ Treg cells, which can produce IL-10 and express AhR. Activation of AhR promotes the differentiation of functional human Tr1 in vitro (81). Furthermore, IL-10 production is mediated via AhR binding with a transcription factor in Tr1 (10).

AhR signaling in the mucosa via dietary ligands and tryptophan catabolism by microbes and host pathways is clearly important in intestinal homeostasis and immunity, as evidenced by recent literature showing a role for AhR in celiac disease (131) and metabolic syndrome (165).

5. EARLY LIFE NUTRITION AND INTESTINAL BARRIER FUNCTION

In this review, we focus on the effect of nutritional components on the intestinal barrier in early life. As such, we describe effects of breast milk, as well as its components that are present in early life nutrition (infant milk formulas). Breastfeeding is recommended by the World Health Organization (WHO) as the best choice of nutrition in early life (269). However, not all mothers can, or choose for other reasons not to, (sufficiently) breastfeed their infants. Cow's milk–based infant milk formulas are a safe alternative.

Nutrition can affect the intestinal barrier at several levels (**Figure 4**):

1. via modulating the composition of microbiota or the formation of bacterial components and metabolites,
2. via a direct effect on the intestinal epithelium, or
3. via an effect on the intestinal immune system.

The effects of food components on the intestinal barrier can be studied by assessing barrier function in vivo via (*a*) the selective uptake of different sugars or the leakage of microbial

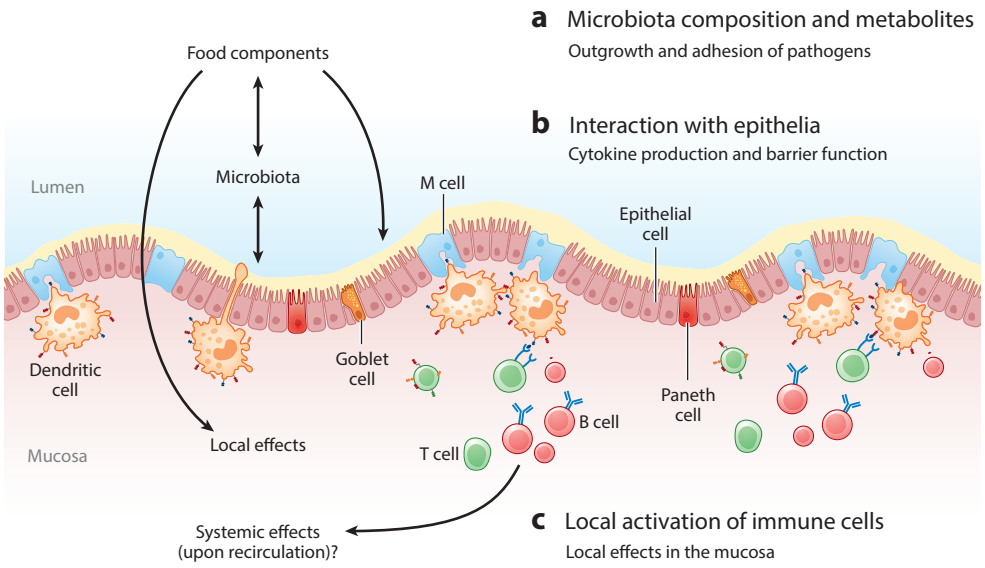

Figure 4

Nutritional components affect intestinal barrier function and immunity on three different levels: (*a*) at the level of the microbiota composition and their production of metabolites, which leads to colonization resistance, preventing outgrowth and adhesion of pathogens—in addition, the microbiota affects the epithelial barrier directly via the production of metabolites such as short-chain fatty acids; (*b*) at the level of the epithelial cell by directly affecting epithelial barrier function and inducing the production of antimicrobial peptides; and (*c*) at the level of the mucosal immune system by interacting directly with the immune cells in the organized immune tissues (Peyer's patches, lymphoid follicles) or with the diffuse immune cells in the mucosa—this interaction occurs through direct uptake of food components across the epithelium by dendrites of dendritic cells (*orange*), by uptake via M cells (*blue*), or by paracellular transport across the epithelium. The mechanisms involved are further illustrated in **Figure 5**. Figure adapted from Reference 249.

components in the circulation and (*b*) the presence or absence of inflammatory cytokines, AMPs, and TJ proteins in fecal samples. The effects of food components on the function of TJ proteins can be studied on biopsy samples in Ussing chambers or in vitro using polarized monolayers of 3D organoid cells or epithelial cell lines by measuring the TEER and permeability of fluorescent markers of different molecular weights (32, 33, 261). In vivo studies in colitis and GI infection models, as well as effects in nutritional intervention studies on infection, inflammation, and barrier function, are also highly informative. As intervention studies in infants are difficult and challenging, in vivo challenge studies in healthy volunteers with nonsteroidal anti-inflammatory drugs (NSAIDs), hydrogen peroxide, or infectious enteropathogens can be used to assess the effects of food components on barrier function (160, 189, 235).

In this section, we describe the effects of breast milk on microbiota and their metabolites, then discuss the direct effect of milk components on barrier function, and finally discuss other components present in early life nutrition that impact barrier function (see **Table 1** and **Figure 5** for overview).

5.1. Milk Components

The beneficial effect of breastfeeding on the development of the infant's gut mucosal barrier and immune system (134) is attributed to the bioactive components of breast milk (245). These

Table 1 Effects of early life nutrition components on intestinal barrier function

Category	Name	Type of component	Effects	Intestinal barrier–related effects (in vivo)	References
Milk proteins	Lactoferrin	Whey protein	Antimicrobial and immunomodulatory effects, iron scavenging	NEC, sepsis, intestinal infection	83, 234 (in vivo, human); 94, 102, 111, 115, 139, 182, 218 (in vitro); 107, 199, 277 (in vivo, piglet); 125 (in vivo, rodent)
	Immunoglobulins (IgG, IgA)	Whey protein	Immune exclusion, prevention of inflammation, epithelial barrier immunomodulation	Intestinal infection, inflammation, compromised barrier function	13, 78, 189, 204, 215 (in vivo, human); 34, 65, 246, 282 (in vitro); 268 (in vivo, piglet); 19, 101, 183 (in vivo, rodent)
	Transforming growth factor β	Anti-inflammatory cytokine	Epithelial barrier strengthening and differentiation	Gut inflammation, NEC, food allergy, compromised barrier function	6, 77, 150 (in vivo, human); 18, 197 (in vitro); 150, 169 (in vivo, piglet); 105, 173, 191, 210 (in vivo, rodent)
	Intestinal alkaline phosphatase	Whey protein	Antimicrobial effect, detoxification of lipopolysaccharide, prevention of barrier function and integrity	Gut inflammation, NEC, sepsis, compromised barrier function	162 (in vivo, human); 130 (in vitro); 3, 24, 56, 244, 263 (in vivo, rodent)
Milk lipids (fat)	Milk fat globular membrane	Polar lipids, proteins, glycoproteins, and sterols	Antimicrobial effect, microbiota modulation, increased epithelial barrier function and integrity	Intestinal infection, compromised barrier function	277 (in vivo, human); 79, 127, 137, 168, 170, 178, 225, 226, 228 (in vitro); 30 (in vivo, rodent)
	PUFAs	(Long-chain) PUFAs	Anti-inflammatory effect, increased epithelial barrier function and integrity	Epithelial damage, compromised barrier function, colitis	22, 70, 265 (in vitro)
	AhR ligands	Fat-soluble AhR ligands; contain diet-derived phytochemicals and tryptophan metabolites	AhR activation, anti-inflammatory effect	Gut inflammation, epithelial damage, compromised barrier function, impaired intestinal homeostasis	72, 121, 132, 159, 221 (in vitro)

(*Continued*)

Table 1 (*Continued*)

Category	Name	Type of component	Effects	Intestinal barrier–related effects (in vivo)	References
Milk oligosaccharides	HMOs	HMOs, nondigestible oligosaccharides	Bifidogenic effect, induction of SCFAs and microbial metabolites, increased epithelial barrier differentiation and function	Intestinal infection, epithelial damage, compromised barrier function	164 (in vivo, human); 36, 87, 98, 104, 126, 145, 166, 181, 220, 229, 233, 242, 258 (in vitro); 152, 196, 220 (in vivo, piglet); 16, 90, 138, 177, 220 (in vivo, rodent)
Milk vesicles	Extracellular vesicles, exosomes	Vesicles secreted from mammary gland epithelial cells; contain polar lipids, proteins, and miRNA	Anti-inflammatory effect; epithelial barrier strengthening, differentiation, and function	Epithelial damage (apoptosis), compromised barrier function, NEC, colitis	5, 8, 26, 58, 82, 113, 140, 142, 154, 198, 255, 267, 272, 278, 283 (in vitro); 25, 27, 82, 140, 161, 188 (in vivo, rodent)
Other components					
Prebiotics	Galacto-oligosaccharides	Nondigestible oligosaccharide	Bifidogenic effect, induction of SCFAs, increased epithelial barrier integrity	Intestinal infection, epithelial damage, compromised barrier function	12, 23, 43, 53, 74, 124, 163, 216 (in vivo, human); 7, 29, 181 (in vitro); 239, 252 (in vivo, piglet)
	Fructo-oligosaccharides	Nondigestible oligosaccharide	Bifidogenic effect, induction of SCFAs, increased epithelial barrier integrity	Intestinal infection, epithelial damage, compromised barrier function	12, 43, 53, 176 (in vivo, human); 106 (in vivo, rodent)
Probiotics	*Lactobacillus* and *Bifidobacterium* species	Microbiota-derived tryptophan metabolites, SCFAs	Enhanced barrier function, effect on microbiota, immune modulation effects (through AhR activation)	Intestinal infection, impaired barrier function	230 (in vivo, human); 72, 121, 159, 260, 264 (in vitro); 89 (in vivo, rodent)
Micronutrients (vitamins and minerals)	Vitamin A, D	Fat-soluble vitamins	Increased epithelial barrier function and integrity, immunomodulation	Intestinal infection, impaired barrier function	93, 206, 214 (in vivo, human); 66, 136, 254 (in vitro); 97, 205 (in vivo, rodent)
	Zinc, calcium	Minerals	Increased epithelial barrier function and integrity, immunomodulation	Intestinal infection, impaired barrier function	37 (in vivo, human); 144, 149, 211 (in vitro); 37 (in vivo, rodent)

Abbreviations: AhR, aryl hydrocarbon receptor; HMO, human milk oligosaccharide; NEC, necrotizing colitis; PUFA, polyunsaturated fatty acid; SCFA, short-chain fatty acid.

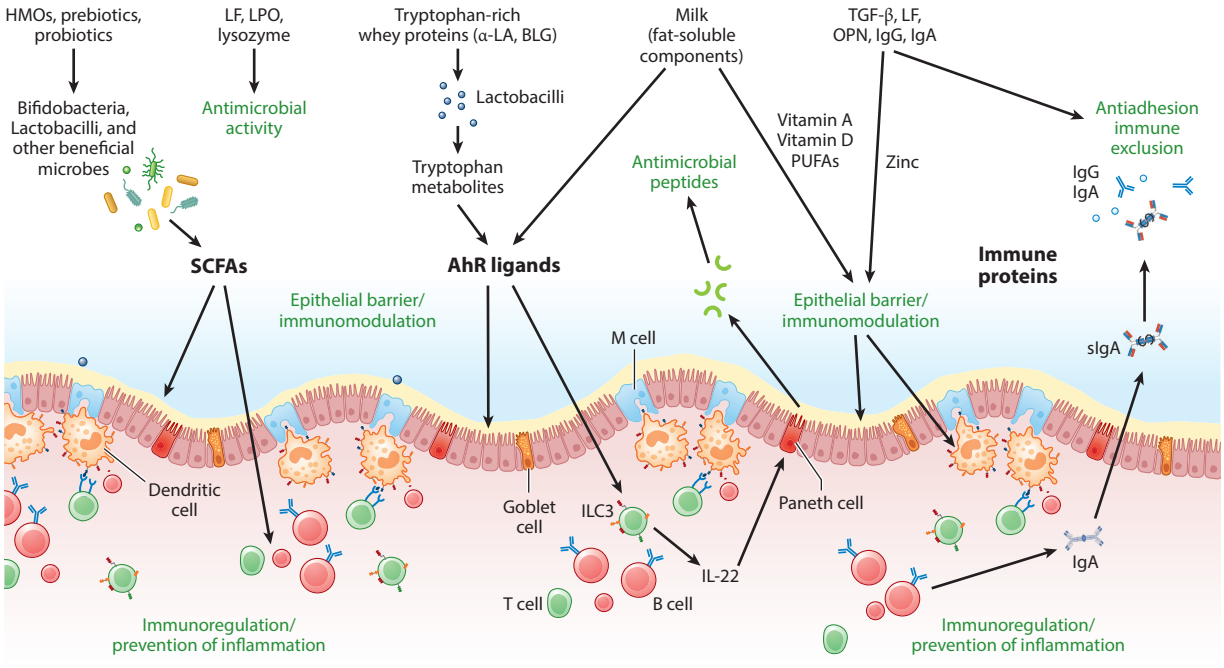

Figure 5

Breast milk and early life nutrition components guard the intestinal barrier and immune system to prevent against infection and inflammation. Milk oligosaccharides, but also pre- and probiotics, can modulate the composition of the intestinal microbiota and lead to the production of SCFAs that promote epithelial barrier function and have immunoregulatory activity. The proteins LF, LPO, and lysozyme have antimicrobial activity. Tryptophan-rich whey proteins such as α-LA and BLG can lead to the production of tryptophan-derived AhR ligands. Other fat-soluble AhR ligands can be provided by milk fat. AhR ligation in ILC3 in the mucosa leads to IL-22 production to stimulate intestinal defenses and epithelial proliferation. Milk fat also contains vitamins A and D as well as PUFAs that have immunoregulatory activities and enhance intestinal barrier function. Finally, milk proteins such as LF, TGF-β, OPN, IgG, and IgA (as well as zinc) lead to immune exclusion of pathogens (IgG and IgA), are immunomodulatory (LF, TGF-β, OPN), prevent inflammation (TGF-β and indirectly IgG and IgA), and can support intestinal barrier function (TGF-β, zinc). Abbreviations: α-LA, α-lactalbumin; AhR, aryl hydrocarbon receptor; BLG, β-lactoglobulin; HMO, human milk oligosaccharide; ILC3, innate lymphoid cell 3; LF, lactoferrin; LPO, lactoperoxidase; OPN, osteopontin; PUFA, polyunsaturated fatty acid; SCFA, short-chain fatty acid; sIgA, secretory IgA; TGF-β, transforming growth factor β.

components include IgA and IgG, lactoferrin (LF), TGF-β, extracellular vesicles (EVs), HMOs, milk lipids such as the milk fat globule membrane (MFGM) and polyunsaturated fatty acids (PUFAs), and micronutrients (vitamins and minerals) (9). Interestingly, most of these bioactive factors are also present in bovine milk, and in most cases their levels are comparable (250).

Interestingly, many of the bovine whey proteins, i.e., the milk proteins minus the caseins, have functional effects on the human immune system (2, 54, 250), and consumption of raw bovine milk is associated with a reduced prevalence of asthma, hay fever, and (in a single study) reduced respiratory tract infections (39, 147, 148, 200, 224, 257).

When breast milk is insufficiently available, bovine milk–based early life nutrition products are given as a safe alternative. The composition of these products is strictly regulated by detailed guidelines to provide optimal nutrition based on current knowledge of breast milk composition and function and the nutritional requirements of infants and toddlers (72, 75, 118).

LF: lactoferrin

MFGM: milk fat globule membrane

With breast milk as the golden standard, this review focuses on the effects of bovine milk–derived components and other ingredients in early life nutrition on intestinal barrier function.

5.1.1. Lactoferrin. LF is an iron-binding glycoprotein that is widely found in mammalian milk (84, 171). LF has an antimicrobial effect on bacteria through the scavenging of iron (115), enhances iron uptake and bioavailability in newborns (231), and has anti-inflammatory properties (4, 139, 218). LF is relatively resistant to digestion, with more than 60% of bovine LF surviving gastric digestion in adults and up to 10% of bovine LF surviving digestion throughout the GI tract in infants (61, 243).

LF can affect intestinal barrier function at several levels. Bovine LF stimulates the proliferation and differentiation of IECs in vitro (44, 94, 111) as well as in vivo in piglets (199). Oral supplementation of bovine LF in piglets increased the area, depth, and width of crypts in the jejunum (273) and resulted in upregulated expression of the TJ protein occludin (107). Likewise, LF restored tumor necrosis factor (TNF)-α-induced reduction in TEER and increased leakage of macromolecules (102).

In addition to these effects on the intestinal epithelial barrier, bovine LF can modulate intestinal immune function by inhibiting the differentiation and maturation of dendritic cells in vitro (182) and by inducing natural killer cell activity in Peyer's patches and mesenteric lymph nodes in mice (125). Bovine LF supplementation has been studied in several studies in infants, especially in low-birth-weight infants who are at risk for severe GI infections. These studies showed that LF reduces the risk of late-onset sepsis, but not that of NEC, in very low birth weight (VLBW) (<1,500-g) infants (83, 234).

In summary, the results of in vitro, preclinical, and dietary supplementation studies suggest that LF plays an important role in supporting intestinal barrier function in early life.

5.1.2. Bovine IgG. Breastfeeding is associated with decreased respiratory and GI infections (69). One of the most important proteins in breast milk is IgA. IgA and IgG antibodies from breast milk can bind to, neutralize, and enhance phagocytosis of intestinal pathogens (167, 282). As infants do not produce sufficient levels of intestinal IgA during the first year of life, they are dependent on the passive delivery of IgA (and IgG) through breastfeeding after birth.

For this reason, bovine IgG, which can bind to a wide range of pathogenic bacteria and viruses, has also been studied as a component to prevent intestinal infections (reviewed in 246). As is the case for LF, orally ingested bovine IgG can survive and maintain its immune activity throughout the GI tract (110).

Bovine colostrum, which is very high in IgG, increases the expression of the TJ protein claudin-2 in a murine model and in Caco-2 cells, suggesting that bovine colostrum can improve intestinal gut barrier integrity (34). Similarly, bovine IgG reduced mucosal expression of proinflammatory cytokines and prevented a reduction in barrier function through preventing the reduction of ZO-1 in a colitis model (183, 184). IgG further contributed to an increase in the number of goblet cells and Muc2 expression (183). In another colitis model, bovine serum–derived IgG reduced the recruitment of T helper lymphocytes and prevented an increase in proinflammatory cytokine production (184). These changes were associated with an increase in Tregs and TGF-β secretion. Similar findings were reported in a bacteria-induced colitis model (101), a chemotherapy-induced mucositis model (19), and a pathogen infection model (108), indicating that oral IgG supplementation can mitigate the severity of colitis in mice.

Supplementation of piglets with bovine IgG or colostrum increased the thickness of the stomach mucosa as well as villus height and crypt depth in the duodenum (268). In addition, bovine colostrum with high IgG levels reduced the NSAID-induced reduction in intestinal

barrier function in animal (189) and human (190) challenge models, and bovine IgG prevented the leakage of bacterial components over IECs in vitro (65).

Bovine IgG from immunized cows has been used to treat and prevent rotavirus and *E. coli*–induced diarrhea in children (reviewed in 246). However, bovine IgG from nonimmunized cows can also improve intestinal infections, as shown in HIV patients before the advent of combination therapies. Several studies have shown that oral supplementation with bovine IgG isolated from colostrum (78, 204, 215) or serum (13) decreases stool frequency.

These studies indicate that bovine IgG, as is already known for IgA in breast milk, can play a role in preventing infection, inflammation, and compromised barrier function.

5.1.3. TGF-β. TGF-β is one of the major cytokines present in both bovine milk and breast milk, particularly in colostrum (85). TGF-β is a key anti-inflammatory cytokine that contributes to Ig isotype switching and the development of Treg cells and that prevents inflammation. There are three forms of TGF-β: TGF-β1, TGF-β2, and TGF-β3. TGF-β2 is the most predominant type in bovine milk and breast milk, followed by TGF-β1 (169). TGF appears in milk as a propeptide that is activated by cleavage under acidic conditions. The amino acid sequence identity of the active form of bovine TGF-β2 and human TGF-β2 is 100% and is 99% for bovine TGF-β1 and human TGF-β1. An important role of TGF-β is to induce Tregs that mediate oral tolerance to dietary antigens (105, 180). For example, the presence of TGF-β in breast milk is crucial for preventing the development of food allergy in mice (105, 251). These studies indicate the potential role of bovine TGF-β in decreasing the risk of food allergy.

In addition, TGF-β supports the function and integrity of the intestinal epithelial barrier. TGF-β1 inhibits cell proliferation in the crypt base (191), where stem cells are located. Similar findings were reported in an IEC line that also became more differentiated in the presence of TGF-β1 (18). Furthermore, TGF-β1 contributes to the migration and differentiation of Paneth cells in the crypts and promotes the rapid regeneration of Paneth cells in vivo (210).

TGF-β2 also inhibits IEC growth and stimulates IEC differentiation, as shown by a reduction in intestinal pathology in an inflammatory bowel disease (IBD) model in mice (173). TGF-β2 moreover reduces both the production of IL-8 and IL-6 in response to IL-1β and lipopolysaccharide (LPS) stimulation of immature human and porcine IECs (169, 197).

Several studies have indicated the potential role of the different TGF-β isoforms in the development of gut immunity and epithelial barrier function in infants and children. In preterm neonates suffering from NEC, TGF-β2 expression in intestinal tissue samples is reduced (150). TGF-β2 suppresses cytokine production by macrophages and protects against NEC-like intestinal injury in mouse pups (150). Therefore, TGF-β was also evaluated for anti-inflammatory action in IBD. Two intervention studies in juvenile Crohn's disease patients showed a clinical remission rate of 75% and 79% after an intervention with a TGF-β2-rich diet (77). The intervention also reduced the levels of proinflammatory IL-1β, IL-8, and interferon (IFN)-γ in the colon and ileum (77). Likewise, the effect of TGF-β was studied in juvenile ulcerative colitis patients aged 10–17, in whom a polymeric diet rich in TGF-β2 reduced inflammation and led to clinical remission in most of the patients (6).

Taken together, these studies indicate that TGF-β1 and TGF-β2 found in milk may contribute to normal development of the intestinal epithelial layer.

5.1.4. Alkaline phosphatase. Another milk protein that may contribute to intestinal barrier function is alkaline phosphatase, an enzyme that is present in breast milk and raw cow's milk. Intestinal levels of alkaline phosphatase shortly after birth were recently shown to be dependent on levels in breast milk (274). This finding suggests that at least part of the enzymatic activity of alkaline phosphatase in the intestine is derived from breast milk. In addition, breast milk contributes

significantly to the levels of alkaline phosphatase as well as beneficial bacteria in feces of preterm infants and has thus been implicated in protection against NEC (162). Alkaline phosphatase is an important enzyme that dephosphorylates LPS (20) and as such can reduce the effects of sepsis. In addition, it plays a crucial role in the development and maintenance of intestinal barrier function. Intestinal alkaline phosphatase is locally expressed and is a marker for the differentiation status of intestinal epithelium, plays an important role in the prevention of inflammation, and supports TJ integrity and barrier function maintenance in vivo (56, 130).

The addition of alkaline phosphatase to pasteurized milk contributes to protection against allergic sensitization against food allergies (3), and alkaline phosphatase prevents severe disease in sepsis models (24, 28), as well as in colitis (244) and NEC (263).

However, alkaline phosphatase is a milk protein that is heat labile and denatures at approximately 62°C. Its activity is often monitored as a means of monitoring the pasteurization efficacy of milk, which means that its activity in early life nutrition is currently limited to breast milk and raw milk.

5.1.5. The milk fat globule membrane. The triglycerides in milk are contained in the MFGM. The MFGM is a highly complex biological structure made up of polar lipids, proteins, glycoproteins, and sterols. The main polar lipids in bovine milk are glycerophospholipids, including phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine; sphingolipids, in particular sphingomyelin; and gangliosides. In addition to lipids, multiple proteins have been identified in the MFGM; these include mucin 1 (MUC1), xanthine dehydrogenase and xanthine oxidase (XO), lactadherin, adipophilin, butyrophilin, and CD36 (151, 158).

The MFGM has antibacterial and antiviral properties and may modulate the intestinal microbiota (225). XO, a major protein in the MFGM, can inhibit bacterial growth by generating nitric oxide (95). MUC1 prevents the adhesion of pathogenic bacteria to the intestinal epithelium in vitro (178, 228) and can inhibit rotavirus in vitro (127). Lactadherin also has antiviral activity against rotavirus (168). Both MUC1 and lactadherin are resistant to digestion in the stomach (185).

Not only the proteins but also the polar lipids in the MFGM can exert antipathogenic activities. Sphingolipids reduce the colonization of *Listeria monocytogenes* in rats (226), possibly by competing with pathogens for cellular binding sites (31). Moreover, the enterotoxin-inhibitory activity of sphingolipids has been demonstrated in vivo and in vitro (128). The lipid components of the MFGM contribute to the inhibition of rotavirus in a dose-dependent manner (79). The role of sphingolipids and its bioactive metabolites can also support infant intestinal development and immunity, as reviewed by Nilsson (170).

In early life, the MFGM may also have a potential role in promoting gut development and intestinal immunity maturation and in improving intestinal integrity (137). A study in infants aged 6 to 11 months demonstrated that an MFGM-enriched complementary food could reduce the prevalence of diarrhea (277). Mechanistically, bovine MFGM–supplemented formula supported the development of the intestinal epithelium by increasing the number of Paneth cells and goblet cells and by upregulating the expression of TJ proteins in rat pups (30).

5.1.6. Polyunsaturated fatty acids. As discussed above, breast milk contains higher amounts of polyunsaturated fatty acids (PUFAs) than does bovine milk, which is why PUFAs are added to infant nutrition. Both the *n*-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) prevent barrier disruption induced in the human IEC line T84 by IL-4 (265). DHA also prevents barrier disruption by the proinflammatory cytokines IL-1β, TNF-α, and IFN-γ in Caco-2 cells (22). In the latter study, DHA also prevented the effects of the proinflammatory cytokines on the expression of the TJ proteins occludin and ZO-1. Supplementation of dietary

n-3 PUFAs protects against experimental colitis in rodents and piglets and promotes intestinal epithelial barrier functions, as recently reviewed by Durkin et al. (70).

5.1.7. Fat-soluble aryl hydrocarbon receptor ligands. In addition to the MFGM and PUFAs, minor fat-soluble components in milk may be important for supporting intestinal barrier function. Interestingly, ligands that bind to AhR have been identified in milk of several species. Two types of AhR ligands—(*a*) phytochemicals and tryptophan metabolites that are present in dietary sources such as cruciferous vegetables and (*b*) microbiota-derived tryptophan metabolites—can be found in milk.

Breast milk contains tryptophan, which can be metabolized by lactic acid bacteria to produce several AhR agonists with different activity (121, 132). Breast milk tryptophan is metabolized by infant-associated *Bifidobacterium* to indole-3 lactic acid, an AhR agonist resulting in anti-inflammatory effects in the immature intestine in vitro (72, 159).

Other food-derived AhR ligands such as quercetin can be present in bovine and breast milk (86, 221), and microbiota-derived tryptophan metabolites are also present in murine milk (89). We recently also showed the presence of AhR ligands in bovine milk fat, although we have not yet identified the specific components (248).

5.1.8. Milk extracellular vesicles. Milk-derived EVs, or exosomes, can play a role in intestinal barrier and immune function as well (1). Milk EVs are vesicles found in bodily fluids, including milk. They range in diameter from 50 to 150 nm and are secreted mainly from mammary gland epithelial cells. The phospholipid bilayer of the EVs protects them from intestinal digestion.

EVs contain many proteins as well as miRNAs (198, 278). miRNAs, short noncoding RNAs containing approximately 19–22 nucleotides, are involved in regulating gene expression as well as protein synthesis in the host cell (8). Milk-derived EVs contain a wide range of immune modulatory miRNAs. Both breast milk (113, 142) and bovine milk (26, 267) exosomes, as well as exosomal miRNAs, survive digestion in the GI tract and can be taken up by human IECs through endocytosis (267). Bovine IgG is present in bovine milk–derived EVs (27), and FcRn was proposed to mediate the absorption of milk exosomes in the infant intestine.

Breast milk–derived exosomes reduce the production of IL-2, IFN-γ, and TNF-α and increase the number of $FoxP3^{+}CD4^{+}$ Treg cells in peripheral blood mononuclear cell culture (5). More recently, Zonneveld et al. (283) proposed a more general inhibition of T cell activation by milk EVs rather than Treg induction. Milk EVs also affect intestinal development in early life. Porcine milk–derived EVs increase villus height, crypt depth, and cell proliferation; these increases were associated with the uptake of milk miRNAs into IECs (58).

Human breast milk–derived EVs also protect IECs from H202-induced apoptosis (154) and enhance wound healing in buccal epithelial cells (283). A possible explanation is that milk exosome–derived miRNAs can downregulate the expression of the p53 gene and protect IECs from apoptosis (272).

Several recent publications have suggested a protective and anti-inflammatory role of milk EVs in preventing intestinal inflammatory diseases in animal models (82, 188, 255). Breast milk–derived EVs increased IEC proliferation and reduced apoptosis in vitro and decreased the incidence of NEC in rat pups (188).

Additionally, both bovine milk– and breast milk–derived EVs prevented intestinal inflammatory injury and reduced proinflammatory cytokines in an experimental colitis model (25, 161), as well as ex vivo in organoids (82). EVs derived from bovine milk increased MUC2 protein expression in a NEC injury model (140) and restored epithelial ZO-1 expression through the activation of NF-κB in a dextran sulfate sodium (DSS)-induced colitis model (25). Taken together, these

results suggest that milk EVs may contribute to intestinal barrier function and may play a role in the prevention of NEC in breastfed infants.

5.1.9. Human milk oligosaccharides. HMOs are also crucial components of breast milk that shape the microbiota composition, lead to the production of microbial metabolites as SCFAs and AhR ligands, and have immunomodulatory activity. However, since HMOs are linked to the microbiota, they are discussed in detail below in Section 5.2.1, which also discusses prebiotics and probiotics. Pre- and probiotics are not present in breast milk but are components that are used in early life nutrition to mimic the functional benefits of HMOs.

GOS: galacto-oligosaccharides

FOS: fructo-oligosaccharides

5.2. Other Early Life Nutrition Ingredients

To achieve a composition similar to that of breast milk, bovine milk, which already contains milk proteins, the MFGM, and EVs, is further supplemented with functional immune-related components like vitamins and minerals, lipids such as PUFAs, prebiotics, and sometimes probiotics to bridge the gap between the composition of bovine milk and that of human milk. Vitamins and minerals are also added to meet nutritional requirements. This section describes the known effects of these components on intestinal barrier function.

5.2.1. Microbiota-related components. Although present regulations state that the addition of nondigestible oligosaccharides (such as prebiotics and HMOs), probiotics, or synbiotics to early life nutrition is not necessary (see 72), they are often supplemented to early life nutrition. In addition, human and animal milk can contain fat-soluble microbial tryptophan metabolites or plant-derived phytochemicals that activate the AhR, which is emerging as an important pathway to promote intestinal barrier function. These components are discussed below.

5.2.1.1. Nondigestible oligosaccharides (prebiotics and human milk oligosaccharides). HMOs are a key constituent of human milk. They are a structurally and biologically diverse group of complex indigestible carbohydrates that survive digestion and reach the colon, where they are fermented by the microbiota (242). More than 200 different oligosaccharides have been identified in breast milk (87), but only approximately 40 different oligosaccharides are reported in bovine milk (233), and at much lower concentrations. The only HMOs found in bovine milk at relatively high concentrations are 3′sialyllactose (3′SL) and 6′SL.

HMOs can be divided into acidic HMOs that have a sialic acid residue, neutral HMOs with fucose, and neutral HMOs without fucose. The quantity and structure of these HMOs differ significantly among women and depend upon secretor and Lewis blood group status (35).

Before 2019, when the first HMO [2′fucosyllactose (2′FL)] became available as an ingredient for infant nutrition, prebiotic oligosaccharides like galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), and inulin were used in human nutrition to provide part of the functionality of HMOs. Both HMOs and prebiotics are typically nondigestible carbohydrates that are fermented by the microbiota in the colon and selectively stimulate the growth and activity of Bifidobacteria and, to a lesser extent, Lactobacilli (23, 163, 258, 275). As a result, HMOs as well as other prebiotics increase the levels of Bifidobacteria and Lactobacilli, which are predominant in the gut microbiota of breastfed infants (133).

The fermentation of oligosaccharides results in the production of SCFAs, which reduce pH in the colon and inhibit growth of pH-sensitive bacteria (23, 216). The indirect effects of HMOs and prebiotics on intestinal barrier and immune function via the production of SCFAs are described in detail elsewhere (242).

HMOs and other oligosaccharides can prevent the adhesion and infection of bacterial and viral pathogens by acting as soluble decoy receptors (36, 145, 242). Both HMOs and other prebiotics

can also support intestinal barrier function via effects on the intestinal epithelium. Acidic and neutral HMOs can inhibit the growth of human IECs and can induce IEC differentiation in vitro (104, 126). This effect has also been shown for GOS and SL, both of which inhibit proliferation of Caco-2 cells and induce expression of genes involved in enterocyte differentiation (181).

2′FL, lacto-*N*-neotetraose (LNnT), and 6′SL dose-dependently inhibited cell proliferation in undifferentiated HT-29 and Caco-2Bbe cultures ($P < 0.05$). In contrast to previous reports, only treatment with 2′FL increased differentiation, as indicated by increased alkaline phosphatase activity, and LNnT increased TEER in differentiated Caco-2Bbe cells (104).

The decrease in TEER and enhanced flux of fluorescein isothiocyanate-dextran 4 kDa (FD4) across human IECs after challenge with TNF-α and IFN-γ were prevented by a mix of HMOs, with 2′FL contributing most to this effect (166), probably via the production of metabolites (229). Similarly, a mix of HMOs as well as 2′FL prevented the LPS-induced production of IL-8 in epithelial T84 cells (98) and in porcine IPEC-J2 epithelial cells (196). In an in vivo study, 2′-FL significantly reduced LPS-induced intestinal permeability and increased the expression of IL-22 (138), which enhances intestinal barrier defenses, as described above.

Mechanistically, GOS upregulate the expression of several goblet cell–related genes, including *MUC2*, which as a result may enhance intestinal barrier function (29), and especially GOS but also FOS can support epithelial integrity by maintaining TJs in vitro, as well as in vivo (7). This role was confirmed in two studies in which expression of occludin and ZO-1 was increased in piglets that received GOS (239, 252). In this study, crypt depth decreased, but villus height was not affected (239). Similarly, GOS increased intestinal barrier function in aspirin-challenged obese adults by reducing sucralose:lactulose ratios in urine (124).

The preventive effects of HMO mixes and single HMOs such as 2′FL on the induction of NEC have also been studied in several newborn animal models. 2′FL prevented the development of NEC in a murine model by reducing proinflammatory cytokines and preserving intestinal histology (90). Likewise, in neonatal rats, breastfeeding supplementation with a pooled HMO mix, 2′FL alone, disialyllacto-*N*-tetraose, or sialylated oligosaccharides, but not with GOS, led to reduced pathology scores relative to nonsupplemented animals after induction of NEC (16). Likewise, 2′-FL and 6′-SL, but not lactose, prevented NEC in both a murine and a piglet model (220). In another study, a 4-HMO mix and a 25-HMO mix could not prevent NEC in preterm pigs (196). In addition to NEC models, 2′FL inhibited enterohemorrhagic *Escherichia coli* colonization and *Campylobacter jejuni*–induced enteric infection and inflammation in mice (256, 276), and both 2′FL and 6′SL reduced food allergy in an animal model (48). This reduction of food allergy by 2′FL and 6′SL was associated with induction of Tregs (48).

Both prebiotic oligosaccharides and HMOs increase the intestinal production of sIgA in mice (106); sIgA levels were also increased in fecal samples of infants that received formula supplemented with a mixture of GOS and FOS (17, 209). Likewise, Estorninos et al. (74) recently showed a similar increase in fecal IgA after supplementation with a milk oligosaccharide–enriched formula. This study also showed increased levels of vaccine-specific IgA. In another study, supplementation with FOS resulted in a nonsignificant increase in fecal antipoliovirus IgA levels (176).

A limited number of studies have described the effects of prebiotics and HMOs on intestinal infection in infants. In 1–2-year-old toddlers attending day care centers, the consumption of a formula with GOS:FOS and *n*-3 PUFAs reduced the occurrence of GI and respiratory infections (53). Similarly, two other studies reported a lower incidence in gastroenteritis (43) and a nonsignificant decrease in GI infections in the first 6 months of life after consumption of GOS:FOS (12).

An analysis of 93 mother-infant pairs studied the association between maternal milk levels of 2′-fucosylated oligosaccharides and the prevention of diarrhea. When maternal breast milk

had high concentrations of 2′-fucosylated oligosaccharides, infants had fewer diarrhea episodes, while low levels of 2′-FL were associated with *Campylobacter* diarrhea, and low levels of lacto-*N*-difucohexaose were associated with calicivirus-induced diarrhea (164).

Taken together, these findings indicate that both prebiotics and HMOs can support intestinal barrier function, either directly or via the induction of microbial metabolites, and can prevent intestinal pathology and infection.

5.2.1.2. Probiotics. The possible occurrence of bacteria in breast milk (discussed above) has been used as an argument to include probiotic bacteria in early life nutritional products (76, 92, 193). Many probiotic products on the market have a long history and track record of safe use in humans and animals. The most commonly used probiotic strains are *Bifidobacterium* and *Lactobacillus* species, which are generally regarded as safe. Clinical and experimental animal studies have proposed different mechanisms of action depending on the probiotic strain and subject population. The proposed mechanisms of probiotics in humans are not understood in precise detail but include enhancement of intestinal barrier function, regulation of TJs, colonization resistance to pathogens and pathobionts present in the microbiome, effects on the microbiota, and immunomodulatory effects on immune cells (187, 260, 264).

Many clinical trials have been performed in very preterm or VLBW infants, with the aim of preventing NEC and death. A recent meta-analysis on 56 trials with probiotic supplements to reduce the risk of NEC and associated morbidity and mortality for very preterm or VLBW infants revealed a moderate to low level of certainty about the effects of probiotics on the risk of NEC (212). However, analyses of other trials to assess randomized controlled trials on VLBW infants indicate that probiotic consumption can significantly reduce the risk of developing medical complications associated with NEC and sepsis, can reduce mortality and length of hospital stay, and can promote weight gain (230). However, the different products or strains used and different durations of supplementation prevented definitive conclusions as to the optimal probiotic, dose, duration, and timing of intervention.

Postbiotics have recently attracted much attention as treatments to prevent the development of diseases by altering the gut microbiome. The International Scientific Association of Probiotics and Prebiotics recently defined a postbiotic as a "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" (207). For example, a postbiotic would not include a live microorganism but could include all the cell-free supernatants containing biologically active metabolites secreted during in vitro culture. This field is relatively new, so further studies on the efficacy and safety of postbiotics are warranted before trials can be considered for infants and neonates.

5.3. Micronutrients

The micronutrients that support the normal functioning of the immune system, among other physiological processes, are an essential part of breast milk nutrients. All micronutrients discussed here are present in breast milk or are fortified in infant milk formulas according to the dietary requirements of infants (71).

5.3.1. Vitamin A. Vitamin A supplementation improved intestinal mucosal barrier function and the expression of TJ proteins in animal models (270). Furthermore, vitamin A and its metabolite, retinoic acid, prevented LPS-induced reduction in TEER via the enhanced expression of claudin-1, occludin, and ZO-1, thereby improving intestinal epithelial barrier integrity (96, 271). In addition to contributing to enhanced barrier integrity, vitamin A protected against the development of NEC in an animal model (271). Both in vivo and in vitro, vitamin A and retinoic acid treatment

increased the expression of claudin-1, occludin, and ZO-1 and reduced the levels of inflammatory factors. The effects of vitamin A on intestinal infections in children were recently reviewed (47, 62).

5.3.2. Vitamin D. In in vitro cell cultures, vitamin D partially prevented a drop in TEER induced by *E. coli* infection. Likewise, vitamin D prevented the LPS-induced decrease in viability of the IEC18 line as well as in intestinal organoids and prevented a decrease in ZO-1 and claudin-2 (136). Moreover, vitamin D directly induced the production of AMPs (253). In a DSS-challenged Caco-2 cell culture model, vitamin D_3 increased TJ proteins and TEER (119). A challenge with ethanol in a similar Caco-2 cell culture model was diminished by vitamin D_3, which prevented a decrease in TEER and a disruption of TJs. The effect of vitamin D supplementation on intestinal barrier function during ethanol exposure was also shown in an in vivo animal model (57).

In a study population with a mean age of 62 years old, a low vitamin D [25(OH)D] status was associated with *Clostridium difficile* infection (206). In weaning mice, vitamin D_3 deficiency increased the severity of *C. rodentium* infection, as shown by increased histological damage, bacterial translocation, and inflammatory cell infiltrates. Thus, vitamin D deficiency may worsen intestinal inflammatory responses and associated tissue damage induced by bacterial infection (97, 205).

In a vitamin D receptor transgenic DSS-induced colitis mouse model, intestinal barrier dysfunction and colitis severity were reduced relative to nontransgenic control mice, suggesting a crucial role for vitamin D receptor signaling in maintaining intestinal barrier function and in protecting against inflammation in colitis models (119, 172, 214). More recently, a role for vitamin D signaling was identified in IBD as well, suggesting that vitamin D supplementation may support intestinal barrier function (66) and may be effective as an adjunct treatment in IBD (93).

5.3.3. Minerals. Most studies into the effects of minerals on intestinal barrier function have focused on zinc. Zinc deficiency induced by ethanol impairs barrier function in experimental animals (208, 280). Zinc deficiency also reduces TEER in human intestinal cells, leads to the decreased presence of TJ proteins (211, 254, 280), and decreases mucus production by intestinal goblet cells (149). In contrast to the case for zinc, the role of calcium in intestinal barrier function has not been extensively studied. However, calcium has been shown to ameliorate experimental *E. coli* infection in rodents (37). The calcium-sensing receptor plays an important role in preserving intestinal homeostasis, as recently reviewed (144).

Together, these studies clearly indicate that vitamin A, vitamin D, zinc, and possibly calcium are key micronutrients that are required for intestinal barrier function.

6. CONCLUDING REMARKS

The intestinal barrier is critical for human health and disease, especially for infants, who are born with an immature GI tract and immune system that are continuously developing during the first years of life. In the intestine, the epithelial layer and gut microbiota have direct interactions with breast milk– or infant milk formula–derived components. Moreover, microbial metabolites produced from this interaction can influence intestinal barrier function. The objective of this review is to discuss the potential effects of nutritional components on barrier function in infancy. Breastfeeding is known to have a protective effect at the intestinal barrier, since breastfeeding is clearly associated with the prevention of diarrhea, intestinal inflammation, and infection in early life.

Over the last decades, many in vitro and animal studies support the hypothesis that nutritional components promote intestinal barrier function in early life. Although no extensive body of evidence exists on the basis of infant feeding studies, the available studies seem to confirm the data from experimental models. Future clinical infant feeding studies are needed to further document

and substantiate the contribution of dietary components to intestinal barrier function and protection against infection in early life.

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AUTHOR CONTRIBUTIONS

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