

Diet and host–microbial crosstalk in postnatal intestinal immune homeostasis

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Abstract | Neonates face unique challenges in the period following birth. The postnatal immune system is in the early stages of development and has a range of functional capabilities that are distinct from the mature adult immune system. Bidirectional immune–microbial interactions regulate the development of mucosal immunity and alter the composition of the microbiota, which contributes to overall host well-being. In the past few years, nutrition has been highlighted as a third element in this interaction that governs host health by modulating microbial composition and the function of the immune system. Dietary changes and imbalances can disturb the immune–microbiota homeostasis, which might alter susceptibility to several autoimmune and metabolic diseases. Major changes in cultural traditions, socioeconomic status and agriculture are affecting the nutritional status of humans worldwide, which is altering core intestinal microbial communities. This phenomenon is especially relevant to the neonatal and paediatric populations, in which the microbiota and immune system are extremely sensitive to dietary influences. In this Review, we discuss the current state of knowledge regarding early-life nutrition, its effects on the microbiota and the consequences of diet-induced perturbation of the structure of the microbial community on mucosal immunity and disease susceptibility.

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Introduction

The ‘hygiene hypothesis’ proposed that an increased predisposition to allergies and the rise in the incidence of atopic diseases was linked to a lack of exposure to infectious agents, microorganisms and parasites during childhood that resulted in the development of the immune system being suppressed.^{1,2} In the past few years, epidemiological studies further showed that children growing up on traditional farms with exposure to livestock and consumption of unprocessed cow’s milk during their early years are resistant to these diseases.³ That errors in the development of the immune system are connected to improved sanitary conditions and the increased use of antibiotics, among other factors, is now evident. The gut microbiota is central to this phenomenon as it responds to changes in the environment and also affects the maturation and function of the immune system. Fluctuations in the composition of this microbiota are also caused by perturbations in diet.^{4,5} This observation has led to the proposal of the ‘diet hypothesis’ that unifies changes in nutrition with gut microbiota and immune health (Figure 1).^{6–8}

Cultural diversity and geographical location contribute to dietary differences that result in distinct patterns of intestinal microbial colonization and disease susceptibility in different populations. The Western diet is generally low in fibre and high in processed foods, which adversely affects the intestinal microbial composition and leads to an obesity-prone metagenome.^{6,8,9} Conversely, the Japanese diet, which includes rice, beans and fermented

foods,^{10,11} and the diet of Eskimos in Greenland (which is typically high in fish and omega-3 fatty acids) promote resistance to chronic inflammatory diseases and heart diseases.^{12–14} In mouse experiments, offspring of mice fed a diet rich in omega-3 fatty acids have an altered gut microbiome and have enhanced production of the anti-inflammatory cytokine IL-10 in the colon and spleen, which protects the mice from an allergic challenge.¹⁵ The influence of nutrition on the microbiome and disease susceptibility is also specific to age. In newborn babies, the establishment and type of feeding has a considerable effect on the composition of the microbial community.¹⁶ In adults, both long-term dietary intake and short-term changes in macronutrients (for example, an animal or plant-product-based diet) influences microbial community structure and microbial gene expression profiles.⁴ The outcomes of the complex dynamic connections between the microbiota and the immune system are most important during the postnatal period and have consequences on host immunity and on metabolic homeostasis that reach well into adulthood.

In this Review, we discuss the effect of diet on host–microbial interactions in early life and highlight the key aspects of nutritional programming during the postnatal period in influencing the lifelong function of the immune system in health and disease.

Nutritional programming

Nutritional deficiencies are a major cause of death in children in developing countries. Globally, undernutrition contributes to ~45% of the deaths of the ~6.6 million

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Competing interests

The authors declare no competing interests.

Key points

- Infant nutrition, including breast-milk, formula milk and solid weaning foods, is a key determinant of early microbial community structure that influences development of protective immunity and seems to affect health throughout life
- Diet-induced dysbiosis changes the species composition of the gut microbiota and leads to immune-mediated inflammatory and metabolic diseases
- Diet influences the postnatal development of innate and adaptive defences at the mucosal barrier surface and affects intestinal barrier function
- A triad of diet, the microbiota and the immune system regulates postnatal intestinal homeostasis and host physiology, which has consequences through to adulthood

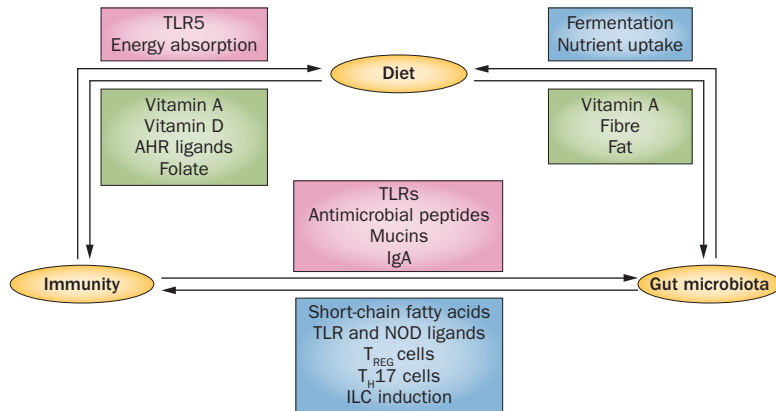


Figure 1 | The ‘diet hypothesis’. Diet, gut microbiota and host immunity are intimately connected and their bidirectional communication is central to maintaining intestinal and metabolic homeostasis. The commensal bacteria determine the nutritional value of food by fermenting dietary components to usable energy sources and by affecting nutrient uptake. Specific bacteria and microbial by-products influence the development and function of key components of mucosal immunity. The mucosal immune system shapes the commensal composition and location. Immune–microbial interactions via pattern-recognition receptors (TLRs, NODs) result in secretion of antimicrobial peptides, mucins and IgA, which maintains intestinal homeostasis and barrier function. The mucosal innate immune system also influences dietary energy absorption. Finally, perturbation of microbial community structure leads to dysbiosis, which can precipitate immune-mediated disorders such as IBD and metabolic diseases such as type 2 diabetes. Abbreviations: ILC, innate lymphoid cell; NOD, nucleotide oligomerization domain; T_H17 cell, type 17 T helper cell; TLR, Toll-like receptor; T_{REG} cell, regulatory T cell.

children <5 years old who die each year.^{17,18} The effects of childhood malnutrition are long-term and can even affect subsequent generations.¹⁹ Nutrition is an important driver of the convergence in gut microbial composition across mammalian phylogeny and within humans.^{20,21} The diet shapes the composition of the gut microbiota and the expression of microbial genes. In turn, the structure and activity of the gut microbiota determines the nutritional value the host will extract from the food they consume. Faecal microbiota profiles in adults with dietary habits that are specific to their culture, region and socio-economic status are fairly stable over time.²¹ By contrast, the composition of the microbiota undergoes considerable changes during the period from birth to weaning.¹⁶ Microarray studies based on small subunit ribosomal DNA have identified intrapersonal and interpersonal variation in the structure of faecal bacterial communities during the first year of life.²² The sterile fetal intestine receives its first inoculum in the hours after birth and the

colonization events that follow shape the structure of the microbial community and influence health outcomes as the child grows up (Figure 2).¹⁶

Early intestinal colonization

Infant nutrition, either breast-milk or formula milk, has a defining role in establishing early colonization patterns of the gut microbiota. In breast-fed infants, the gut microbiota is dominated by species from the *Bifidobacterium* family, whereas formula-fed babies harbour a more diverse microbiome containing *Escherichia coli*, *Clostridium difficile*, *Bacteroides* spp. and *Lactobacillus* spp.²³ In general, a step-wise progression towards adult-like enterotypes has been documented, with initial colonization primarily by Firmicutes and aerobic and facultative anaerobic bacteria such as *Staphylococcus*, *Streptococcus* and Enterobacteriaceae.^{24,25} These species are followed by colonization by *Enterococcus* and *Lactobacillus*-like species, which generate an anaerobic intestinal environment that provides favourable conditions for subsequent anaerobic colonizers.²⁶ After 1 week of life, *Bifidobacterium*, *Bacteroides* and *Clostridium* are detected in the faeces; the dominance of *Bifidobacterium* species in breast-fed infants is usually accompanied by a decrease in the amount of Enterobacteriaceae.^{16,27}

Breast-milk contains functional nutrients such as carbohydrates, nondigestible carbohydrates, fatty acids and lactoferrin that provide the microenvironment for gut protection and maturation.^{28,29} The oligosaccharides found in human breast-milk are nondigestible carbohydrates that are fermented in the colon and stimulate the growth of specific bacteria, including *Bifidobacterium* and *Bacteroides*, that contribute to the development of the immune system.^{27,30} Compared with that of adults, the infant metabolome is rich in acetate but deficient in propionate and butyrate,³¹ which correlates with the established microbial ecosystem as *Bifidobacterium* spp. and lactic acid bacteria do not produce butyrate. The infant intestinal environment is adapted to this butyrate deficit and enterocytes probably utilize alternative energy sources (these sources have yet to be identified).³² In addition to encoding genes whose products are involved in carbohydrate metabolism, bifidobacteria also express genes involved in folate biosynthesis,³³ which contribute to the unique nutritional requirement for the development of infants’ intestine and immune system. Human milk is also a source of live bacteria such as staphylococci, streptococci, bifidobacteria and lactic acid bacteria.^{34–36}

The introduction of solid foods containing insoluble nondigestible carbohydrates, and weaning from breast-milk or formula milk at age 4–6 months introduces another shift in the composition of the intestinal microbiome with increased counts of *Bacteroides*, *Clostridium* and anaerobic streptococci.^{37–39} This stage in the development of infants also marks the point when adult-like microbes that express genes involved in the degradation of xenobiotic compounds and vitamin biosynthesis are detectable. The gut microbiota begins to reach adult-like composition by the time an infant is 1 year old,⁴⁰ and is stable and firmly established by 2–3 years of age.^{16,41}

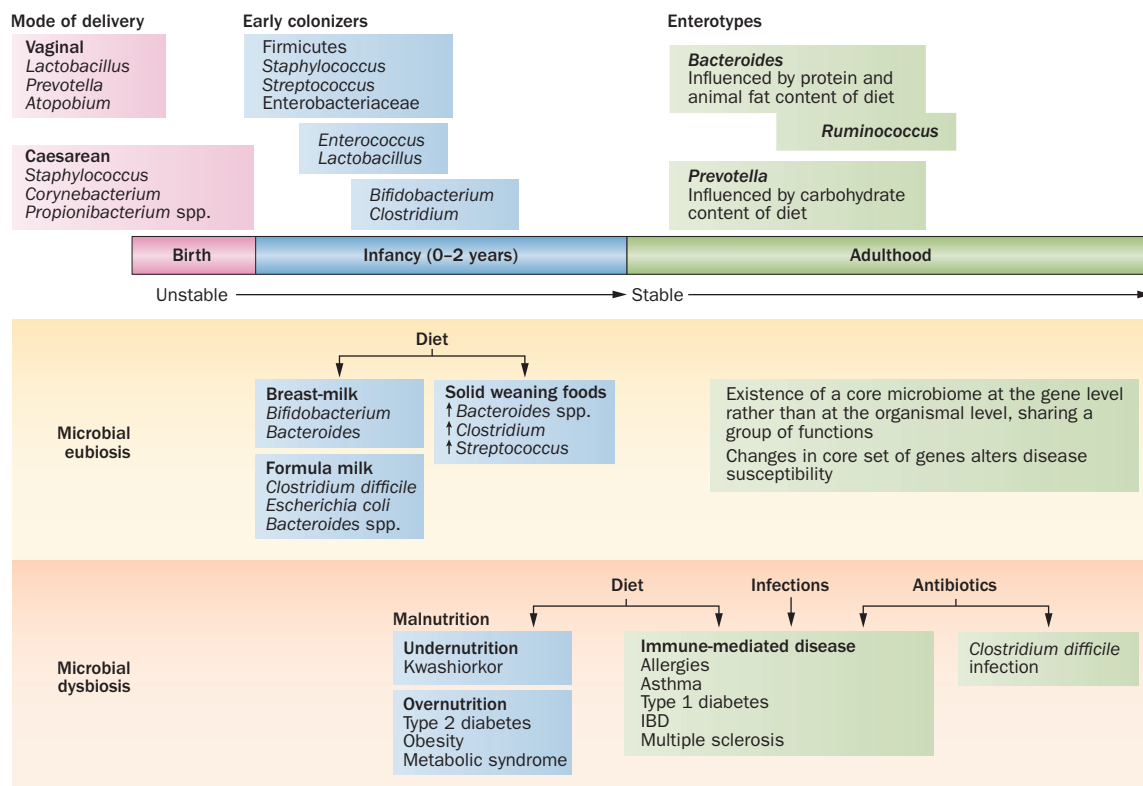


Figure 2 | Diet, gut microbiota and dysbiosis. Several features regulate the establishment and composition of the microbiota and their effect on the health and immune function of the host. Eubiosis or a normal microflora structure that protects against infections educates the immune system and contributes to nutrient digestion. Energy harvest is established by early intestinal colonization with specific microbes immediately after birth. An ordered process of subsequent colonization and expansion shaped by diet results in the establishment of distinct ‘enterotypes’, or clusters of microbial communities, that remains fairly stable in adults. Perturbations in the microbial community structure or dysbiosis are induced by factors such as diet, use of antibiotics or infection, which can alter susceptibility to several diseases.

However, in a paper published in 2012, it was suggested that the population of adult-type anaerobes might reach densities similar to those seen in adults within 1 week of birth and that the switch from facultative to strict anaerobes in healthy breast-fed neonates might occur earlier than previously assumed.⁴²

Bacterial enterotypes

The adult human gut microbiota can be clustered into three enterotypes of co-occurring microbial species defined by the levels of bacterial genera—*Bacteroides*, *Prevotella* and *Ruminococcus*.⁴³ The separation between the *Ruminococcus* cluster and the *Bacteroides*-led enterotype does not seem to be very discrete; however, the two groups remain distinct from the *Prevotella*-driven group.⁴⁴ These enterotypes are strongly associated with long-term diets; high protein and animal fat content of the diet increases levels of *Bacteroides* whereas a high carbohydrate content leads to dominance of *Prevotella*.^{9,44} Metagenomic studies comparing the gut microbiomes of members of agrarian Malawian⁴¹ and Bangladeshi⁴⁵ societies to the gut microbiomes of residents of the USA also found a similar inverse relationship between *Bacteroides* and *Prevotella* genera, underscoring a link between diet and structure of the gut microbiota. Whether these enterotypes can be used to predict disease incidence and

outcome and whether long-term dietary interventions can stably switch individuals to a disease-resistant microbiome will be important to determine. Furthermore, the origin of these enterotypes has not yet been characterized. A longitudinal study of the faecal microbial composition of Danish infants found that the establishment of the enterotype occurred between 9 and 36 months of age and correlated with the time of cessation of breastfeeding.⁴⁶ Patterns of initial intestinal colonization that are influenced by early childhood nutrition might be involved in the establishment of adult enterotypes.

Dysbiosis and disease

The composition of the microbial community is dynamic in early life but can also shift in adulthood under the influence of diet, antibiotic use or infection with invasive pathogens (Figure 2). These changes in the gut microbiota, referred to as dysbiosis, can disturb the homeostasis of microorganisms in the intestine and favour the outgrowth of potentially pathogenic constituents. Ultimately, dysbiosis can perturb the regulatory circuits of the immune system that restrain intestinal inflammation, leading to immune-mediated diseases directed against antigens of the gut microbiota.⁴⁷ As discussed earlier, the postnatal microbiota is not stable and is profoundly influenced by diet. However, the role of the

Box 1 | Gnotobiotic mice for investigating host–microbiota relationships

Germ-free animals are reared in a sterile environment (special positive-pressure isolators that maintain a ‘germ-free’ environment) and can be used to establish simplified microbial ecosystems to study the effect of eubiosis and dysbiosis on host immune function and physiology. The absence of a microbiota affects several developmental and physiological aspects in these mice as outlined below.⁵³

Developmental defects**Intestine**

- IECs with altered patterns of microvilli formation and decreased rates of turnover
- Reduced capillary network formation
- Fewer and less cellular Peyer’s patches
- Thinner and less cellular lamina propria
- Fewer plasma cells in germinal centres
- Smaller and less cellular isolated lymphoid follicles

Secondary lymphoid organs

- Spleen and lymph nodes lack structure and have poorly formed B-zones and T-zones
- Abnormal high-endothelial venule morphology
- Mesenteric lymph nodes with smaller germinal centres that are less cellular with fewer plasma cells

Immune defects

- Increased susceptibility to infection by certain bacteria, viruses and parasites (*Shigella flexneri*, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serova Typhimurium)
- Display atopy with a characteristic T_H2 -cell gene signature, airway eosinophilia and increased IgE production upon antigen challenge
- Fewer CD8⁺ intraepithelial lymphocytes
- Fewer CD4⁺ lamina propria lymphocytes; decreased levels of T_H17 cells in small intestines
- Fewer FOXP3⁺ T regulatory cells in mesenteric lymph nodes with decreased suppressive capacity
- Reduced IgA production by B cells
- Reduced expression of REG3 γ and angiogenin-4 (both antimicrobial peptides) by Paneth cells
- Altered gene expression profiles of IECs
- Reduced expression of major histocompatibility complex class II, Toll-like receptor 9 and IL-25 by IECs

Metabolic defects

- Decreased levels of ATP in intestines
- Altered glycosylation pattern of lumenally exposed surface proteins on IECs
- Reduced energy absorption capacity

Abbreviations: FOXP3, forkhead box P3; IEC, intestinal epithelial cell; REG3, regenerating islet-derived protein 3; T_H , T helper.

evolving and fluctuating neonatal microbiota in influencing the development and function of the immune system is still unclear. Furthermore, the degree of involvement of the neonatal immune system in shaping the composition of the gut microbiota is also unknown.

Basis for and effect of dysbiosis

Dysbiosis is characterized by a reduction in taxonomic diversity and species representation of the gut microbiota,⁴⁸ and can be accompanied by a decrease in bacterial biomass, depending on the nature of the infection and inflammatory insult.^{49,50} Pathogenic bacteria exploit conditions of dysbiosis, including decreased and altered metabolic capacity of the intestine,⁵¹ and gain access to intestinal nutrients and niches, displacing commensal bacteria and leaving the host increasingly susceptible to both pathogens and pathobionts. The developing microbiota in early postnatal life is fragile and extremely susceptible

to external influences that affect overall host health. For instance, a correlation exists between the use of antibiotics during infancy and childhood and increased incidences of asthma, atopic dermatitis, multiple sclerosis and IBD.⁵²

The effects of dysbiosis on host physiology have been studied extensively in gnotobiotic mice (Box 1).^{53,54} Faecal microbial communities and the abundance of bacterial genes in experimentally colonized germ-free mice are highly sensitive to different foods.⁵⁵ Furthermore, dysbiosis induced by antibiotics in newborn mice leads to alterations in the development of the immune system that are similar to those observed in germ-free mice,^{56–58} with reduced numbers of intestinal regulatory T cells (T_{REG} cells),⁵⁹ increased colonic infiltration of invariant natural killer T cells^{60,61} and increased susceptibility to allergen-induced airway hyper-reactivity and colitis.⁶⁰ Thus, host–microbe interactions at the postnatal stage are critical for the establishment of protective immunity. Disruption of this process by changes in diet, the use of antibiotics or by infectious inflammation might alter the susceptibility to immune-mediated diseases in adulthood.

Diet, dysbiosis and disease

Malnutrition (which includes overnutrition and undernutrition) is a global health problem and a major contributor to childhood morbidity and mortality. Food insecurity has been linked to this epidemic,⁶² but it is becoming increasingly evident that the gut microbiota is closely connected to the problem of malnutrition. In developing countries, inadequate nutrition and micronutrient deficiencies delay the growth of children and result in stunting that affects a staggering 27% of children <5 years old.⁶³ Interestingly, discordance for moderate to severe malnutrition between twins (monozygotic and dizygotic) within the same household and fed similar diets is only ~40%, which suggests that additional factors such as individual gut community configurations might influence the severity of undernutrition.⁶⁴ By contrast, in developed countries, modern societal practices such as improved hygiene, reduced family size, an increased incidence of Caesarean deliveries, reduced rates of breastfeeding and widespread antibiotic use in children affects the transmission and maintenance of the indigenous gut microbiota.⁶⁵ The consequent effect on the development and function of the immune system has contributed to the increase in chronic inflammatory and autoimmune disorders.⁶⁶ Thus, in addition to a genetic predisposition and biological mechanisms, a ‘shift’ in the resident microbiota from a ‘healthy’ to a ‘diseased’ state underlies disease development and progression.

In a study published in 2013, the gut microbiota was implicated in the propagation of kwashiorkor, a form of severe acute malnutrition.⁶⁴ Longitudinal studies in Malawian twin pairs showed that the overall gene content of the faecal microbiota in children with kwashiorkor did not develop with increasing age.⁶⁴ In addition, the disease phenotype, including weight loss, was transmissible to germ-free mice that received faecal transplants from sick children but not from healthy ones.⁶⁴ Importantly, treatment with ready-to-use therapeutic food did not change the

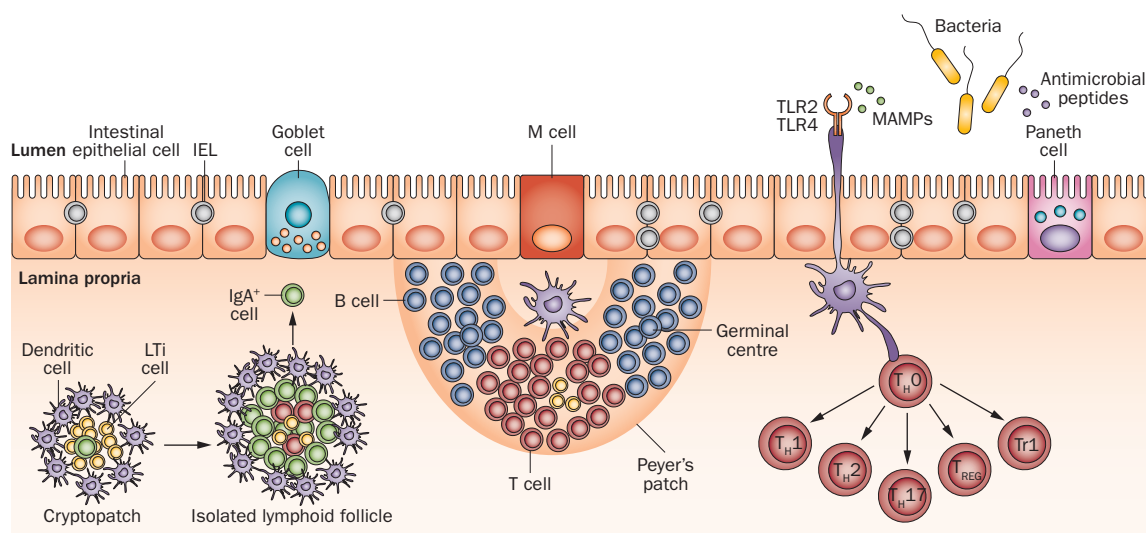


Figure 3 | The intestinal barrier. The intestinal barrier is made up of a single layer of epithelium consisting of intestinal epithelial cells and specialized Goblet cells, M cells and Paneth cells (present only in small intestine). Peyer's patches (found specifically in the ileum) and mesenteric lymph nodes develop prenatally when LTi are recruited to sites of the developing intestines called cryptopatches. Cryptopatches mature into isolated lymphoid follicles when pattern-recognition receptors (TLRs) are triggered by MAMPs, which then release IgA-producing plasma cells into the lamina propria. Dendritic cells in the Peyer's patches access microbes through M cells or directly from the lumen by extending dendrites through intestinal epithelial cells. These antigen-loaded dendritic cells can induce T-cell differentiation or T-cell-dependent B-cell maturation into germinal centres. Naive T cells (T_H0 cells) can differentiate into effector T_H1 , T_H2 or T_H17 cells or into regulatory FOXP3⁺ T_{REG} cells or Tr1 cells. Microbial sensing by intestinal epithelial cells also marks the release of antimicrobial peptides and stimulation of intestinal epithelial cell proliferation in crypts. Abbreviations: IEL, intraepithelial lymphocyte; LTi, lymphoid tissue inducer cell; MAMP, microbe-associated molecular pattern; M cell, microfold cell; T_H , T helper cell; TLR, Toll-like receptor; Tr1, type 1 regulatory T cell; T_{REG} , regulatory T cell.

microbial community profiles of affected children in the long term, supporting the notion that multiple determinants shape the fitness landscape of the gut; this landscape can be used to predict disease severity and progression.

Not surprisingly, the microbiota and excessive caloric intake have also been linked to obesity, the metabolic syndrome and type 2 diabetes. For example, the intestinal microbial profile of obese mice is considerably different to that of normal-weight mice with over-representation of *Firmicutes* instead of *Bacteroidetes* that results in increased harvesting of energy from the diet of the host.⁶⁷ This disease trait is transmissible, as colonization of germ-free mice with an 'obese microbiota' results in increased body fat compared with colonization with 'lean microbiota'.⁶⁷ In addition, germ-free mice are resistant to diet-induced obesity, but show increased adiposity when colonized with gut flora from leptin-deficient *ob/ob* mice.⁶⁸ Similarly, studies of twin cohorts in the USA revealed a distinct bacterial phylogenetic composition and representation of microbial genes involved in nutrient metabolism in lean versus obese twin pairs.⁶⁹ Interestingly, the immune system might itself regulate obesity as mice deficient in Toll-like receptor (TLR) 5 have an increased incidence of the metabolic syndrome, which has been linked to their unique gut microbiota and outgrowth of *E. coli*.^{70,71} Other studies have, however, challenged the magnitude of the effect TLR signalling has on changes to the microbial composition⁷² and have raised awareness of effects of animal husbandry conditions and lineage or legacy effects in microbiome studies in rodents.⁷³

Substantial changes to the commensal gut microbiota have been observed in patients with type 2 diabetes. These patients had decreased levels of bacteria from the phylum *Firmicutes* and strains of *Clostridia* that produce short-chain fatty acids as well as increased levels of the *Bacteroidetes* and *Prevotella* groups that correlated with high plasma concentrations of glucose.^{74–76} In addition, a low-grade systemic and chronic inflammation that results from reduced transepithelial integrity and increased metabolic endotoxaemia that is linked to altered gut microbial profiles has been associated with the development of type 2 diabetes.⁷⁷ Increased serum levels of lipopolysaccharide and translocation of commensal *E. coli* have also been observed in mouse models of type 2 diabetes induced by a high-fat diet.^{78,79}

Changes in diet and the composition of the microbiota also contribute to susceptibility to autoimmune diseases such as type 1 diabetes.^{66,80} For example, low gut microbial diversity has been linked with the development of type 1 diabetes in children.^{81–83} The presence of segmented filamentous bacteria has a protective effect on the incidence of type 1 diabetes in mice in disease-susceptible female nonobese diabetic mice.⁸⁴ A meta-analysis of 43 studies found that breast-feeding might offer some limited protection against the development of type 1 diabetes,⁸⁵ whereas exposure to cow's milk protein through infant formula might increase the risk of developing β -cell autoimmunity.⁸⁶ These are as yet unsettled issues, as other studies found no effects of breast-feeding on the susceptibility to developing type 1 diabetes.⁸⁰

Box 2 | Composition of the intestinal epithelium

- Enterocytes: Absorptive cells that secrete hydrolases and absorb nutrients, ions and fluids
- Goblet cells: Produce mucin that makes up the mucous layer
- Paneth cells: Found primarily in small intestines and secrete antimicrobial peptides such as cryptidins, defensins and enzymes such as lysozyme
- Microfold (M) cells: Specialized cells found in epithelium of Peyer's patches that transport organisms and particles from the gut lumen to immune cells deep in the epithelial barrier
- Enteroendocrine cells: Secrete hormones such as serotonin, substance P and secretin

After weaning, the introduction of solid foods, including excessive intake of cow's milk and consumption of gluten-containing cereals has also been associated with the development of type 1 diabetes.^{87,88}

Thus, dietary patterns and microbiota shape host physiology and effect disease susceptibility at mucosal barrier and systemic sites. The components of the host barrier and immune system that are affected by diet and the microbiota are discussed in the next section.

Mucosal barrier function

The intestinal tract is the largest barrier in the human body. It is made up of a single layer of epithelial cells (intestinal epithelial cells [IECs]) that confines the gut microbiota and other potentially harmful substances to the lumen whilst regulating the flow of solutes, nutrients and ions into the underlying mucosa.^{89–91} Highly specialized barrier defences have evolved that include multiple layers of resistance, incorporating a stratified mucous layer, a fairly impenetrable but responsive epithelium and a lamina propria that contains innate and adaptive immune cells that maintain intestinal homeostasis (Figure 3). In the event of a barrier breach, all these components are poised to mount antimicrobial clearance responses and to rapidly reseal and repair tissue after injury to restore barrier function. The development and maturation of the intestinal mucosa and its gut-associated lymphoid tissue, including Peyer's patches, isolated lymphoid follicles and mesenteric lymph nodes, is initiated by and dependent upon intestinal microbial colonization.⁹⁰

The intestinal epithelium contains five main types of cells, each of which is responsive to and conditioned by the gut microbiota (Box 2). The intestinal epithelium of a newborn baby is immature and therefore ingested foreign particles and bacteria readily gain access to the lymphatic system and bloodstream during this period.⁹² Maturation changes are rapidly triggered by microbial colonization,⁹³ as well as by dietary substances such as breast-milk or formula milk,⁹⁴ which prepares the infant intestine to accommodate dense bacterial colonization (Figure 4). This process might take several weeks, and is usually prolonged in infants born prematurely.⁹⁵ In premature neonates, immaturity of the intestinal barrier can result in devastating diseases such as necrotizing enterocolitis,⁹⁶ toxicogenic diarrhoea and intestinal allergies.⁹⁷

Toll-like receptors and barrier function

The IECs express a wide array of receptors such as TLRs and C-type lectin receptors that reside in the endosome or on the cell surface, and cytosolic nucleotide oligomerization domain-like receptors that sense microbe-associated molecular patterns (MAMPs) expressed by commensal and pathogenic microbiota.⁹⁸ Recognition of MAMPs by neonatal IECs is necessary for stimulating the development of isolated lymphoid follicles and the formation of lymphoid structures that are capable of supporting maturation of B cells and production of sIgA.^{99–103} A singular example of the sensitivity and responsiveness of the infant gut to microbial colonization is regulation of the expression of TLR4 (the receptor for the Gram-negative bacterial product lipopolysaccharide). In mouse IECs, expression of TLR4 is high before intestinal colonization at birth.¹⁰⁴ Within hours of exposure to the gut microbiota, the expression of TLR4 in IECs is rapidly downregulated with a concomitant attenuation of components of the TLR4 signalling pathway such as inhibition of IRAK-1 translation by the microRNA miR-146a.¹⁰⁵ This desensitization of a microbial sensor is an adaptation to mounting microbial loads, and failure to temper TLR4 signals has been linked to the development of necrotizing enterocolitis, particularly in preterm infants.¹⁰⁶

Neonatal intestinal maturation and barrier function is modified by breast-feeding. Intestinal immunomodulation that is mediated by breast-milk might result in subclinical infections that gradually stimulate immunological memory to pathogens while reducing inflammation.¹⁰⁷ Breast-milk modulates the expression of TLRs, as well as the signalling pathways they are involved in. For example, soluble TLR2 that is found in human milk can competitively inhibit signalling through membrane TLR2 and helps restrict innate immune stimulation in the neonatal gut.¹⁰⁸ Furthermore, reduced TLR2 activity at birth could facilitate the normal establishment of *Bifidobacterium* in the intestine.¹⁰⁸ Immune cells such as macrophages that are present in colostrum and mature breast-milk persist in the neonatal intestine and also translocate to systemic sites where they form a first line of defence against invading pathogens.¹⁰⁹ Intestinal growth, maturation and function are also promoted by various maternal factors such as epidermal growth factor and transforming growth factor β , which are found at high levels in the colostrum and mature breast-milk.¹¹⁰

Tight junctions

The barrier function of the intestinal epithelium is maintained by the apical junction complex, which is composed of tight junctions and adherence junctions.¹¹¹ Adherence junctions and desmosomes provide the strong adhesive bonds between IECs and participate in intercellular communication. Tight junctions, which encircle the apical ends of the lateral membranes of IECs, mediate the selective permeability and paracellular transport function of the epithelial barrier. The permeability of tight junctions is regulated by several factors, including local cytokine milieu,¹¹² age,¹¹³ the composition of the commensal bacteria and infection.¹¹⁴ Treatment

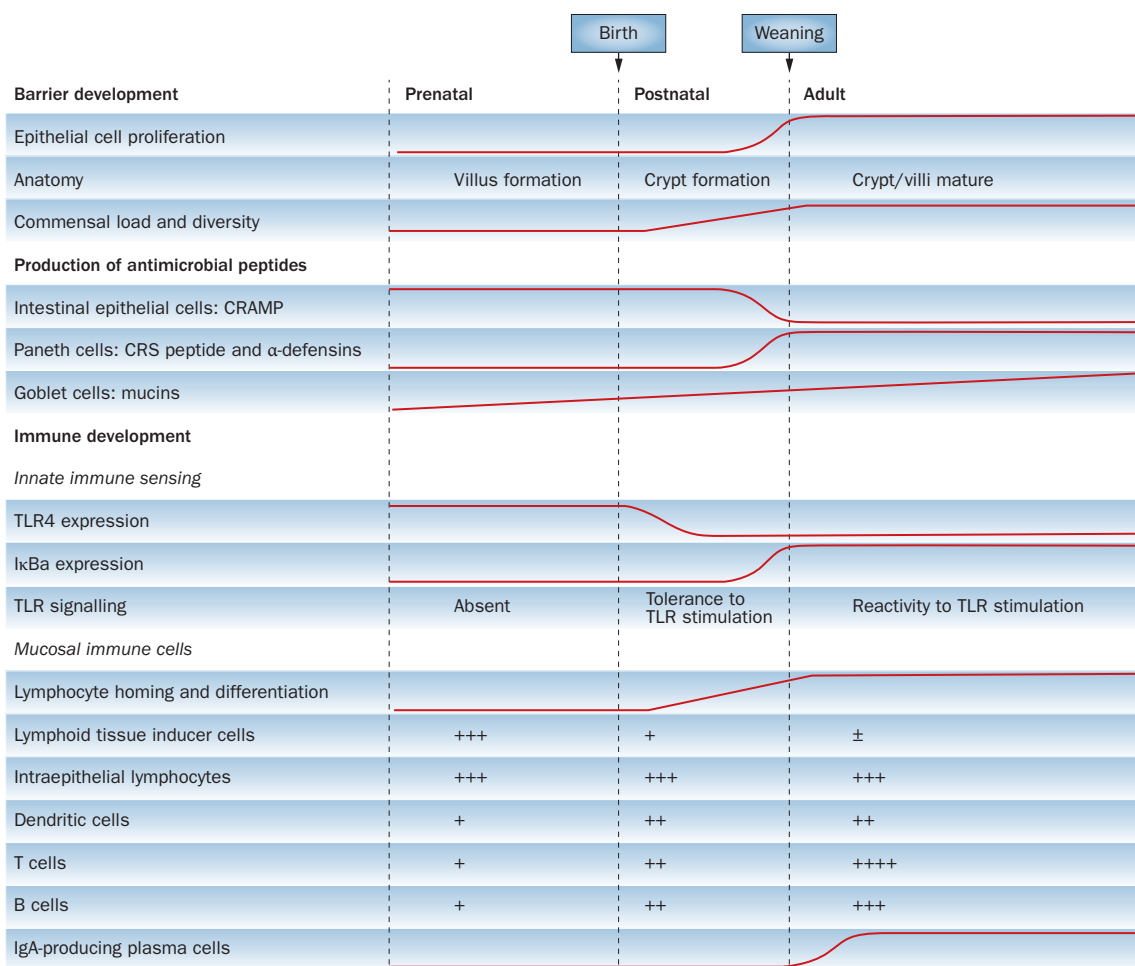


Figure 4 | Development and maturation of the intestinal mucosal barrier and mucosal immune system. Developmental changes in the prenatal, postnatal and adult intestine are summarized. Intestinal microbial colonization, as well as dietary components, induces maturation of the intestinal epithelium and initiates development of the mucosal immune system. Complex bidirectional interactions between gut microbiota, diet and the immune system itself regulate the establishment and maintenance of intestinal homeostasis and barrier function. Abbreviations: CRAMP, cathelin-related antimicrobial peptide; CRS, cryptidin-related sequence; TLR, Toll-like receptor; ±, cells might or might not be present; +, ++, +++, +++++, relative numbers of indicated cells.

of epithelial cell monolayers with cytokines such as IFN- γ and TNF increases the permeability of the tight junctions, which can be reversed by commensal bacteria such as *Bacteroides thetaiotaomicron*, as well as by probiotic *Streptococcus thermophilus* and *Lactobacillus acidophilus*.¹¹⁵ The mechanisms by which commensal microbiota protect barrier function include modulation of the expression of the occludin and claudin proteins and utilization of epithelial cell signalling machinery such as Rho GTPases, PKC and MAPK pathways to enhance barrier integrity.¹¹⁴

Enteric pathogens such as *Vibrio cholerae*¹¹⁶ and enteropathogenic *E. coli*¹¹⁷ disrupt the tight junction barrier, which leads to abnormal electrolyte and fluid transport and tissue inflammation. Impaired function of the tight junctions has been observed in several autoimmune diseases, including coeliac disease and type 1 diabetes.¹¹⁸ In preterm infants, failure of tight junctions and barrier function has been implicated in the development of necrotizing enterocolitis.¹¹⁹ Finally, tight junctions are

also influenced by dietary components, but more work needs to be done to firmly establish the cause–effect relationship.¹¹⁴ Glutamines, polyphenols and probiotics enhance and protect tight junction barrier integrity, whereas alcohol and its metabolites impair tight junction barrier function.¹¹⁴

Immune system development

The mucosal tissues of the gastrointestinal tract harbour more cells from the immune system than all the secondary lymphatic tissues combined. Over the past few years, it has become apparent that the complex microbiota (including commensals, symbiotic bacteria and pathogens) influences immune reactivity in the intestine as well as at extraintestinal sites such as the pancreas⁸⁴ and the brain.^{120,121} The infant mucosal immune system matures over several months after birth and the process is closely tied with the development and establishment of the gut microbiota and the quality of dietary nutrients and commensal-derived metabolites (Figure 3).^{122,123}

Organized lymphoid tissues

The development of the mucosal immune system begins at the fetal stage. At this stage in mice, the actions of a subset of innate lymphoid cells (ILCs) called lymphoid tissue inducer cells initiates prenatal organization of secondary lymphoid tissues including Peyer's patches and mesenteric lymph nodes.^{101,124} Additional tertiary lymphoid tissue structures such as the isolated lymphoid follicles, cryptopatches in the small intestine and colonic patches in the large intestine develop after birth under the influence of lymphoid tissue inducer cells.¹²⁴ Cryptopatches consist of a cluster of lymphoid tissue inducer cells that express the transcription factor nuclear receptor ROR- γ t, are surrounded by dendritic cells that express CD11c (also known as integrin- α X) and are positioned below the single layer of intestinal epithelium. Colonization by gut microbiota is required before cryptopatches develop into isolated lymphoid follicles.⁹⁰ Aryl hydrocarbon receptor ligands (which are derived from cruciferous vegetables such as broccoli) provide important signals for the development and maturation of the immune system.^{125,126} The aryl hydrocarbon receptor is expressed in ILCs positive for nuclear receptor ROR- γ t and has an essential role in their function and maintenance.^{126–128} *Ahr*-deficient mice lack postnatally imprinted cryptopatches and isolated lymphoid follicles but not Peyer's patches, as these develop in the embryo.¹²⁷ Furthermore, these mice are highly susceptible to infection with the intestinal pathogen *Citrobacter rodentium*, which emphasizes the role of dietary components in controlling mucosal immunity.

Myeloid cells

Myeloid cells, such as dendritic cells positive for integrin- α E, dendritic cells negative for integrin- α E and positive for CX3CR1 as well as macrophages, are strategically positioned below the intestinal epithelium and act as cellular mediators that translate microbial signals into intestinal homeostasis. Dendritic cells loaded with microbial antigens interact with lymphocytes to induce T-cell differentiation and T-cell-dependent maturation of B cells in the germinal centres.⁹⁰ CX3CR1⁺ dendritic cells make direct contact with luminal antigens by extending processes through the tight junction of IECs in a TLR-dependent manner (Figure 3).¹²⁹ This process is important for tolerance to orally ingested antigens.¹³⁰ Microbiota-induced production of IL-23 by dendritic cells also recruits ILCs to the sites of mucosal immune responses during infections.¹³¹

In newborn mice, delayed developmental maturation of dendritic cells and a reduced number of antigen-presenting cells results in compromised immunity in the first week of life.¹³² However, the function and homeostasis of myeloid cells in the infant intestine is less clear. An important role for dietary micronutrients in regulating mucosal myeloid cell homeostasis is emerging. In mice, migratory dendritic cells that are positive for integrin- α E have high basal expression of *Aldh1a2*.¹³³ This gene encodes the enzyme retinal dehydrogenase 2 that synthesizes retinoic acid (a vitamin A metabolite), which imprints gut homing potential on T cells and B cells by inducing expression of migration receptors α 4 β 7 and CCR9.^{134,135} Thus, these

myeloid cells are central to regulation of mucosal immunity mediated by vitamin A. Vitamin A deficiency is a common micronutrient deficiency in children¹³⁶ and its effect on intestinal myeloid cell homeostasis and function in the young remains to be determined.

Lymphoid cells

Several subsets of lymphocytes reside throughout the intestinal epithelium (intraepithelial lymphocytes [IELs]) and the lamina propria (lamina propria lymphocytes). IELs are heterogeneous populations of antigen-experienced T cells that express either the α β or γ δ T-cell receptor (TCR).¹³⁷ As IELs have direct contact with enterocytes and close proximity to antigens in the gut lumen, they are geared to provide immediate and heightened immune protection to halt the initial entry and spread of pathogens. At the same time, IELs are under tight regulatory control, as an overzealous inflammatory response could jeopardize barrier integrity. As with other cell types in the gut, the development and function of IELs are also influenced by dietary components such as aryl hydrocarbon receptor ligands¹²⁵ and vitamin D.¹³⁸ Mice deficient in vitamin D receptor have reduced numbers of CD8 α α ⁺ IELs, which was associated with low levels of IL-10 in the small intestine and increased inflammation in wild-type animals.¹³⁹

Lamina propria lymphocytes are enriched in differentiated TCR α β ⁺ CD4⁺ T cells, including the proinflammatory type 17 T helper (T_H17) cell subset and suppressor T_{REG} cells. Segmented filamentous bacteria are key to the differentiation and accumulation of T_H17 cells in the terminal ileum of mice,^{140,141} where they are critical for host defence against infections with extracellular bacteria and fungi.¹⁴² T_H17 cells have also been associated with numerous autoimmune and chronic inflammatory diseases. For example, increased numbers of T_H17 cells have been found in patients with IBD^{143,144} and polymorphisms in the *IL23R* gene (which is important for the maintenance and pathogenicity of T_H17 cells) are linked to susceptibility to IBD.¹⁴⁵ In addition, numerous studies in mouse models of IBD have demonstrated the involvement of T_H17 cells in the pathogenesis of IBD.^{146,147} As a result of the tremendous pathogenic potential of T_H17 cells, several mechanisms have evolved to restrain and limit their function and prevent chronic inflammatory episodes. T_{REG} cells expressing the transcription factor FOXP3 and type 1 T_{REG} cells that produce IL-10 are key to this activity and suppress intestinal inflammation.^{148,149} Genome-wide association studies have linked *IL10* polymorphisms (IL-10 produced by T_{REG} cells is critical for suppressive effects by T_{REG} cells¹⁵⁰) to susceptibility to IBD.¹⁵¹ In addition, mutations in *IL10*, *IL10RA* or *IL10RB* as well as IL-10R signalling components *STAT3*, *TYK2* and *JAK2* lead to the development of early-onset IBD.^{152,153}

Vitamin A is a fat-soluble essential micronutrient whose metabolite, retinoic acid, has several crucial roles in intestinal immunity, including regulating the balance between intestinal T_H17 and T_{REG} cells. Retinoic acid promotes the extrathymic generation of T_{REG} cells and induction of mucosal and oral tolerance.^{154,155} Interestingly, mice fed a diet deficient in vitamin A also have diminished numbers

of T_H17 cells in the small intestine at steady state, and differentiation of T_H17 cells in the presence of low physiological concentrations of retinoic acid can promote their trafficking to the gut.^{156,157} Products of bacterial fermentation such as short-chain fatty acids regulate the balance of inflammatory and regulatory responses in the colon of mice.^{158–160} A reduced intake of plant dietary fibres and use of antibiotics negatively affect levels of short-chain fatty acids, which alters intestinal immune regulation by reducing the number of colonic T_{REG} cells.¹⁶¹

ILCs include a variety of specialized cells that secrete a suite of effector cytokines and chemokines to combat infection and repair tissue at mucosal barriers.¹⁶² The ILC subsets have considerable developmental and functional plasticity, which might be instructed by the needs of their local microenvironment. Furthermore, ILCs are also influenced by dietary factors and act as sensors of dietary stress. Vitamin A deficiency negatively affects the development of group 3 ILCs, which results in compromised immunity to acute bacterial infection.¹⁶³ However, this phenomenon was compensated for by the dramatic expansion of group 2 ILCs (which produce IL-13), resulting in resistance to nematode infection in mice.¹⁶³ In addition, maternal levels of dietary retinoids control the size of secondary lymphoid organs and the efficiency of immune responses in their adult offspring by regulating the differentiation of group 3 ILCs (lymphoid tissue inducer cells) *in utero*.¹⁶⁴

Conclusions

The relationship between diet, microbiota and host immunity is being rapidly unravelled using a combination of epidemiological, immunological, metagenomic and metabolomic approaches. These studies are most pertinent at the postnatal period when dietary intake is closely tied to the development of both the gut microbiota and the immune system. In a study published in 2014, a prenatal placental microbiome was described that could be a source of the infants' first bacterial inoculum via intra-uterine seeding.¹⁶⁵ Whether and how this low abundance yet metabolically rich microbiome directs the development of the immune system and the microbial community structure during gestation, as well as the effect of maternal nutrition on these processes, remains to be determined. A systems approach involving both animal studies and

analysis of human cohorts are needed to unravel the complexities of microbiota–host crosstalk in early life. Animal models are invaluable and have provided a plethora of information and insights into the interplay between the immune system and host microbiota. However, it is important to caution that a direct correlation from animal studies to humans is not possible, particularly when interrogating immune developmental events in early life. For instance, in humans, αβ TCR⁺ T cells are seen in peripheral tissue at 10–12 gestational weeks; however, in mice, peripheral T cells are undetected in the fetus and their numbers only increase after birth,¹⁶⁶ which is suggestive of distinct developmental cues and immune requirements in human versus mouse neonates.

The interdependence of diet, immune and microbiota interactions and communications between the elements of this triad dictate intestinal mucosal homeostasis as well as metabolic well-being. The mechanisms by which these dialogues occur are only now being elaborated on and major gaps remain in our understanding of how specific nutrients and microbial metabolites regulate microbial composition, host metabolism and immunity. The use of specific dietary components in modulating the gut microbiota and subsequent immune function offers an attractive approach to deliver health benefits to a vulnerable population, such as paediatric and geriatric populations. As such, probiotics and prebiotics are being increasingly used to prevent and treat a variety of gastrointestinal and systemic diseases in infants.^{167,168} Discoveries aimed at establishing specific features of the immune–microbiota crosstalk will provide useful insights for the development of preventive and therapeutic agents of multiple infectious, autoimmune and metabolic disorders.

Review criteria

PubMed was searched using key words, including “microbiota/gut microbiota” AND “diet/nutrition” AND “neonate/pediatric/adult” OR “immune system development/immune/mucosal immunity/autoimmunity” OR “enterotype/obesity/malnutrition/barrier function/epithelial barrier”. Due to the broad scope of the Review and limitations on allowable length, not all relevant original publications were cited and readers have been directed to excellent comprehensive reviews where available.

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