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# Spatial and temporal key steps in early-life intestinal immune system development and education

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## Keywords

antigen sampling; breast milk; maternal factors; microbiota; neonatal immunity; weaning

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Education of our intestinal immune system early in life strongly influences adult health. This education strongly relies on series of events that must occur in well-defined time windows. From initial colonization by maternal-derived microbiota during delivery to dietary changes from mother's milk to solid foods at weaning, these early-life events have indeed long-standing consequences on our immunity, facilitating tolerance to environmental exposures or, on the contrary, increasing the risk of developing noncommunicable diseases such as allergies, asthma, obesity, and inflammatory bowel diseases. In this review, we provide an outline of the recent advances in our understanding of these events and how they are mechanistically related to intestinal immunity development and education. First, we review the susceptibility of neonates to infections and inflammatory diseases, related to their immune system and microbiota changes. Then, we highlight the maternal factors involved in protection and education of the mucosal immune system of the offspring, the role of the microbiota, and the nature of neonatal immune system until weaning. We also present how the development of some immune responses is intertwined in temporal and spatial windows of opportunity. Finally, we discuss pending questions regarding the neonate particular immune status and the activation of the intestinal immune system at weaning.

## Introduction

The adult gut mucosa is well equipped to tolerate the enteric microbiota while protecting against enteropathogenic infections or food-borne toxins. Barrier-strengthening defense mechanisms promote homeostasis by keeping microorganisms at bay. In the small and large intestine, the secretion of antimicrobial peptides (AMPs) by Paneth cells and enterocytes creates a chemical barrier [1–3]. In addition, goblet cells

generate a mucus layer that forms a physical barrier to withhold luminal bacteria, especially in the colon, where commensal bacteria exhibit the highest density [4,5]. Below this mucus layer, a thick glycocalyx enables access of soluble molecules but not virus or bacterium-sized microparticles to the intestinal epithelial cells (IECs) [6,7]. Moreover, tight junctions seal the epithelial cell–cell contacts and regulate the

## Abbreviations

AMP, antimicrobial peptides; cDC, conventional dendritic cell; CRAMP, cathelicidin-related antimicrobial peptide; CTL, cytotoxic T cells; EGF, epidermal growth factor; EPEC, enteropathogenic *Escherichia coli*; FAE, follicle-associated epithelium; GAP, goblet cell-associated antigen passage; GC, germinal center; HMO, human milk oligosaccharides; IEC, intestinal epithelial cell; ILC3, type 3 innate lymphoid cells; ILF, isolated lymphoid follicle; LOS, late-onset sepsis; MLN, mesenteric lymph node; NEC, necrotizing enterocolitis; pIgR, polymeric Ig receptor; PP, Peyer's patch; SARS-CoV2, severe acute respiratory syndrome coronavirus type 2; SCFA, short-chain fatty acid; sIgA, secretory immunoglobulin A; Tfh, follicular helper T cell; TLR, Toll-like receptor; Treg, regulatory T cells.

paracellular passage of solutes [8]. Finally, the intestinal immune system comprises many mechanisms of surveillance, defense, and tolerance, which provide a tailored response to encountered antigens [9,10]. In the small intestine, the immune system is compartmentalized between organized inductive sites, that is, the isolated lymphoid follicles (ILFs) and Peyer's patches (PPs), and diffuse effector sites, that is, the lamina propria (LP) and the epithelium. In absence of infection, the intestinal immune system promotes tolerance to soluble antigens transported by LP migratory conventional dendritic cells (cDCs) through afferent lymphatics to the draining mesenteric lymph nodes (MLNs) [11–13]. By contrast, PPs do not have afferent lymphatics but are in direct contact with luminal antigens [14]. PPs consist of clustered B-cell follicles forming domes covered by the follicle-associated epithelium (FAE) containing M cells that are specialized in the transcytosis of luminal particulate antigens [15–17]. These antigens are then taken up by subepithelial macrophages and specialized monocyte-derived DCs termed LysoDCs [18]. Upon stimulation, subepithelial LysoDCs migrate to the T-cell zones of PPs to induce an immune response [19]. In addition, subepithelial phagocytes interact with and activate naïve B cells [20]. Due to continuous access to luminal antigens, PPs serve as the main inductive sites for B-cell priming and maturation, leading to their homing to the LP where they differentiate into immunoglobulin A (IgA)-secreting plasma cells [21,22]. Secreted IgA, transported by villus epithelial cells into the lumen via the polymeric Ig receptor (pIgR), plays a pivotal role in homeostasis and during infection, notably by excluding pathogens and neutralizing toxins, but also by regulating the microbiota composition [23–26].

In neonates, the situation largely differs. At birth, the neonatal gastrointestinal tract is rapidly colonized by trillions of bacteria, which initiate the development of the mucosal immune system [27], while breastfeeding provides neonates with factors regulating the microbiota composition and the immune response but also protecting against infections [28]. At weaning, cessation of breastfeeding and the resulting change in diet now containing nutrients with a complex composition induce a diversification of the microbiota and trigger the maturation of the mucosal immune system, notably leading to the production of endogenous IgA. During the last decade, several early-life key age-related events that are essential for the development of optimal intestinal immunity in adults have been described, introducing and supporting the concept of time windows of opportunity [27,29,30]. These events are not only dependent on time but also on the sites where

they occur. Thus, sampling of luminal antigens is tightly regulated both temporally and spatially in early life as is the proximity between the microbiota and the mucosa and the type of luminal antigen encountered, with a clear distinction between the small intestine and the colon [31]. In this review, we first discuss the susceptibility of neonates to infections and inflammatory diseases as an initial evidence that intestinal immunity in neonates is not absent or immature but actually strongly different from that of adults. Then, we briefly summarize the multistep process of microbiota assembly as a key element of intestinal immune education, before focusing on maternal factors that are the primary actors to be involved in neonatal protection and mucosal immune system education. We also describe neonatal defense mechanisms and immune responses of neonates. Finally, we discuss how microbiota is involved in several spatial and temporal key steps of immune education and imprinting. Altogether, this review highlights the microbiota, breast milk factors, and the host's response to environmental changes as three major factors supporting the multistep evolution of early-life homeostasis.

## **Susceptibility of the neonatal host to inflammation and infection**

Clinical studies describe the neonatal immune status as hyperinflammatory, that is, prone to an inappropriate inflammatory reaction. At the same time, the neonatal host is generally more susceptible to infection although exceptions have been described. The molecular mechanisms that determine these age-dependent differences are only beginning to be unraveled but they likely include differences in the organization and gene expression of stromal cells, particularities in the differentiation and adaptation of innate and adaptive immune cells as well as the reduced diversity and density of the neonatal microbiota.

One example for the hyperinflammatory status of the neonatal immune system is necrotizing enterocolitis (NEC), a severe inflammation of the colonic tissue affecting mainly preterm infants [32]. The inflammatory response is thought to be driven by the early bacterial colonization of the intestinal tract and caused by the immature mucosal tissue in preterm infants, unable to cope with postnatal bacterial colonization. Certain pathobionts have been associated with NEC. Their expansion in the neonatal intestine is facilitated by the lack of a dense and diverse microbiota that limits pathobiont growth by competition. Consistently, antibiotic treatment that reduces microbiota diversity is associated with an enhanced risk of developing NEC

(Fig. 1) [33,34]. Indeed, studies comparing the fecal microbiota between preterm infants indicate a lower bacterial diversity and certain alterations of the microbiota composition in infants developing NEC [32,35]. Hence, the use of probiotics (single or combination of beneficial live bacteria), especially the administration of strains of *Lactobacillus* spp. and *Bifidobacterium* spp., has been linked to the prevention of NEC in preterm neonates [36–38]. Probiotics enhance the abundance of beneficial bacteria, reduce pathobiont numbers, and lower the compositional variation. In addition, they exert an anti-inflammatory effect, decreasing the expression of innate immune receptors and increasing expression of the anti-inflammatory cytokine IL-10 in the small intestine [39].

Another example is neonatal sepsis. In the first days after birth, neonatal sepsis, either as early-onset sepsis developing within 72 h after delivery or as late-onset sepsis (LOS) developing between 72 h and 28 days after delivery, is associated with a severe systemic inflammation and represents a leading cause of neonatal death [40]. The commensal bacterium of the maternal vaginal tract *Streptococcus agalactiae* (also called group B streptococci, GBS) and *Escherichia coli* K1 represent typical pathogens linked to neonatal sepsis. In developing countries, systemic neonatal infections with enteric *Salmonella* are also increasingly recognized [41]. Similar to NEC, a low bacterial diversity of the early postnatal microbiota has been associated with a higher risk of developing neonatal sepsis (Fig. 1) [42]. A low microbiota complexity facilitates the persistence and expansion of sepsis-causing pathobionts in the gut. In a mouse model of LOS driven by systemic dissemination of the opportunistic pathogen *Klebsiella pneumoniae*, Singer *et al.* [43] demonstrated that neonatal microbiota composition influences sepsis outcome. Oral vancomycin treatment promoted high abundance of naturally vancomycin-resistant members of the *Lactobacillus* genus and protected against LOS, whereas gentamicin treatment decreased the relative abundance of *Lactobacillus* and aggravated disease severity.

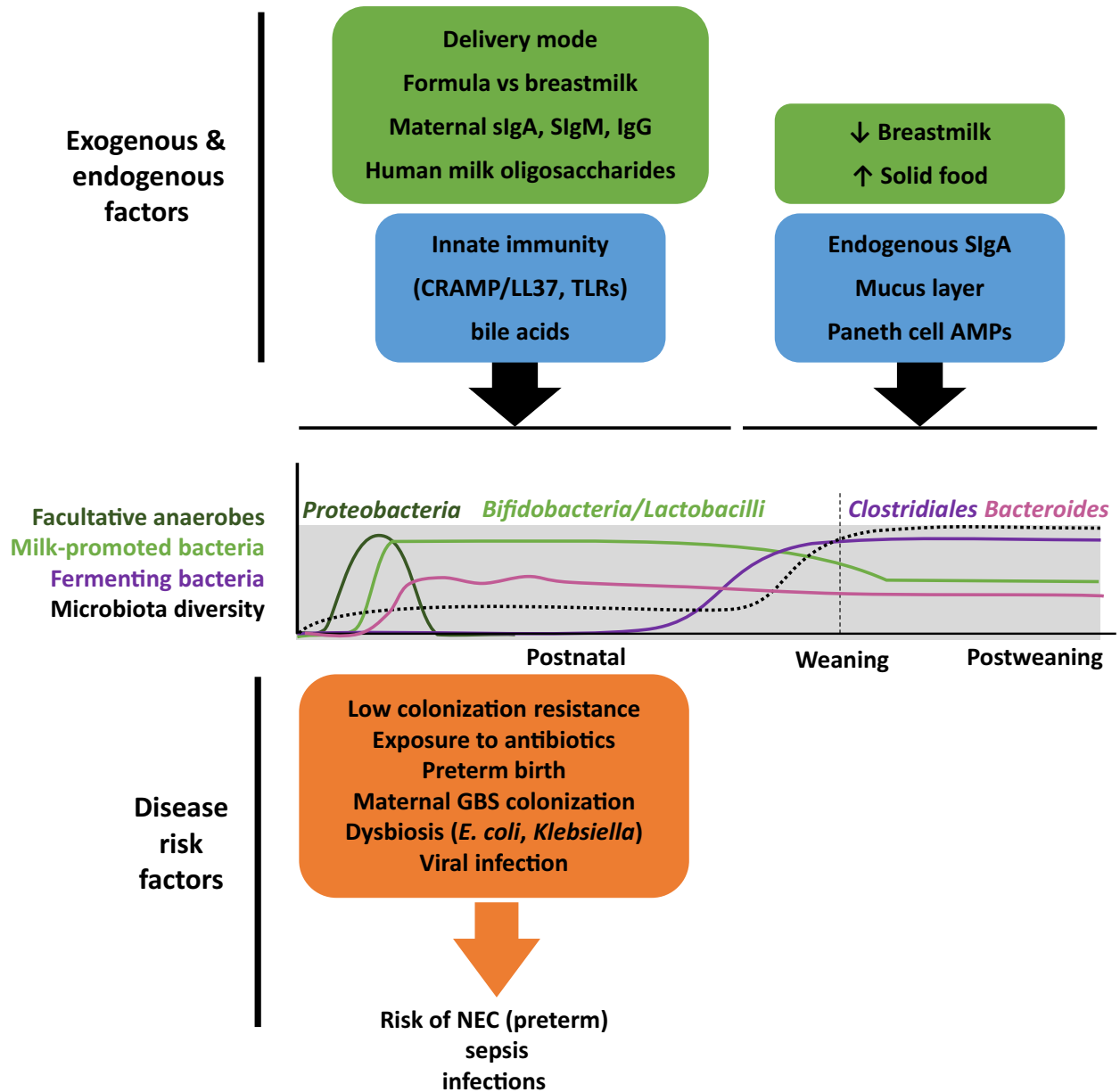
On the other hand, neonates exhibit an enhanced susceptibility to infections, in particular caused by pathogens that target the mucosae of the respiratory and gastrointestinal tract (Fig. 1) [44]. Infections of the respiratory tract, for example by respiratory syncytial virus, or of the gastrointestinal tract by rotavirus, cryptosporidium, enteropathogenic *E. coli* (EPEC), or *Shigella* spp., represent the most prominent causes of infant morbidity and mortality worldwide [44].

The intestinal parasite *Cryptosporidium parvum* persistently infects young mammals, leading to watery

diarrhea [45]. In mice, symptomatic infection is limited to the neonatal period. It increases intestinal permeability, due on one hand to *C. parvum* disrupting enterocyte tight junctions, and on the other hand to the recruitment of monocytes that secrete the pro-inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$ . *Cryptosporidium parvum* also reprograms LP CX<sub>3</sub>CR1<sup>high</sup> macrophages from expressing high levels of the anti-inflammatory cytokine IL-10 to the pro-inflammatory TNF- $\alpha$ . Infected enterocytes upregulate several chemokines, especially CXCL9 and CXCL10, attracting LP CD103<sup>+</sup> cDCs, which control parasitic infection through secretion of IL-12 p40 and IFN- $\gamma$  [46].

Viral infection with rotavirus still represents one of the leading causes of diarrhea-associated mortality in children under five [47]. In the mouse model of rotavirus infection, neonates exhibit a high viral load and diarrhea, whereas adult mice remain asymptomatic [48]. This age-dependent infection susceptibility is related to the limited expression of Toll-like receptor (TLR) 3 by IECs between birth and postnatal day 10 (P10). TLR3 recognizes double-stranded RNA molecules and its increased expression after P10 dampens the susceptibility to rotavirus infection through induction of a protective type III interferon-mediated antiviral host response [49]. Of note, endoscopic biopsies from healthy human infants below the age of 6 years also revealed lower TLR3 expression levels.

Oral exposure of adult mice to EPEC is not associated with signs of infection. However, neonates infected with EPEC before P10 exhibit the typical attachment and effacement (A/E) lesions with EPEC tightly attached to the apical plasma membrane of IECs [50]. The close adherence to IECs stimulates immunoregulatory protein serum amyloid A3 expression and enhances Reg3 $\gamma$  secretion by goblet cells. Neonatal infection resolves spontaneously during the weaning period when the microbiota composition changes. This phenomenon, known as colonization resistance, is conferred by the highly competitive dense and diverse microbiota of the adult host. Also, some individual species such as bacteria belonging to the *Clostridium* clusters IV and XIVa may play a particularly strong protective role against enteropathogens [51]. Although germ-free or antibiotic-treated adult mice are readily colonized by EPEC, they fail to develop A/E lesions, whereas EPEC microcolonies are present in infected germ-free neonates, indicating that the microbiota is not involved in lesion formation at that age. Thus, lack of colonization resistance facilitates neonatal infection but age-dependent host factors independent of the microbiota play a critical role in EPEC pathogenesis [50]. In the adult murine small



**Fig. 1.** Multistep assembly of the postnatal enteric microbiota and influencing factors. Maternal fecal bacteria represent the primary source of the neonate's early bacterial colonization. The composition of the initial microbiota depends on the mode of delivery, diet (breast milk vs. formula), and environmental exposure (upper left green box). Aerobic conditions initially favor facultative anaerobic species, such as *Escherichia coli*, that subsequently lower oxygen levels and facilitate colonization by microaerophilic and anaerobic bacteria (middle panel). Breast milk constituents such as lactose, sIgA, and HMOs direct the composition of the early human microbiota characterized by Lactobacilli and *Bifidobacteria* (upper left green box). Mucosal host factors influence the postnatal microbiota in an age-dependent manner (Blue boxes). Although the murine neonatal epithelium is characterized by the expression of the cathelicidin CRAMP, TLR5-mediated Reg3 $\gamma$  expression, TLR4 tolerance, and low mucin production, the adult intestine secretes defensins and TLR5 expression is restricted to Paneth cells. Oral acquisition of pathogens such as GBS at birth can lead to systemic infection (sepsis), whereas an inappropriate mucosal inflammation to dysbiotic colonization in the preterm host can cause NEC. At weaning, the uptake of solid food with complex carbohydrates and fibers and the reduction of breast milk-derived factors induce a major change in the composition of the microbiota, favoring the expansion of fermenting bacteria such as members of the Clostridiales phylum (upper right green box). Endogenously generated sIgA, Paneth cell-derived defensins, and the mucus layer stabilize the microbiota composition in the adult host (upper right blue box).

intestine, *Salmonella* targets M cells of terminal ileum PPs to facilitate infection and systemic dissemination [52]. In contrast, they readily invade IECs in neonatal mice, proliferate intracellularly, and disseminate to liver and spleen [53]. *Salmonella* translocates a number of virulence factors via its pathogenicity island (SPI) 1-encoded type 3 secretion system to modulate its intraepithelial compartment and form so-called *Salmonella*-containing vacuoles. Interestingly, enterocyte invasion and intraepithelial proliferation resemble *Salmonella* infection as it occurs in humans. It therefore provides a relevant *in vivo* model to study bacterial effector molecules involved in the *Salmonella*-IEC interaction [54].

Although neonates are highly susceptible to many infectious diseases, there are notable exceptions to the rule. Oral infection with the Gram-negative bacterium *Yersinia enterocolitica* triggers a strong immune response that increases the survival rates in 1-week-old mice as compared to adults [55]. Indeed, neonates exhibit an early recruitment of macrophages and neutrophils as well as an enhanced expression of pro-inflammatory genes in the MLNs upon *Yersinia* infection [56,57]. Besides the strong activation of innate immunity, infected neonates efficiently develop *Yersinia*-specific memory B cells along with IgG1 and IgG2a antibodies [56]. This enhanced protection requires IFN- $\gamma$  secretion in two sequential waves: The early protection depends on its secretion by CD8<sup>+</sup> T cells [58], whereas the late protection is ensured by CD4<sup>+</sup> T cells [56]. Another example where children seem to respond to a pathogen in a more appropriate manner and are able to balance pathogen clearance vs. immune-mediated pathology in a more beneficial way than adults is the infection with the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV2). Children are susceptible to the infection [59] and contain comparable amounts of the virus in the nasopharynx [60]. However, the morbidity and mortality are strongly reduced in the pediatric population even though the development of the multisystem inflammatory syndrome has been documented as a rare complication in children previously infected with SARS-CoV2 [59,61]. Interestingly, children display lower levels and a narrower repertoire of neutralizing antibodies after infection suggesting a more efficient pathogen control at the innate level [62]. Natural cross-reactive antibodies have been suggested to contribute to the successful disease control in children [63]. Further studies need to elucidate the age-dependent differences in the immune response over the course of a SARS-CoV2 infection.

In conclusion, neonates generally present an increased susceptibility to infectious diseases relying on an easier access to the subepithelial tissue and a

reduced or altered antimicrobial host defense. However, they are not devoid of defense mechanisms and can mount specific immune responses following infection with bacterial, viral, or parasitic pathogens. In fact, their ability to mount a strong inflammatory response makes them, under certain conditions, susceptible to inappropriate inflammation and functional organ impairment, as exemplified by NEC and neonatal sepsis. The need to maintain immune homeostasis particularly at mucosal surfaces draws attention to the process of postnatal colonization and establishment of the enteric microbiota.

### Multistep assembly of the microbiota

Although experimental evidence strongly supports the sterile womb hypothesis, the presence of a placental microbiome is still debated [64–66]. During delivery, bacteria are transferred from the mother to the child. Vaginally delivered neonates receive a microbiota closely related to the maternal fecal microbiota [67]. In contrast, the primary microbiota of newborns delivered by caesarian section is closer to skin microbiota (e.g., *Staphylococcus*, *Corynebacterium*) [68]. The initial microbiota exhibits a low bacterial diversity, little difference between the different parts of the gastrointestinal tract and its composition is influenced by environmental and geographical cues [68,69].

The empty niche of the neonatal gut favors colonization by fast-growing, aerotolerant bacteria such as members of the Enterobacteriaceae family that includes *E. coli* or the *Enterococcus* genus (Fig. 1) [69–72]. This initial colonization decreases oxygen availability within the gut lumen and makes it suitable for anaerobic genera, for instance *Clostridium* and *Bifidobacterium*. Thus, initially dominated by members of the phyla Proteobacteria and Actinobacteria, the healthy microbiota diversifies as bacterial species from the Firmicutes and Bacteroidetes phyla expand, turning the tide in dominant phyla. Besides the mode of delivery and its circumstances, the neonatal diet (i.e., breast milk vs. formula) shapes the microbiota composition [73]. Breast milk carries microorganisms from the mother, thus directly transferring bacteria to newborns [74]. In addition, breast milk in humans is highly enriched in human milk oligosaccharides (HMO) that put a selection pressure favoring bacterial species able to catabolize these complex sugars, especially *Bifidobacteria* (Fig. 1) [75]. Moreover, immunoglobulins present in breast milk shape the neonatal gut microbiota and influence intestinal homeostasis until adulthood [76]. Breastfeeding also has an influence on the virome that is established in two sequential steps [77].

First, in parallel with bacterial colonization, viral sequences are detected in newborn stool and their abundance further increases with age. These viruses are mainly prophages that integrate in bacterial genomes, for instance, belonging to *Bifidobacterium* and *Lactobacillus* genera. Secondly, at 4 months of age viruses that infect human cells are increasingly found in fecal samples. However, their abundance is lower in breastfed as compared to formula-fed children.

Weaning represents an important step into microbiota diversification and selection. Weaning is characterized by a change in diet, from breast milk to solid food, restricting breast milk-derived immunomodulatory factors and changing nutrient availability ultimately reshaping microbial communities (Fig. 1) [31,78]. The solid diet favors bacterial species able to metabolize nondigestible fibers and produce short-chain fatty acids (SCFAs), whereas the 'milk-oriented microbiota' decreases [72]. In humans, from weaning (at approximately 6 months) until 2–3 years of age, the bacterial diversity increases, and the temporal variability of the microbiota composition gradually decreases until resembling the bacterial composition and richness of adults. In mice, cessation of breastfeeding and weaning occurs at 21 days. This time point marks a major shift in the microbiota composition and initiates the formation of an organ-specific microbiota in small and large intestine. Bacterial richness continues to rise thereafter until approximately 6 weeks of age [69,79–82].

Prolonged maintenance of an enteric microbiota with low microbial diversity may have long-term consequences. The western lifestyle, characterized by high fat low-fiber diet, antibiotic use, few contacts with animals, fewer infections, and birth by caesarian section, has been associated with changes in the enteric microbiota composition leading to a lower bacterial diversity as compared to the one found in inhabitants of rural areas or indigenous tribes [83–86]. The 'hygiene hypothesis' suggests a link between western lifestyle factors and an impaired education of the tolerance-inducing arm of the immune system, leading to an increased prevalence of allergies as well as inflammatory and auto-immune diseases [87,88]. The 'disappearing microbiota' hypothesis further adds insights on the consequences of the loss of bacterial species [89]. For instance, the prevalence of *Helicobacter pylori*, the main bacterial species found in the stomach, has been greatly declining over the last century. *Helicobacter pylori* has both detrimental and beneficial effects. On the one hand, it can cause gastritis and duodenal ulcers and represents a contributing factor to stomach cancer. Notably, the age of the host at the

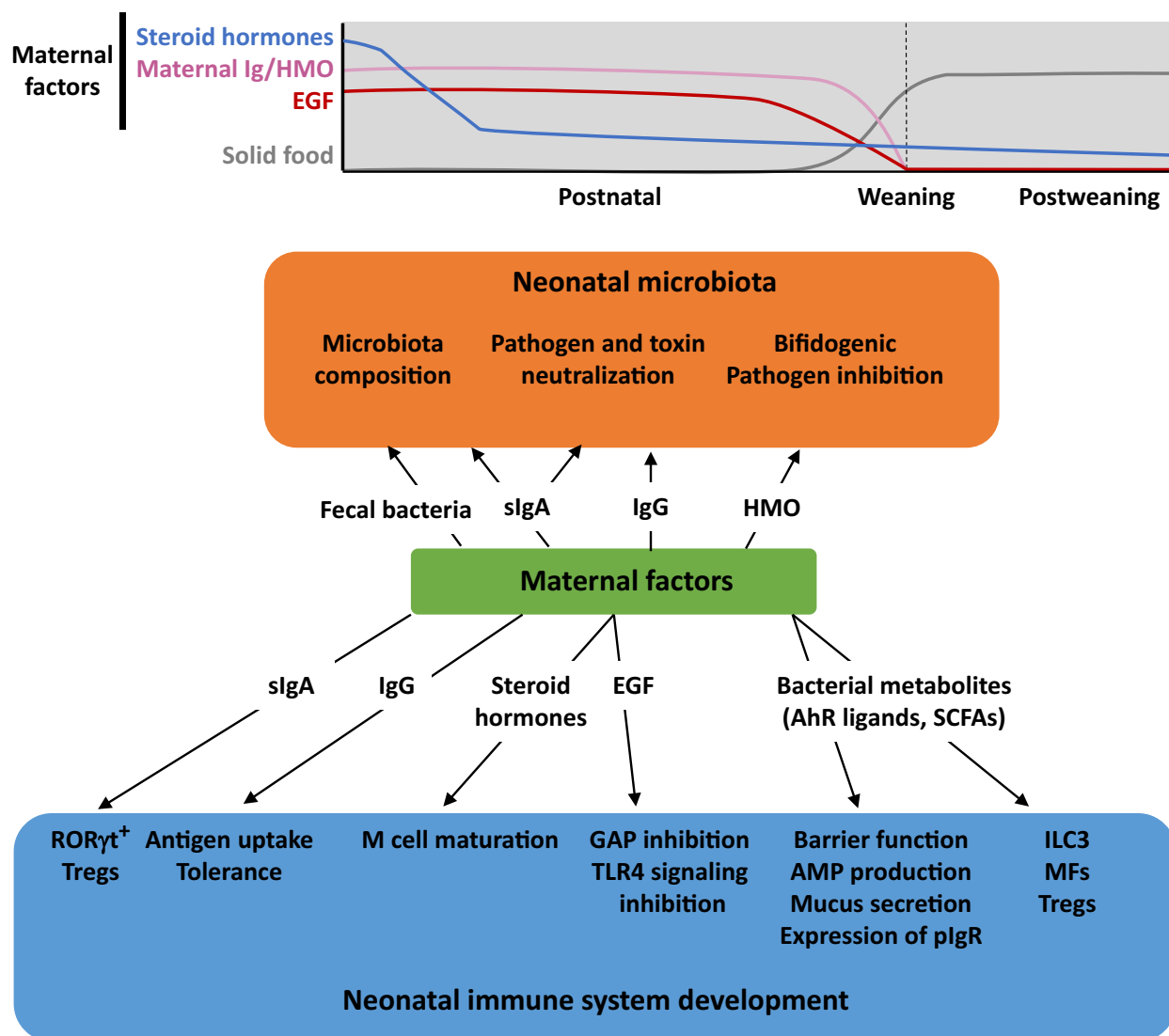
time of initial infection may account for the susceptibility to *H. pylori*-associated disease manifestations [90]. On the other hand, *H. pylori* is linked to increased levels of regulatory T cells (Tregs) promoting immune tolerance [91,92]. Thus, the disappearance of *H. pylori* may affect the host's health both in a positive and negative way. In addition, individual bacterial species also affect each other by competing for ecological niches. Thus, the disappearance of a commensal bacterium may provide a niche for an enteric pathogen.

### **With a little help from your mother: maternal factors involved in neonatal protection and mucosal immune system education**

#### **The maternal microbiota**

In addition to providing the newborn with beneficial commensal bacteria upon delivery, the influence of the maternal microbiota on the fetus significantly impacts both intestinal innate immunity and energy metabolism in the offspring [93,94]. Using a model of transient colonization of germ-free dams with an auxotroph *E. coli* strain, Gomez de Agüero *et al.* [93] showed that transient bacterial colonization of the dam increases intestinal type 3 innate lymphoid cells (ILC3) and macrophages in the gut of the offspring. It also stimulates expression of genes related to mucosal immunity, such as members of the antibacterial Reg3 protein family, lysozyme, pIgR, and mucus secretion-inducing genes. Altogether, these factors reinforce mucosal integrity and reduce translocation of bacteria to the neonatal MLNs. Mechanisms of protection by the maternal microbiota rely on the transfer of microbial-derived metabolites, such as aryl hydrocarbon receptor ligands, from the mother to the offspring (Fig. 2). This transfer seems partly dependent on maternal antibody-mediated retention and transmission of microbial-derived products during pregnancy but also lactation [93]. Among these metabolites, SCFAs stimulate the free fatty acid G protein-coupled receptors 41 and 43 in the embryo and neonate, promoting the development of thymic and peripheral Tregs [95] and conferring protection against metabolic disorder, obesity, and asthma in the adult offspring [94,96].

Hence, the maternal microbiota during pregnancy and lactation produces factors that reach the offspring and shape intestinal homeostasis by promoting enterocyte maturation, epithelial barrier formation, intestinal innate immunity, and metabolism (Fig. 2).



**Fig. 2.** Maternal factors influencing neonatal microbiota composition and immune maturation. Even prior to birth, bacterial metabolites from the maternal intestine reach the fetus and influence the maturation of ILC3 and macrophages (MFs). Maternal factors such as steroid hormones, breast milk-derived immunoglobulins (Ig), HMO, and EGF are provided during breastfeeding (upper panel) and influence the neonatal microbiota composition (orange box) and mucosal immune maturation (blue box) after birth. Breast milk secretory IgA (sIgA) protects neonates via immune exclusion and toxin neutralization and influences the development of ROR $\gamma$ t<sup>+</sup> Tregs. IgG promotes epithelial FcRn-mediated antigen uptake and immune tolerance. Breast milk HMO are bifidogenic promoting a healthy ‘milk-oriented microbiota’. High EGF concentrations in breast milk inhibit acetylcholine-mediated activation of the GAPs in the small intestine and colon. Decrease of EGF allows temporal GAP-mediated antigen sampling in the colon between postnatal day 10 and 21, which facilitates tolerance induction. Decreasing concentrations of the maternal steroid hormones estradiol and progesterone in the neonates over time may favor PP M cell maturation after 1 week of life.

### Maternal secreted immunoglobulins

The passive immunity, transmitted from the mother to the newborn through breastfeeding, greatly influences neonatal health. In the lactating mother, intestinal plasma cells migrate in a CCR10/CCL28-dependent manner to the mammary glands where they secrete

immunoglobulins into breast milk [97]. Those maternal antibodies include mostly secretory IgA (sIgA) followed by IgM and IgG, and reflect specificity to the maternal microbiota. As vaginally delivered newborns have a primary microbiota similar to the maternal fecal microbiota [67], transfer of these specific antibodies gives a clear advantage to control bacteria in the



neonatal gut [28]. Thus, passive immunity provides protection to neonates by shaping the microbiota and keeping it at bay from the sterile tissue, thus preventing invasion of pathobionts and inflammation (Fig. 2). Weanling mice nursed by dams deficient for Ig secretion (pIgR<sup>-/-</sup>), compared to those nursed by wild-type dams, have a different microbiota composition and deregulated colonic epithelial cell gene expression leading to an impaired barrier function, with increased translocation of enteric bacteria, such as *Ochrobactrum anthropi*, but also worsened inflammation in dextran sodium sulfate-induced colitis [76]. However, it is not clear whether IgA has a direct effect on colonic epithelial cells or an indirect effect through alteration of the microbiota. Passive immunity also prevents early activation of the adaptive immune system. An early germinal center (GC) activation and IgA production in PPs at P13 was observed in pups nursed by females deficient in adaptive immunity [98]. Moreover, pups nursed by dams deficient in antibody production have increased levels of activated T cells and IgA<sup>+</sup> plasma cells [99]. This premature development is likely induced by an increased bacterial translocation across the neonatal intestinal epithelium due to a deficit in bacterial coating by maternal antibodies, leading to an early activation of the mucosal immune system. Upon challenge with *Enterobacter cloacae*, a fast-growing facultative anaerobe of the adult microbiota, these prematurely produced neonatal IgA appear as effective as maternal antibodies in preventing bacterial translocation to the MLNs [99]. The induction of endogenous IgA in neonates in the absence of breast milk Ig is likely a downstream effect of a premature activation of follicular helper T cell (Tfh). Indeed, Koch *et al.* [100] demonstrated that transferred maternal IgG2b and IgG3 limit activation of Tfh cells as well as GC expansion in pup PPs and MLNs. Early activation of the adaptive immune system also occurs during infectious challenge. Thus, neonates develop reovirus-specific antibody-secreting cells in PPs and MLNs upon challenge at P10 [101]. However, this early virus-specific IgA response is abrogated when pups receive passive immunity from an immunized foster dam, probably due to an efficient neutralization of the virus by maternal antibodies. Passive transfer of maternal pathogen-specific antibodies does indeed provide protection in neonates against rotavirus [102], poliovirus [103], or HIV [104] through their neutralizing activity. In humans, high titers of maternal-specific IgA also correlate with a better protection of neonates against diarrheagenic enteropathogens such as *E. coli*, *Vibrio cholerae*, *Shigella*, and *Campylobacter* [105]. However, immunoglobulins from unimmunized mothers

can also protect against enterotoxigenic *E. coli* infection through IgG coating, suggesting a contribution of polyreactive and nonspecific immunoglobulins [106]. Overall, these studies show that the transfer of maternal secreted immunoglobulins, either polyreactive (IgG) or antigen-specific (IgG and IgA), plays an important role in neonatal protection by exerting their immune exclusion activity, allowing symbiosis with the microbiota, and preventing infection (Fig. 2). Nevertheless, it is important to keep in mind that immunized mothers confer a transient immunity to neonates that vanishes after the breastfeeding period, thus supporting the need for the establishment of endogenous active immunity by the young host (e.g., vaccination) [107].

Lastly, passive immunity is also involved in shaping the neonatal adaptive immunity and thus might aid to set a trajectory for health and disease in later life. This is facilitated through the bidirectional transport of maternal IgG between intestinal lumen and the mucosal tissue via epithelial expression of the neonatal Fc receptor (FcRn) [108]. In humans, IgG transfer from mother to offspring is largely limited to the fetal period via placental transfer. In rodents, however, this transfer continues during the neonatal period in which IEC expresses extremely high levels of FcRn, enabling the translocation of milk IgG from the gut lumen to the mucosal tissue [108]. Yoshida *et al.* [109] showed that weanling mice are less susceptible to *Citrobacter rodentium* infection when FcRn is expressed in IEC. FcRn allows the delivery of IgG-bound bacterial antigen to LP CD11c<sup>+</sup> cells, leading to specific T-cell activation in the MLNs. Thus, IgG transport from the intestinal lumen to the mucosal tissue supports the transfer of antigens and their presentation, leading to an appropriate immune response (Fig. 2). In addition to initiating adaptive immune responses against pathogens, transfer of breast milk IgG immune complexes can induce tolerance to food allergens in the offspring. A thorough study by Ohsaki *et al.* demonstrated that breast milk ovalbumin immune complexes are delivered to LP CD11c<sup>+</sup> cells through the transport of IgG by FcRn [110]. These phagocytes then present the antigen and activate specific Tregs protecting the offspring from allergy-mediated immunopathology. Interestingly, this also involves expression of FcRn in CD11c<sup>+</sup> cells.

Passive immunity can also shape the B-cell receptor repertoire, likely by interfering with presented epitopes and limiting long Tfh-GC B-cell interactions, thus reducing plasma cell generation [111]. Recently, Ramanan *et al.* [112] demonstrated an unprecedented mechanism of immune profile transmission across multiple generations regulated by levels of breast milk IgA

rather than through genetic background or microbiota composition. Taking advantage of immune phenotypic traits specific to C57BL/6 or BALB/c mouse strains, that is, high levels of colonic ROR $\gamma$ t<sup>+</sup> Tregs and high levels of IgA, respectively, they studied the transmission of these traits across generations by cross-fostering pups with dams of the opposite genetic background. They showed that variations in breast milk IgA production condition colonic ROR $\gamma$ t<sup>+</sup> Treg proportions later in life. High levels of IgA-coated bacteria affect antigen detection and limit expansion of ROR $\gamma$ t<sup>+</sup> Tregs. On the contrary, low levels of IgA lead to higher frequencies of ROR $\gamma$ t<sup>+</sup> Tregs, which in turn suppress B-cell activation and IgA induction. It is noteworthy that this mechanism is active during the first week of life, long before endogenous plasma cells and ROR $\gamma$ t<sup>+</sup> Tregs appear in the offspring. This timeframe suggests that maternal IgA influences the intestinal microenvironment, either by acting on the microbiota, the nonhematopoietic, or hematopoietic compartment, leading to an early immune imprinting.

In summary, in addition to protection against pathogens, passive immunity impacts neonatal immune cell development and the establishment of immune homeostasis with consequences far beyond the postnatal phase (Fig. 2).

### Maternal leukocytes

Besides immunoglobulins, murine and human breast milk contains immune cells, of which approximately 80% are myeloid cells and 20% are lymphocytes, mainly T cells [113]. The findings on the fate and function of these cells are controversial. Maternally derived lymphocytes have been reported in mouse offspring tissue although other studies were unable to confirm this finding [113,114]. Nevertheless, the possibility exists that maternal immune cells are able to survive the adverse environment of the upper gastrointestinal tract and penetrate the epithelium. This would lead to microchimerism and provide neonatal tissues with mature and active leukocytes [113,115]. For instance, using GFP expressing dams nursing WT neonates, it was shown that from P6, milk CD8<sup>+</sup> cytotoxic T cells (CTL) expressing the gut homing proteins  $\alpha$ 4 $\beta$ 7 and CCR9 engraft in neonatal PPs, and to a lesser extent in the MLNs and the villus LP [116]. Their number was reported to decline after weaning. These maternal CTL may be active and functional, as they express granzyme B, TNF- $\alpha$ , IFN- $\gamma$ , and IL-18.

Although it is not possible to track maternal cell transfer in the tissues of human neonates, immune cells have been characterized in human breast milk. For

instance, a recent study identified MAIT cells,  $\gamma\delta$  T cells, ILC1, and ILC3 in breast milk [117]. Interestingly, breast milk also contains pathogen-specific lymphocytes. Thus, breast milk of HIV-infected mothers can contain HIV-specific CD8<sup>+</sup> T cells, which exhibit a memory T-cell phenotype and express the gut homing receptor CCR9 [118]. One could hypothesize that these activated CTL take part in preventing HIV transmission in newborns and that other types of pathogen-specific lymphocytes could be transmitted from infected mothers. As described above, plasma cells migrate during lactation to the mammary glands where they mainly secrete IgA. Human breast milk also contains  $\alpha$ 4 $\beta$ 7<sup>+</sup> plasma cells and plasmablasts [119]. However, only one study performed in mice reported maternal IgG-secreting plasma cells in the spleen and bone marrow of B-cell-deficient ( $\mu^{-/-}$ ) pups [120].

In summary, the breast milk-mediated transfer of intact maternal immune cells may help to maintain gut barrier function, promote the establishment of symbiosis, and protect against pathogens. The exact contribution of maternal cells, however, remains to be established.

### Maternal soluble factors

Highly abundant in human breast milk, HMOs influence the neonatal host-microbial interaction in several ways [121,122]. Indigestible for the infant host, they serve as substrate for bacteria such as *Bifidobacterium* and *Bacteroides* [123]. HMOs prevent bacterial adhesion and act as a nonimmune exclusion factor (Fig. 2). HMOs can also be recognized by different families of immune cell receptors, such as C-type lectins and sialic-acid-binding immunoglobulin-like lectins (Siglecs). Thus, HMOs can bind the C-type lectin receptor DC-SIGN on human monocyte-derived dendritic cells [124,125]. It has been proposed that such binding could promote tolerogenic properties and/or block pathogen interactions with these dendritic cells [124,126]. However, the exact role of these interactions is still unclear.

A large number of milk-derived peptides and proteins harbor antimicrobial and immunomodulatory activity. For instance, the iron-binding glycoprotein lactoferrin efficiently decreases the growth of bacteria associated with LOS [127]. The murine cathelicidin-related antimicrobial peptide (CRAMP) or the human homologue LL-37 inhibits growth of *Staphylococcus aureus*, *E. coli*, and *Streptococcus pyogenes* (group A streptococci) [128]. Similar to  $\alpha$ -defensins,  $\beta$ -defensins,  $\beta$ -casein, and lysozyme, these antimicrobial molecules have therapeutic potential to prevent

neonatal infection [129,130]. In addition, human breast milk contains soluble CD14 (sCD14) that binds lipopolysaccharide (LPS) from Gram-negative bacteria [131]. Milk-derived sCD14 promotes LPS recognition by TLR4 leading to the secretion of pro-inflammatory cytokines [132]. Finally, human breast milk (especially the breast milk produced during early lactation called colostrum) contains additional unidentified proteins that enhance innate immune recognition by neonatal cells [133].

Along with antimicrobial molecules and sCD14, many cytokines, such as IL-10, TGF- $\beta$ 1, TGF- $\beta$ 2, IL-6, TNF, and IFN- $\gamma$ , are transferred through breastfeeding. They promote oral tolerance as well as IEC proliferation and survival, thus supporting mucosal barrier function [134].

Finally, breast milk-derived epidermal growth factor (EGF) plays a critical role in maintaining epithelial barrier integrity and orchestrating the establishment of neonatal mucosal immunity (Figs 2 and 3) [135]. Interestingly, milk-borne EGF is at its highest concentration during early lactation and decreases over the lactation period (Fig. 2). During the early postnatal phase, EGF inhibits goblet cell-associated antigen passage (GAP) formation, prevents bacterial translocation, inhibits TLR4 signaling, and is protective in experimental models of NEC and LOS [31,136,137]. The decline of EGF facilitates GAP-mediated translocation of antigens and immunostimulatory molecules prior to weaning, which is critical for the initiation of intestinal immune system maturation [31,78,135,138].

In summary, many milk-derived compounds promote pathogen detection and killing, intestinal immune system maturation and oral tolerance development, maintenance of gut barrier function, and modulation of the microbiota composition in neonates (Fig. 2).

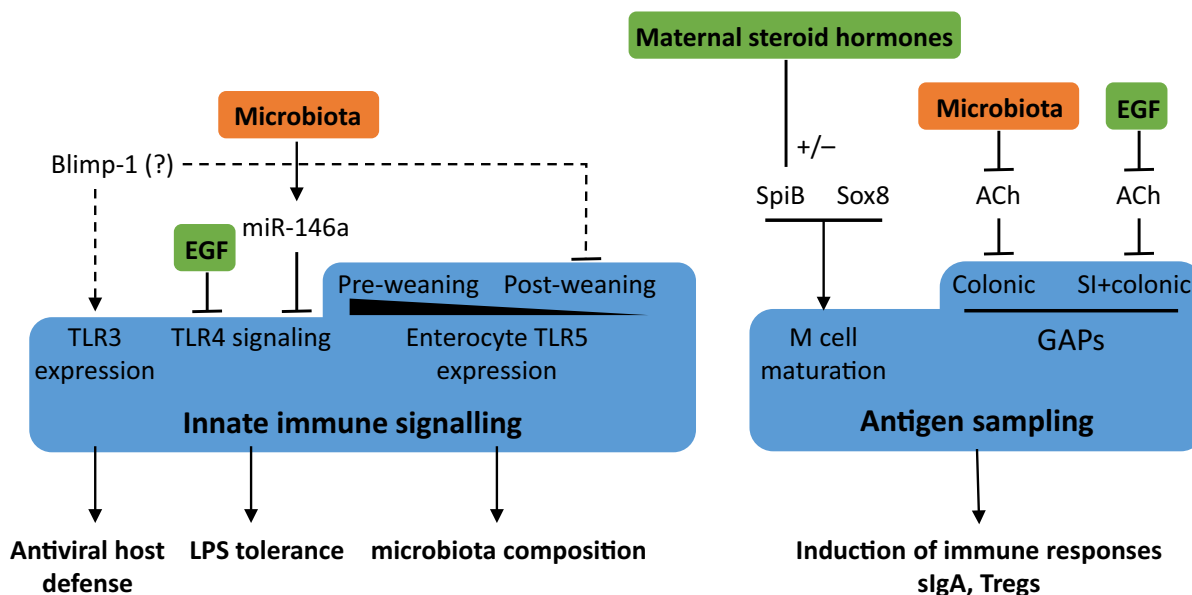
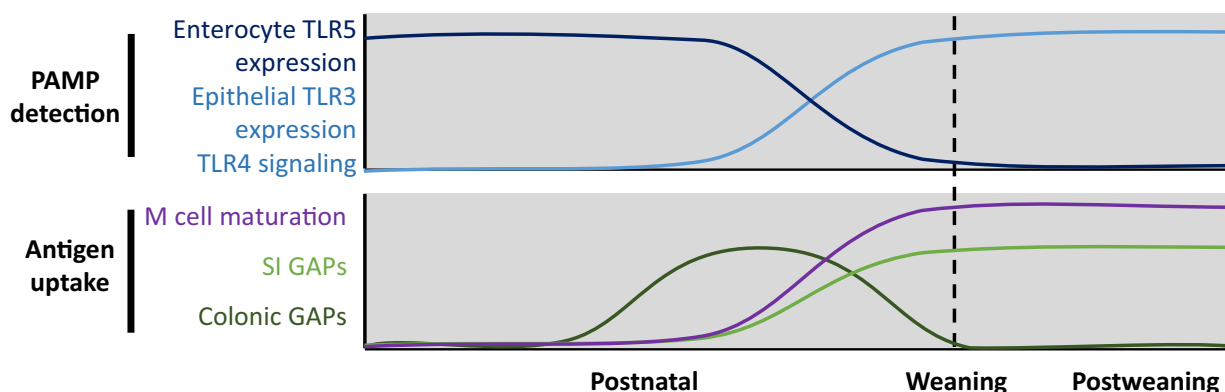
## Early-life defense mechanisms and immune responses

### Postnatal intestinal tissue development

Mice have a short gestation period of only 21 days and exhibit substantial postnatal tissue maturation. Although small intestinal villi develop prenatally at E15 [139], intestinal crypts develop only from P12 to P15 [140]. Neonatal IECs exhibit a low proliferation rate, and the continuous renewal of the epithelium is not initiated until 10–12 days after birth. Crypt-villus structures elongate from birth in the duodenum and from P4 in the ileum until P16 [141]. Mature Paneth cells appear upon crypt formation around 2 weeks after birth [142]. Colonization by the microbiota

significantly influences IEC maturation as illustrated in germ-free mice; within 2 days of conventionalization, colonic enterocyte proliferation intensifies and crypts elongate [143]. During the suckling period, enterocytes are equipped to metabolize milk nutrients [144]. Their neonatal phenotype is mainly regulated by expression of Blimp-1, a transcriptional repressor that prevents adult-like enterocyte differentiation until weaning. Consistently, transgenic mice with epithelial cell deletion of Blimp-1 (Blimp-1<sup>ΔIEC</sup>) are born with continuous epithelial cell turnover, the presence of small intestinal crypts and increased numbers of Paneth cells. IECs from Blimp-1<sup>ΔIEC</sup> neonates also exhibit a transcriptional profile similar to the adult epithelium, that is, low expression of enzymes involved in milk constituent degradation and elevated expression of enzymes that allow degradation of complex carbohydrates such as starch [144,145]. This adult-like epithelium makes the neonate ill-adapted to neonatal life and results in reduced weight gain and increased mortality [144]. The secretion of mucins by goblet cells is also markedly reduced in neonates, leading to a less prominent mucus layer. Consequently, colonizing bacteria get in closer contact with the mucosal tissue surface. This spatial proximity may contribute to the enhanced susceptibility of neonate mice to *E. coli* K1 infection [146]. Zhang *et al.* [53] showed that mucin gene expression and mucus layer thickness in mice increase at P15. Similarly, the small intestinal mucus layer of neonate rats increases until it covers the villi during the first 10 days postpartum. Thus, age-dependent differences between the adult and neonatal epithelium such as reduced IEC proliferation and exfoliation, lack of mature Paneth cells and Paneth cell-derived AMPs and a reduced mucus layer may contribute to the enhanced susceptibility of the neonate host to infection.

Mouse PPs and ILFs, which are gut immune inductive sites containing B-cell follicle(s), develop at different time points pre- and postnatally, respectively. Prenatal formation of PP anlagen is initiated at embryonic day 12.5 (E12.5) as VCAM-1<sup>+</sup> stromal cells begin to recruit cKit<sup>+</sup>LT $\beta$ <sup>+</sup>CD11c<sup>+</sup> cells to the intestinal tissue via the receptor tyrosine (RET) kinase -RET ligand axis [147]. Next, recruitment of lymphoid tissue inducer (LTi) cells that are essential for the formation of secondary lymphoid organs occurs through several signals. In fetal gut tissue, neuronal stimulation triggers expression of retinoic acid, which induces the expression of the chemokine ligand CXCL13 on stromal cells, attracting ROR $\gamma$ <sup>+</sup> LTi cells forming clusters at E15.5 [148,149]. Additionally, IL-7 expression in the tissue leads to the upregulation of lymphotoxin (LT)



**Fig. 3.** Innate immune recognition and antigen sampling in neonates. Pathogen-associated molecular pattern (PAMP) detection and antigen uptake are under strict temporal regulation (upper panels). Epithelial TLR3 expression is increased at weaning, likely through downregulation of the transcriptional regulator Blimp-1, and contributes to the antiviral host response (lower left blue box). Microbiota-derived endotoxin induces epithelial expression of the microRNA miR-146a that reprograms TLR4 signaling and promotes LPS tolerance (lower left blue box). TLR4 signaling is also regulated by maternal EGF. TLR5 is expressed by the postnatal intestinal epithelium and via Reg3 $\gamma$  influences the colonization efficacy of flagellated bacteria (lower left blue box). After weaning, TLR5 expression is restricted to crypt Paneth cells. Concentrations of maternal steroid hormones in neonates after 1 week of life may favor Spi-B expression and the postnatal maturation of M cells, responsible for luminal sampling of particulate antigen in PPs (lower right blue box). Antigen uptake and tolerance induction by GAP are inhibited by high concentrations of EGF during early lactation. The decline of breast milk EGF transiently activates GAP in the colon before it is inhibited again at weaning by the increasing bacterial load (lower right blue box). In contrast, GAP and antigen sampling in the small intestine (SI) is only facilitated at weaning and remains functional thereafter.

$\alpha 1\beta 2$  in ROR $\gamma$ t<sup>+</sup> LTi cells, necessary for PP formation [150,151]. At E17.5, the interaction between LT $\alpha 1\beta 2$ <sup>+</sup> LTi cells and LT $\beta$ R<sup>+</sup> mesenchymal cells leads to the development of a microenvironment enriched in VCAM-1<sup>+</sup>ICAM-1<sup>+</sup> mesenchymal cells expressing the chemokines CXCL13, CCL19, and CCL21, which promote the recruitment of lymphocytes to the PP anlagen [152–155]. Four days after birth, lymphocytes seed

the anlagen and a mature adult-like lymphoid structure of the PPs is detected at P7, with a compartmentalized distribution of T cells in the interfollicular region (IFR), B cells and follicular dendritic cells in follicles, and high endothelial venules in the parafollicular areas [156]. In contrast to lymph nodes, the main route of antigen transport to PPs is carried out by M cells. At birth, the FAE typically lacks mature M cell

markers that gradually appear during postnatal development (Fig. 3) [53]. Recently, Hays *et al.* [157] showed that maternal steroid hormones that reach the newborn could be involved in the regulation of neonatal M cell maturation. Indeed, maternal estradiol and progesterone concentrations decrease after birth to reach levels at P7 that favor M cell maturation (Figs 2 and 3). Spi-B represents the master regulator of M cell maturation [158]. *Spib*-deficient mice lack expression of typical mature M cell markers, such as GP2, a surface receptor for type-I-piliated bacteria, and CCL9, a chemokine involved in the recruitment of CCR1-expressing immune cells, whereas early markers, such as *Marks11* and *Anxa5*, are still expressed. In neonates, expression of *Spib* is only detected from P8, followed by GP2 and CCL9 expression at P15 [53]. Finally, glycoconjugates with  $\alpha$ -linked fucose residues recognized by the lectin UEA1 start to be expressed at P21 [53]. Another regulator of M cell differentiation, *Sox8*, could also play a role in the delayed maturation of M cells in neonates [159]. Transcytosis of luminal particulate material is ineffective in immature M cells, which limits antigen transport to the subepithelial dome until the PP immune system is ready to handle pathogens. Consistently, invasiveness of *S. agalactiae* (GBS), responsible of many LOS cases, increases in adults after treatment with estradiol and progesterone concentrations that mimic neonate hormonal levels reached at P7 [157]. Such hormonal concentrations were shown to promote M cell maturation and transcytosis of the bacteria.

Cryptopatches, the small clusters of lymphoid cells giving rise to ILFs, are detected at the base of intestinal villi between crypts 2 weeks after birth [160]. Their postnatal development requires  $ROR\gamma^+IL-7R\alpha^+$  LTi cells and IL-7 signaling. Diet-derived aryl hydrocarbon receptor ligands are needed for their maintenance, and diet-derived vitamin A and its metabolite, retinoic acid, have also been shown to influence  $ROR\gamma^+$  ILC3 cell numbers and the further development of cryptopatches [161,162]. Thus, murine gut-inductive sites undergo important structural changes postnatally and their architecture is completed only within 2 weeks postpartum.

Unlike rodents, the long gestational period of humans allows the gut mucosa to develop during fetal life. The human gut mucosal tissue development is completed by 19 weeks of gestation with its final architecture already in place at birth [140]. Thus, the human newborn intestine contains PPs [163], but their size and number gradually increase until puberty [164]. ILFs, with similar structure and immune cell composition as in mice, are present in human small intestine

and colon [165]. However, whether human ILF development is similar to mice remains to be determined. Human cryptopatches have been reported in only one study using CCR6 to identify putative clusters of LTi cells [166]. Nevertheless, humanized mice reconstituted with human hematopoietic stem cells that express IL-2R $\gamma$  are able to develop cryptopatches, whereas the same mice deficient for IL-2R $\gamma$  are not, suggesting that cryptopatches can also develop in humans [167].

### Antimicrobial peptide production and innate immune stimulation in neonates

While still developing, the neonatal mucosal immune system faces two challenges: firstly, to avoid any inappropriate inflammatory response that could compromise the gut barrier integrity and, secondly, to protect the organism from infection by enteropathogens. The lack of mature Paneth cells and AMP secretion (e.g.,  $\alpha$ -defensins and lysozyme) in the small intestine before P14 makes murine neonates more vulnerable to infection [168]. Instead, neonatal IECs express the CRAMP during the first 2 weeks of life. CRAMP expression does not depend on the presence of the microbiota, which suggests a strictly developmental regulation. CRAMP exhibits antimicrobial activity against pathogenic bacteria (e.g., *Salmonella* Typhimurium, *E. coli* K1) but also against some commensal bacteria (e.g., *Lactobacillus reuteri* or *Lactobacillus murinus*). *Cramp*<sup>-/-</sup> neonates are more susceptible to *Listeria monocytogenes* infection, thus illustrating the role of CRAMP to prevent infection *in vivo* [168].

Two weeks after birth,  $ROR\gamma^+$  ILCs populate the small intestinal tissue in higher numbers and with a higher expression of IL-22 than in adults [169].  $ROR\gamma^+$  ILC3-derived IL-22 promotes Reg3 $\gamma$  expression and epithelial barrier function. Although the function of ILC3s in neonates remains to be established, they most likely contribute to the mucosal host defense during the late neonatal period.

Microbial detection through epithelial pattern recognition receptors must be tightly regulated to avoid inappropriate innate immune stimulation and inflammation-driven disruption of mucosal barrier integrity (Fig. 3). This particularly accounts for the neonate intestine characterized by reduced mucus production, AMP secretion, and epithelial cell regeneration. During the first 3 weeks of murine life, epithelial TLR4 signaling is reprogrammed by enhanced production of the small hairpin mRNA miR-146a, which inhibits transcription of the TLR signaling molecule interleukin 1 receptor-associated kinase 1 (IRAK1) [170]. This mechanism facilitates LPS tolerance by

preventing the secretion of pro-inflammatory mediators and mucosal damage until weaning [171]. Interestingly, miR-146a induction and neonatal epithelial TLR4 tolerization depend on microbial signals (Fig. 3), since TLR4 deficient mice fail to develop epithelial innate immune tolerance [170]. This may explain why milk proteins have been shown to enhance TLR4 recognition ensuring miR-146a upregulation and TLR4 tolerization [133]. On the other hand, caesarian section-born mice maintain epithelial IRAK1 protein expression promoting epithelial stimulation and cell death [170]. Interestingly, NEC in human pre-term infants has been linked to an inappropriate TLR4 stimulation [37,39,172].

TLR5, the receptor for bacterial flagellin, is highly expressed by neonatal IECs prior to weaning (Fig. 3) [173]. TLR5 expression during the neonatal period limits colonization by flagellated bacteria and has a long-term impact on the microbiota composition [173]. After weaning, its expression is restricted to Paneth cells [174]. Conversely, intestinal epithelial TLR3 expression is found only after weaning and confers protection against viral infection (Fig. 3) [48]. This age-dependent expression does not depend on microbial signals but may be linked to IEC maturation through downregulation of the transcriptional regulator Blimp-1 [144].

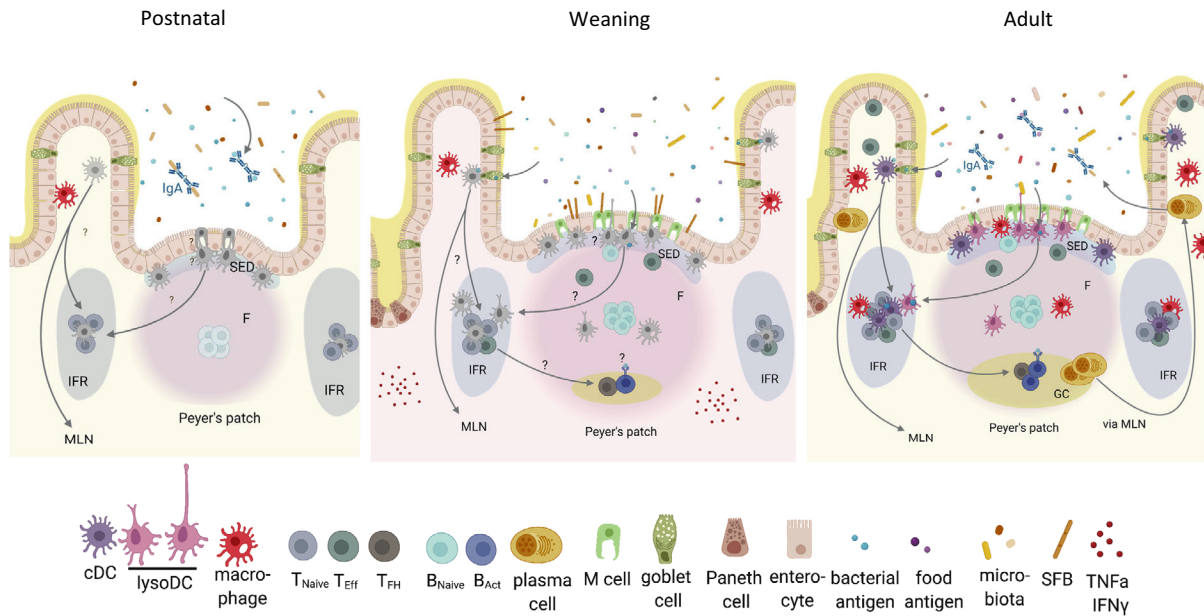
In summary, the intestinal epithelium gets in close contact with microorganisms during the neonatal period. A shift in the AMP spectrum and changes in the expression and function of innate immune receptors may help to establish postnatal host-microbial homeostasis.

### Neonatal immune responses

At steady state, the adult small intestinal tissue is populated with numerous conventional and nonconventional lymphocytes and most lymphocyte populations reflect a continuous cross talk with the microbiota as germ-free mice have greatly reduced numbers of these cell populations. The murine gut is devoid of adaptive immune cells at birth and is seeded during the first week of life. Interestingly, although the neonate is rapidly colonized after birth by commensal bacteria, T and B cells during the postnatal period display a naïve phenotype and are only found in the gut-associated lymphoid tissues but not the LP (Fig. 4) [114]. Activated lymphocytes are only detected after weaning, both in gut-associated lymphoid tissues and at effector sites but they reach adult levels long after [114,175]. Thus, there is a relatively long period, during which the adaptive immune system of the gut under

homeostatic conditions remains oblivious to microbial, food, and environmental stimulation (Fig. 4).

Induction of tolerance to orally ingested molecules requires antigen delivery to cDCs, which is tightly regulated during the neonatal period. GAP-mediated transport of luminal antigens represents a substantial sampling mechanism taking place at the small intestinal and colonic epithelium [176,177]. In early life, GAPs are inhibited by breast milk-derived EGF, limiting antigen exposure and preventing bacterial translocation in colon and small intestine (Fig. 3) [31,137]. In the preweaning period, the decline of EGF in breast milk limits EGFR activation and allows acetylcholine signaling that leads to GAP formation in the colon, thereby promoting transport of luminal antigens exclusively to the colonic LP. Antigens delivered by GAPs are preferentially taken up by CX<sub>3</sub>CR1<sup>+</sup>CD103<sup>+</sup> cDCs [177], which are known to promote Treg expansion and tolerance [178,179]. As such, preweaning induction of colonic GAP triggers the activation of T cells and preferential development of Tregs specific for bacterial antigens in the colon-draining MLNs followed by their migration in the colon LP where they establish tolerance to the microbiota [31]. Around weaning, the detection of the increased bacterial load by colonic goblet cells via a Myd88-dependant pathway inhibits colonic GAPs whereas small intestinal GAPs start to form, are maintained throughout life, and by contrast play a key role in the tolerance to food antigens [180,181] (Fig. 3). Therefore, GAP formation is tightly regulated both temporally and spatially in early life to support induction and maintenance of tolerance in adults and to avoid immune responses to gut microbiota in the colon and to food antigens in the small intestine. In adult mice, the induction of Tregs and tolerance rely on the ability of CD103<sup>+</sup> cDCs to metabolize vitamin A into retinoic acid through their RALDH activity [178,179]. In contrast, in 1-week-old mice, MLN CD103<sup>+</sup> cDCs exhibit weak RALDH activity as compared to 3-week-old mice and are consequently less efficient in activating antigen-specific T cells, rendering neonates more prone to developing allergy [182]. Nevertheless, upon diet supplementation with vitamin A, neonatal MLN CD103<sup>+</sup> cDCs mature and secrete retinoic acid. In this model, neonatal tolerance is induced through expansion of IFN- $\gamma$  producing T helper cells in contrast to the induction of FoxP3<sup>+</sup> Tregs in the adult [182]. Recently, it was demonstrated that during the first 2 weeks of life, neonates have myeloid-derived suppressor cells that are induced by breast milk-derived lactoferrin [183]. These cells can inhibit T-cell expansion in co-culture via secretion of prostaglandin PGE<sub>2</sub>, nitric oxide, S100A8, and



**Fig. 4.** Age-dependent immune maturation of the PPs and villus LP. During postnatal development (left panel), commensal bacteria and bacterial antigen as well as breast milk IgA are found in the intestinal lumen. GAPs and M cells that allow antigen uptake in the adult have not developed/matured, yet. The mononuclear phagocyte (MNP) compartment is ill-defined. The follicle (F) and IFR are present in PPs but B and T lymphocytes within are not primed and do not migrate to the LP under homeostatic conditions. At weaning (mid-panel), solid food and food antigens are found in the luminal content whereas maternal IgA disappears and is not yet replaced by self-production. Microbiota displays significant dynamics and undergoes diversification due to the complex and diverse nutrients of the solid food. The status of the MNP compartment is still ill-defined. GAPs and M cells are present and allow for the uptake of luminal antigens that are then presented to T cells and a homeostatic/tolerogenic bias is imprinted. The pro-inflammatory cytokines of the weaning reaction  $IFN\gamma$  and  $TNF\alpha$  are transiently present and shape the immune responses, ultimately leading to the adult-state homeostasis. T follicular helper cells primed at weaning aid the activation of B cells that recognize luminal antigen. In the adult (right panel), the organization of the MNP compartment is well characterized with regional specificities in PP. Thus, cDCs are enriched in the IFR where naïve T cells reside, whereas LysoDC and macrophages are enriched in the subepithelial dome (SED) where constant uptake of luminal antigen occurs. Presentation of these antigens leads to the presence of antigen-experienced T cells in the LP and PPs. In GC, B cells undergo homeostatic activation and differentiate into IgA-producing plasma cells that migrate through the MLNs to the LP where they secrete IgA into the intestinal lumen and control microbial composition, localization, and density. Created with BioRender.com.

S100A9. *In vivo*, myeloid-derived suppressor cells improve the outcome of NEC in preterm neonates by promoting Tregs over Th17 cells, leading to decreased intestinal permeability and inflammation, and increased survival.

Neonatal T cells have long been considered immature but this concept is an oversimplification and poorly reflects the plethora of data on neonatal T-cell responses collected to this point (thoroughly reviewed by [184]). Regarding their phenotype and location, murine T cells found in the neonatal intestine are more similar to those found in sterile secondary lymphoid organs than in their adult intestinal counterparts. In contrast to the situation in mice, activated T cells have been found in human fetal intestine devoid of living microorganisms, but their origin and function require further investigations [185,186]. Neonatal T cells are

able to respond to stimulation, but they do so in a different fashion as compared to adult T cells. This reflects their adaptation to the neonatal environment and their origin, since neonatal T cells by definition represent recent thymic emigrants [187]. Historically, one of the neonatal features that has been first described is their Th2 bias during secondary responses and thus a limited Th1 response in the first week of life [188]. This bias is mediated by neonatal type 1 cDCs (cDC1) that are phenotypically and functionally distinct from their adult counterparts [189]. Neonatal cDC1 produce IL-10 to limit cytotoxic T-cell activation but can prime *bona fide* protective Th1 responses after stimulation via TLR3 [190]. Despite their Th2 bias, a type 1 response can still be elicited in newborns under specific settings. For example, DNA vaccines promote Th1 cell expansion in neonatal mice, whereas

vaccination with a live virus leads to Th2 responses [191].

### **Microbiota imprinting: the neonatal windows of opportunity**

In recent years, several studies have highlighted that the long-term development of a mature and homeostatic intestinal immune system entails a crosstalk with the microbiota in specific time windows of opportunity starting even prior to birth [27,29,30].

During pregnancy, maternal microbiota-derived metabolites shape the neonate's innate immune system and this influence extends to the time of breastfeeding [93]. Dietary fibers strongly influence the nature of these maternal microbiota-derived metabolites, notably SCFAs. Maternal SCFAs promote the development of Tregs [95] and confer protection against metabolic dysregulation and obesity in the adult offspring [94]. They also protect the offspring against house dust mite-induced asthma, regardless of the age at the time of sensitization [96].

At birth, the exposure to the microbiota is crucial to induce the secretion of TNF- $\alpha$  by spleen myeloid cells that in turn is necessary to induce the differentiation of neonatal IL-10-producing pre-cDC1 into IL-12-p40-producing cDC1 [192]. Importantly, this maturation of pre-cDC1 allows neonates to generate a protective cytotoxic T-cell response against *L. monocytogenes*.

The crucial role of host-microbiota interactions during early life was demonstrated by colonization of germ-free mice at different time points after birth. Although many phenotypical traits were reversed by conventionalization of adult germ-free animals [193], other immune parameters were found to require the presence of the microbiota during a postnatal time window. For example, only microbial exposure during early life but not in adults is able to inhibit the expression of CXCL16 in lung and colonic mucosa, allowing the subsequent control of invariant natural killer T (iNKT) cell numbers, which is protective against immune-mediated diseases (e.g., colitis) in later life [194]. The intestinal expansion of iNKT cells during the neonatal period is controlled by sphingolipids derived from *Bacteroides fragilis*, a member of the commensal microbial community [195]. Similarly, early microbial exposure is required to control the levels of IgE [196] and the influx of Tregs to the neonate's skin [197]. Another study showed that exposure to bacterial species able to synthesize riboflavin, such as the pathobiont *Prevotella mirabilis*, during the second week of life promotes the expansion of MAIT cells, especially in the skin where they favor tissue repair [198].

Finally, GAP-mediated antigen sampling in the colon must happen during the second week of life to ensure appropriate immune responses in adulthood [31]. Inhibition of colonic GAP formation in the preweaning phase or their maintenance after weaning lead to impaired Treg development and worsen inflammation in the DSS-induced colitis model.

### **Weaning-induced immunity**

#### **The weaning reaction, the last window of opportunity**

At weaning, the passive protection provided by breast milk-derived proteins is replaced by a stepwise maturation and establishment of the endogenous adaptive immune system. At the same time, the introduction of solid food in the diet reshapes the microbiota composition and promotes its diversification, reprogramming bacterial metabolic pathways [145]. Thus, major changes occur in the gut at weaning leading to the final education of the intestinal immune system during what has been called a weaning reaction [78]. The opening of this last window of opportunity at 3 weeks of age in mice is regulated by the decreasing concentration of breast milk EGF and is characterized by a strong and transient upregulation of IFN- $\gamma$  and TNF- $\alpha$  expression [78]. Similarly, during the first week after weaning, piglet intestinal tissues undergo an inflammatory response, notably with the upregulation of IL-6, IL-1 $\beta$ , and TNF- $\alpha$  [199]. Although TNF- $\alpha$  expression induced by microbial colonization at birth is restricted to the spleen and promotes pre-cDC1 maturation [192], TNF- $\alpha$  expression induced by the weaning reaction occurs in the gut [78,199]. In adult mice, the gut TNF- $\alpha$  expression that follows TLR7 stimulation is known to induce a massive and rapid migration of cDCs from the villus LP to the MLN and from the PP dome-associated villus LP to the IFRs enriched in naïve T cells [200,201]. Whether such migration occurs during the weaning reaction-induced TNF- $\alpha$  expression in infant animals remains to be established. Anyhow, in weanling mice microbial antigens, along with SCFAs and retinoic acid promote the expansion of colonic ROR $\gamma$ t<sup>+</sup> Tregs that enhance protection against DSS-induced colitis in adults [78]. Mice that missed the weaning reaction are ill-equipped to adequately respond to pathological inflammations such as colitis, allergy, or cancer in adulthood. The critical role of innate immune signals during this transition has been highlighted in humans by the work of Vatanen *et al.* [202]. They showed that the production of highly stimulatory hexa-acylated LPS by members of the infant



microbiota was associated with protection from allergic disease.

### Mucosal immune cell changes induced during weaning

A major change driven by weaning is the expansion and activation of mucosal B cells, inducing the maturation of PPs, ILFs, and cryptopatches and marking the start of endogenous IgA production, crucial for maintaining intestinal barrier, controlling the microbiota, and fighting against pathogens. In the small intestine, peptidoglycan from Gram-negative bacteria is recognized by epithelial nucleotide-binding oligomerization domain-containing protein 1 (NOD1) [203], which leads to the expression of  $\beta$ -defensin 3 and CCL20. In turn, CCL20 recruits CCR6-expressing B cells into cryptopatches. B-cell expansion induces the maturation of cryptopatches into ILF through TLR2- and TLR4-mediated microbial recognition. Consecutively, ILF B cells modulate the commensal microbial community through IgA induction.

Germinal center formation and the subsequent generation of antigen-specific IgA characterize PP maturation (Fig. 4). Lack of receptor activator of nuclear factor kappa-B ligand (RANKL) expression in IEC blocks M cell differentiation at a very early stage [204]. In the absence of M cells, the uptake of particulate antigens is strongly inhibited and a delay in GC formation and plasma cell generation after weaning leads to decreased levels of IgA in the intestinal lumen and reduction of IgA-coated bacteria that persist in adulthood. The transcription factor Sox8 is expressed downstream of RANKL signaling and involved in the late stage of M cell maturation (Fig. 3) [159]. Impaired antigen uptake in Sox8-deficient mice leads to delayed GC reaction and IgA induction at weaning that do not persist into adulthood. Other mechanisms for soluble and particulate antigen uptake that contribute to the establishment of intestinal homeostasis in absence of mature M cells remain to be identified and the stage of the immune development during which those mechanisms are activated needs to be defined. Moreover, the relative contribution of the different PP phagocyte subsets to the weaning-induced immune reaction has to be determined. In adults, subepithelial LysoDCs and macrophages are the main phagocyte subsets involved in particulate antigen uptake (Fig. 4) [19,205,206]; whether it is also the case in neonates and weanling mice and whether they can be activated the same way than in adults is currently unknown.

In mice and humans, B-cell clones that are generated in early life persist into adulthood, even those

specific of bacterial species encountered at weaning but lost afterward due to the diversification of the microbiota [207]. Activation of B cells in the PPs may be influenced significantly by dietary antigens, as rats that are colonized with a commensal microbiota but are kept on a protein-depleted diet have smaller PPs mainly containing immature B cells, a phenotype that can be rescued when complementing the diet with casein [208].

Many changes in the intestinal immune system occur at weaning with the induction of microbially induced ROR $\gamma$ t<sup>+</sup> Tregs and IgA being the best-characterized examples. CX<sub>3</sub>CR1<sup>+</sup> macrophages make up a great proportion of the gut immune cell compartment and react very rapidly to changes in homeostasis. In the adult, intestinal macrophages are renewed relatively quickly and differentiate from incoming monocytes that were generated in the bone marrow [209]. The neonatal colonic tissue, however, contains macrophages that mainly stem from embryonic hematopoiesis [209]. From weaning onwards, there is an influx of CCR2-dependent monocytes, which with time replace embryonic-derived macrophages and constantly replenish the intestinal macrophage pool. The seeding of the colon with an adult macrophage compartment requires microbial exposure as this process is significantly dampened in germ-free mice. Interestingly, fetal-derived macrophages residing far from the intestinal lumen and its content do not completely disappear in the adult tissue. A small subset of T-cell immunoglobulin and mucin domain containing 4 (TIM-4)-expressing macrophages are indeed long-lived and derived from fetal hematopoiesis, whereas the TIM-4-negative macrophages derived from CCR2-dependent monocytes, quite rare at birth, increase in frequency at 4 weeks of age, and further expand in adults [210]. Another study showed that whereas macrophages close to the gut lumen are rapidly renewed, a large part of muscularis and submucosal macrophages, distant from the lumen, are long-lived, self-maintaining cells barely replaced by circulating monocytes [211]. These macrophages are associated with and support neurons and gut vasculature and their loss typically results in vascular leakage and reduced transit.

Similar to their adult counterparts, neonatal colonic macrophages display an anti-inflammatory profile that is critical for gut homeostasis. IL-10 receptor signaling is essential to induce these anti-inflammatory properties in macrophages and its loss in mouse and man results in spontaneous colitis and severe early-onset inflammatory bowel disease, respectively [212,213]. Loss of autocrine IL-10 signaling in intestinal macrophages actually reshapes them into IL-23-producing

cells, which leads to IL-22-driven colitis [214]. Interestingly, unlike the colon where IL-10 production by macrophages as well as Treg expansion and maintenance depend on microbial exposure, macrophage production of IL-10 and the generation of Tregs in the small intestine require dietary amino acids [215,216], indicating a regionalized regulation of intestinal immunity based on intestinal lumen content sampling, possibly by GAPs [31,180].

## Conclusions and perspectives

The results discussed in this review illustrate that the neonatal mucosal tissue and immune system undergo gradual maturation processes within defined time windows and depend on developmental programs and/or exogenous signals such as microbial exposure. This assigns the neonatal period a critical and nonredundant role in the individual's life to set the stage for future host-microbial interactions and to establish immune homeostasis. This relates to cell homing and early suppression of immune activation, the acquisition of tolerance to innocuous antigens during a time-window controlled sampling activity, and, finally, the generation of large amounts of effector T lymphocytes and plasma cells. The discovery of the succession of necessary and nonredundant phases during ontogeny has given rise to the concept of layered immunity [217], which combined with the concept of windows of opportunity, may help to identify important endogenous and exogenous stimuli that act on the host during the different phases and ultimately allow to better understand the situation of the adult host [30]. It may also help to define the cause of immune- and inflammation-mediated diseases of the intestinal tract and identify possible strategies to reinstall immune homeostasis and preserve health.

However, important questions remain. For example, what are the functional mechanisms that explain the prolonged period of immune naïveté of the healthy murine neonate? The bacterial colonization of the mucosal surfaces starts at birth and quickly reaches high bacterial numbers. However, the adaptive immune system in the small intestine is maintained in a naïve, silent state until after weaning (Fig. 4). In addition, what are the mechanisms that suppress early immune priming? First, the microbial antigen diversity and antigen concentration in the intestinal lumen of neonates may—despite high bacterial density—be too low and fail to reach the threshold required to allow cognate antigen recognition to occur. A combined approach using metagenomics, metaproteomics, and antigen prediction might help to answer that question.

Second, the mechanisms that allow antigen uptake, processing, and presentation may not be fully functional at birth. The main routes of antigen sampling may indeed be less functional since GAPs are inhibited in neonates and mature M cells only appear in PPs shortly before weaning [31,53]. Moreover, subepithelial phagocytes may be unable or less efficient to prime B and T cells before weaning. Third, breast milk factors strongly involved in the regulation of the neonatal intestinal immune response [31,78,137] may restrain PP activation before weaning. Lastly, neonatal T-cell intrinsic features may preclude effective activation under homeostatic conditions. Consistently, neonatal T lymphocytes by definition are recent thymic emigrants that have been shown to exhibit significant differences after activation [187,218].

Other open questions relate to the emergence of immune activation at weaning. In particular, the role of specific commensal bacteria involved in this process such as the so-called segmented filamentous bacteria (SFB) that intimately interact with the apical plasma membrane of the intestinal epithelium in the ileum and the FAE overlaying the PPs (Fig. 4) [219,220]. Notably, SFBs show the highest abundance at 3 weeks after birth [221,222] and exhibit strong immunomodulatory properties characterized by a strong IgA and Th17 inducing activity in PPs or ILFs [223–225]. In PPs they also stimulate the expression of IL-21, aiding the induction of commensal-specific IgA and creating a regulatory loop controlling SFB abundance [226]. Another candidate might be *Alcaligenes* spp. that reside within subepithelial phagocytes in PPs and induce IgA, Th17, and ILC3 responses [227,228]. Besides specific viable microorganisms, bacterial metabolites have also been identified as potent inducers of immune responses [229]. They are also involved in PP activation and might initiate immune priming at weaning. Finally, although the landscape of PP mononuclear phagocytes has been well characterized in the adult [18] their presence, location, and function during the postnatal development and weaning remains to be established (Fig. 4).

A better understanding of the neonatal and infant mucosal immunity has important medical implications. Children exhibit the highest risk of enteric infections, and diarrheic diseases significantly contribute to childhood mortality worldwide. A mechanistic understanding of the influence of maternal and dietary factors as well as microbial metabolites and immunostimulatory molecules is instrumental to improve oral vaccines and develop therapeutic strategies to prevent early-life infections. Given the life-long priming during the neonatal window of opportunity, this time period

might also represent the prime period for interventions that aim to prevent inflammatory and immune-mediated diseases in later life.

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## Conflict of interest

The authors declare no conflict of interest.

## Author contributions

CW, NT, MWH, and HL wrote the manuscript. CW and NT designed the figures.

## References

- Dupont A, Heinbockel L, Brandenburg K & Hornef MW (2014) Antimicrobial peptides and the enteric mucus layer act in concert to protect the intestinal mucosa. *Gut Microbes* **5**, 761–765.
- Gallo RL & Hooper LV (2012) Epithelial antimicrobial defence of the skin and intestine. *Nat Rev Immunol* **12**, 503–516.
- Mukherjee S, Vaishnava S & Hooper LV (2008) Multi-layered regulation of intestinal antimicrobial defense. *Cell Mol Life Sci* **65**, 3019–3027.
- Bergstrom K, Shan X, Casero D, Batushansky A, Lagishetty V, Jacobs JP, Hoover C, Kondo Y, Shao B, Gao L *et al.* (2020) Proximal colon-derived O-glycosylated mucus encapsulates and modulates the microbiota. *Science* **370**, 467–472.
- Johansson MEV, Phillipson M, Petersson J, Velcich A, Holm L & Hansson GC (2008) The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci USA* **105**, 15064–15069.
- Frey A, Giannasca KT, Weltzin R, Giannasca PJ, Reggio H, Lencer WI & Neutra MR (1996) Role of the glycocalyx in regulating access of microparticles to apical plasma membranes of intestinal epithelial cells: implications for microbial attachment and oral vaccine targeting. *J Exp Med* **184**, 1045–1059.
- Mantis NJ, Frey A & Neutra MR (2000) Accessibility of glycolipid and oligosaccharide epitopes on rabbit villus and follicle-associated epithelium. *Am J Physiol Gastrointest Liver Physiol* **278**, G915–G923.
- Luissint A-C, Parkos CA & Nusrat A (2016) Inflammation and the intestinal barrier: leukocyte-epithelial cell interactions, cell junction remodeling, and mucosal repair. *Gastroenterology* **151**, 616–632.
- Garrett WS, Gordon JI & Glimcher LH (2010) Homeostasis and inflammation in the intestine. *Cell* **140**, 859–870.
- Hooper LV & Macpherson AJ (2010) Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol* **10**, 159–169.
- Mowat AM (2018) To respond or not to respond — a personal perspective of intestinal tolerance. *Nat Rev Immunol* **18**, 405–415.
- Schulz O, Jaensson E, Persson EK, Liu X, Worbs T, Agace WW & Pabst O (2009) Intestinal CD103<sup>+</sup>, but not CX3CR1<sup>+</sup>, antigen sampling cells migrate in lymph and serve classical dendritic cell functions. *J Exp Med* **206**, 3101–3114.
- Worbs T, Bode U, Yan S, Hoffmann MW, Hintzen G, Bernhardt G, Förster R & Pabst O (2006) Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J Exp Med* **203**, 519–527.
- Jung C, Hugot J-P & Barreau F (2010) Peyer's patches: the immune sensors of the intestine. *Int J Inflamm* **2010**, 1–12.
- Ermund A, Gustafsson JK, Hansson GC & Keita ÅV (2013) Mucus properties and goblet cell quantification in mouse, rat and human ileal Peyer's patches. *PLoS One* **8**, e83688.
- Jinnohara T, Kanaya T, Hase K, Sakakibara S, Kato T, Tachibana N, Sasaki T, Hashimoto Y, Sato T, Watarai H *et al.* (2017) IL-22BP dictates characteristics of Peyer's patch follicle-associated epithelium for antigen uptake. *J Exp Med* **214**, 1607–1618.
- Mabbott NA, Donaldson DS, Ohno H, Williams IR & Mahajan A (2013) Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium. *Mucosal Immunol* **6**, 666–677.
- Da Silva C, Wagner C, Bonnardel J, Gorvel J-P & Lelouard H (2017) The Peyer's patch mononuclear phagocyte system at steady state and during infection. *Front Immunol* **8**, 01254.
- Wagner C, Bonnardel J, Silva CD, Spinelli L, Portilla CA, Tomas J, Lagier M, Chasson L, Masse M, Dalod

- M *et al.* (2020) Differentiation paths of Peyer's patch LysoDCs are linked to sampling site positioning, migration, and T cell priming. *Cell Rep* **31**, 107479.
- 20 Reboldi A, Arnon TI, Rodda LB, Atakilit A, Sheppard D & Cyster JG (2016) IgA production requires B cell interaction with subepithelial dendritic cells in Peyer's patches. *Science* **352**, aaf4822.
  - 21 Craig SW & Cebra JJ (1971) Peyer's patches: an enriched source of precursors for IgA-producing immunocytes in the rabbit. *J Exp Med* **134**, 188–200.
  - 22 Lycke NY & Bemark M (2012) The role of Peyer's patches in synchronizing gut IgA responses. *Front Immunol* **3**, 00329.
  - 23 Johansen F-E, Pekna M, Norderhaug IN, Haneberg B, Hietala MA, Krajci P, Betsholtz C & Brandtzaeg P (1999) Absence of Epithelial immunoglobulin a transport, with increased mucosal leakiness, in polymeric immunoglobulin receptor/secretory component-deficient mice. *J Exp Med* **190**, 915–922.
  - 24 Macpherson AJ, Yilmaz B, Limenitakis JP & Ganai-Vonarburg SC (2018) IgA function in relation to the intestinal microbiota. *Annu Rev Immunol* **36**, 359–381.
  - 25 Pabst O, Cerovic V & Hornef M (2016) Secretory IgA in the coordination of establishment and maintenance of the microbiota. *Trends Immunol* **37**, 287–296.
  - 26 Sutherland DB, Suzuki K & Fagarasan S (2016) Fostering of advanced mutualism with gut microbiota by Immunoglobulin A. *Immunol Rev* **270**, 20–31.
  - 27 Gensollen T, Iyer SS, Kasper DL & Blumberg RS (2016) How colonization by microbiota in early life shapes the immune system. *Science* **352**, 539–544.
  - 28 Parigi SM, Eldh M, Larssen P, Gabrielsson S & Villablanca EJ (2015) Breast milk and solid food shaping intestinal immunity. *Front Immunol* **6**, 415.
  - 29 Al Nabhani Z & Eberl G (2020) Imprinting of the immune system by the microbiota early in life. *Mucosal Immunol* **13**, 183–189.
  - 30 Hornef MW & Torow N (2020) 'Layered immunity' and the 'neonatal window of opportunity' – timed succession of non-redundant phases to establish mucosal host–microbial homeostasis after birth. *Immunology* **159**, 15–25.
  - 31 Knoop KA, Gustafsson JK, McDonald KG, Kulkarni DH, Coughlin PE, McCrate S, Kim D, Hsieh C-S, Hogan SP, Elson CO *et al.* (2017) Microbial antigen encounter during a preweaning interval is critical for tolerance to gut bacteria. *Sci Immunol* **2**, ea01314.
  - 32 Neu J & Walker WA (2011) Necrotizing enterocolitis. *N Engl J Med* **364**, 255–264.
  - 33 Alexander VN, Northrup V & Bizzarro MJ (2011) Antibiotic exposure in the newborn intensive care unit and the risk of necrotizing enterocolitis. *J Pediatr* **159**, 392–397.
  - 34 Cotten CM, Taylor S, Stoll B, Goldberg RN, Hansen NI, Sánchez PJ, Ambalavanan N & Benjamin DK (2009) Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. *Pediatrics* **123**, 58–66.
  - 35 Hosny M, Cassir N & La Scola B (2017) Updating on gut microbiota and its relationship with the occurrence of necrotizing enterocolitis. *Hum Microbiome J* **4**, 14–19.
  - 36 van Best N, Trepels-Kottek S, Savelkoul P, Orlikowsky T, Hornef MW & Penders J (2020) Influence of probiotic supplementation on the developing microbiota in human preterm neonates. *Gut Microbes* **12**, 1826747.
  - 37 Hunter C, Dimaguila MAV, Gal P, Wimmer JE, Ransom JL, Carlos RQ, Smith M & Davanzo CC (2012) Effect of routine probiotic, *Lactobacillus reuteri* DSM 17938, use on rates of necrotizing enterocolitis in neonates with birthweight < 1000 grams: a sequential analysis. *BMC Pediatr* **12**, 142.
  - 38 Morgan RL, Preidis GA, Kashyap PC, Weizman AV, Sadeghirad B, Chang Y, Florez ID, Foroutan F, Shahid S & Zeraatkar D (2020) Probiotics reduce mortality and morbidity in preterm, low-birth-weight infants: a systematic review and network meta-analysis of randomized trials. *Gastroenterology* **159**, 467–480.
  - 39 Liu Y, Fatheree NY, Mangalat N & Rhoads JM (2011) *Lactobacillus reuteri* strains reduce incidence and severity of experimental necrotizing enterocolitis via modulation of TLR4 and NF- $\kappa$ B signaling in the intestine. *Am J Physiol Gastrointest Liver Physiol* **302**, G608–G617.
  - 40 Shane AL, Sánchez PJ & Stoll BJ (2017) Neonatal sepsis. *Lancet* **390**, 1770–1780.
  - 41 Feasey NA, Dougan G, Kingsley RA, Heyderman RS & Gordon MA (2012) Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. *Lancet* **379**, 2489–2499.
  - 42 Madan JC, Salari RC, Saxena D, Davidson L, O'Toole GA, Moore JH, Sogin ML, Foster JA, Edwards WH, Palumbo P *et al.* (2012) Gut microbial colonisation in premature neonates predicts neonatal sepsis. *Arch Dis Child Fetal Neonatal Ed* **97**, F456–F462.
  - 43 Singer JR, Blosser EG, Zindl CL, Silberger DJ, Conlan S, Laufer VA, DiToro D, Deming C, Kumar R, Morrow CD *et al.* (2019) Preventing dysbiosis of the neonatal mouse intestinal microbiome protects against late-onset sepsis. *Nat Med* **25**, 1772–1782.
  - 44 Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, Rudan I, Campbell H, Cibulskis R, Li M *et al.* (2012) Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet* **379**, 2151–2161.
  - 45 de Sablet T, Potiron L, Marquis M, Bussière FI, Lacroix-Lamandé S & Laurent F (2016)

- Cryptosporidium parvum* increases intestinal permeability through interaction with epithelial cells and IL-1 $\beta$  and TNF $\alpha$  released by inflammatory monocytes. *Cell Microbiol* **18**, 1871–1880.
- 46 Lantier L, Lacroix-Lamadé S, Potiron L, Metton C, Drouet F, Guesdon W, Gnahoui-David A, Vern YL, Deriaud E, Fenis A *et al.* (2013) Intestinal CD103+ dendritic cells are key players in the innate immune control of *Cryptosporidium parvum* infection in neonatal mice. *PLoS Pathog* **9**, e1003801.
- 47 Troeger C, Khalil IA, Rao PC, Cao S, Blacker BF, Ahmed T, Armah G, Bines JE, Brewer TG, Colombara DV *et al.* (2018) Rotavirus vaccination and the global burden of rotavirus diarrhea among children younger than 5 years. *JAMA Pediatr* **172**, 958–965.
- 48 Pott J, Stockinger S, Torow N, Smoczek A, Lindner C, McInerney G, Bäckhed F, Baumann U, Pabst O, Bleich A *et al.* (2012) Age-dependent TLR3 expression of the intestinal epithelium contributes to rotavirus susceptibility. *PLoS Pathog* **8**, e1002670.
- 49 Pott J, Mahlaköiv T, Mordstein M, Duerr CU, Michiels T, Stockinger S, Staeheli P & Hornef MW (2011) IFN- $\lambda$  determines the intestinal epithelial antiviral host defense. *Proc Natl Acad Sci USA* **108**, 7944–7949.
- 50 Dupont A, Sommer F, Zhang K, Repnik U, Basic M, Bleich A, Kühnel M, Bäckhed F, Litvak Y, Fulde M *et al.* (2016) Age-dependent susceptibility to enteropathogenic *Escherichia coli* (EPEC) infection in mice. *PLoS Pathog* **12**, e1005616.
- 51 Kim Y-G, Sakamoto K, Seo S-U, Pickard JM, Gilliland MG, Pudlo NA, Hoostal M, Li X, Wang TD, Feehley T *et al.* (2017) Neonatal acquisition of Clostridia species protects against colonization by bacterial pathogens. *Science* **356**, 315–319.
- 52 Jones BD, Gori N & Falkow S (1994) *Salmonella typhimurium* initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. *J Exp Med* **180**, 15–23.
- 53 Zhang K, Dupont A, Torow N, Gohde F, Leschner S, Lienenklaus S, Weiss S, Brinkmann MM, Kühnel M, Hensel M *et al.* (2014) Age-dependent enterocyte invasion and microcolony formation by *Salmonella*. *PLoS Pathog* **10**, e1004385.
- 54 Zhang K, Riba A, Nietschke M, Torow N, Repnik U, Pütz A, Fulde M, Dupont A, Hensel M & Hornef M (2018) Minimal SPII-T3SS effector requirement for *Salmonella enterocyte* invasion and intracellular proliferation *in vivo*. *PLoS Pathog* **14**, e1006925.
- 55 Echeverry A, Schesser K & Adkins B (2007) Murine neonates are highly resistant to *Yersinia enterocolitica* following orogastric exposure. *Infect Immun* **75**, 2234–2243.
- 56 Echeverry A, Saijo S, Schesser K & Adkins B (2010) *Yersinia enterocolitica* promotes robust mucosal inflammatory T-cell immunity in murine neonates. *Infect Immun* **78**, 3595–3608.
- 57 Siefker DT, Echeverry A, Brambilla R, Fukata M, Schesser K & Adkins B (2014) Murine neonates infected with *Yersinia enterocolitica* develop rapid and robust proinflammatory responses in intestinal lymphoid tissues. *Infect Immun* **82**, 762–772.
- 58 Siefker DT & Adkins B (2017) Rapid CD8+ function is critical for protection of neonatal mice from an extracellular bacterial enteropathogen. *Front Pediatr* **4**, 141.
- 59 Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z & Tong S (2020) Epidemiology of COVID-19 among children in China. *Pediatrics* **145**, e20200702.
- 60 Jones TC, Mühlemann B, Veith T, Biele G, Zuchowski M, Hofmann J, Stein A, Edelmann A, Corman VM & Drosten C (2020) An analysis of SARS-CoV-2 viral load by patient age. *medRxiv* 2020.06.08.20125484. <https://doi.org/10.1038/s41564-021-00904-0>
- 61 Cheung EW, Zachariah P, Gorelik M, Boneparth A, Kernie SG, Orange JS & Milner JD (2020) Multisystem inflammatory syndrome related to COVID-19 in previously healthy children and adolescents in New York City. *JAMA* **324**, 294.
- 62 Pierce CA, Preston-Hurlburt P, Dai Y, Aschner CB, Cheshenko N, Galen B, Garforth SJ, Herrera NG, Jangra RK, Morano NC *et al.* (2020) Immune responses to SARS-CoV-2 infection in hospitalized pediatric and adult patients. *Sci Transl Med* **12**, eabd5487.
- 63 Carsetti R, Quintarelli C, Quinti I, Mortari EP, Zumla A, Ippolito G & Locatelli F (2020) The immune system of children: the key to understanding SARS-CoV-2 susceptibility? *Lancet Child Adolesc Health* **4**, 414–416.
- 64 de Goffau MC, Lager S, Sovio U, Gaccioli F, Cook E, Peacock SJ, Parkhill J, Charnock-Jones DS & Smith GCS (2019) Human placenta has no microbiome but can contain potential pathogens. *Nature* **572**, 329–334.
- 65 Rackaityte E, Halkias J, Fukui EM, Mendoza VF, Hayzelden C, Crawford ED, Fujimura KE, Burt TD & Lynch SV (2021) Corroborating evidence refutes batch effect as explanation for fetal bacteria. *Microbiome* **9**, 1–4.
- 66 Kennedy KM, Gerlach MJ, Adam T, Heimesaat MM, Rossi L, Surette MG, Sloboda DM & Braun T. Fetal meconium does not have a detectable microbiota before birth. *Nat Microbiol.* 2021;1–9. <http://dx.doi.org/10.1038/s41564-021-00904-0>.
- 67 Mitchell C, Hogstrom L, Bryant A, Bergerat A, Cher A, Pochan S, Herman P, Carrigan M, Sharp K, Huttenhower C *et al.* (2020) Delivery mode impacts newborn gut colonization efficiency. *bioRxiv* [PREPRINT].

- 68 Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N & Knight R (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci USA* **107**, 11971–11975.
- 69 Yatsunenkov T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP *et al.* (2012) Human gut microbiome viewed across age and geography. *Nature* **486**, 222–227.
- 70 Adlerberth I, Lindberg E, Åberg N, Hesselmar B, Saalman R, Strannegård I-L & Wold AE (2006) Reduced enterobacterial and increased *Staphylococcal* colonization of the infantile bowel: an effect of hygienic lifestyle? *Pediatr Res* **59**, 96–101.
- 71 Derrien M, Alvarez A-S & de Vos WM (2019) The gut microbiota in the first decade of life. *Trends Microbiol* **27**, 997–1010.
- 72 Robertson RC, Manges AR, Finlay BB & Prendergast AJ (2019) The human microbiome and child growth – first 1000 days and beyond. *Trends Microbiol* **27**, 131–147.
- 73 Guaraldi F & Salvatori G (2012) Effect of breast and formula feeding on gut microbiota shaping in newborns. *Front Cell Infect Microbiol* **2**, 94.
- 74 Fernández L, Pannaraj PS, Rautava S & Rodríguez JM (2020) The microbiota of the human mammary ecosystem. *Front Cell Infect Microbiol* **10**, 689.
- 75 Pacheco AR, Barile D, Underwood MA & Mills DA (2015) The impact of the milk glycobiome on the neonate gut microbiota. *Annu Rev Anim Biosci* **3**, 419–445.
- 76 Rogier EW, Frantz AL, Bruno MEC, Wedlund L, Cohen DA, Stromberg AJ & Kaetzel CS (2014) Secretory antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and host gene expression. *Proc Natl Acad Sci USA* **111**, 3074–3079.
- 77 Liang G, Zhao C, Zhang H, Mattei L, Sherrill-Mix S, Bittinger K, Kessler LR, Wu GD, Baldassano RN, DeRusso P *et al.* (2020) The stepwise assembly of the neonatal virome is modulated by breastfeeding. *Nature* **581**, 470–474.
- 78 Al Nabhani Z, Dulauroy S, Marques R, Cousu C, Al Bounny S, Déjardin F, Sparwasser T, Bérard M, Cerf-Bensussan N & Eberl G (2019) A weaning reaction to microbiota is required for resistance to immunopathologies in the adult. *Immunity* **50**, 1276–1288.e5.
- 79 Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H *et al.* (2015) Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe* **17**, 690–703.
- 80 van Best N, Rolle-Kampczyk U, Schaap FG, Basic M, Olde Damink SWM, Bleich A, Savelkoul PHM, von Bergen M, Penders J & Hornef MW (2020) Bile acids drive the newborn's gut microbiota maturation. *Nat Commun* **11**, 3692.
- 81 Hill CJ, Lynch DB, Murphy K, Ulaszewska M, Jeffery IB, O'Shea CA, Watkins C, Dempsey E, Mattivi F, Tuohy K *et al.* (2017) Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome* **5**, 4.
- 82 Vatanen T, Plichta DR, Somani J, Münch PC, Arthur TD, Hall AB, Rudolf S, Oakeley EJ, Ke X, Young RA *et al.* (2019) Genomic variation and strain-specific functional adaptation in the human gut microbiome during early life. *Nat Microbiol* **4**, 470–479.
- 83 Clemente JC, Pehrsson EC, Blaser MJ, Sandhu K, Gao Z, Wang B, Magris M, Hidalgo G, Contreras M, Noya-Alarcón Ó *et al.* (2015) The microbiome of uncontacted Amerindians. *Sci Adv* **1**, e1500183.
- 84 Filippo CD, Cavalieri D, Paola MD, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G & Lionetti P (2010) Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA* **107**, 14691–14696.
- 85 Martínez I, Stegen JC, Maldonado-Gómez MX, Eren AM, Siba PM, Greenhill AR & Walter J (2015) The gut microbiota of rural papua new guineans: composition, diversity patterns, and ecological processes. *Cell Rep* **11**, 527–538.
- 86 Tito RY, Knights D, Metcalf J, Obregon-Tito AJ, Cleland L, Najjar F, Roe B, Reinhard K, Sobolik K, Belknap S *et al.* (2012) Insights from characterizing extinct human gut microbiomes. *PLoS One* **7**, e51146.
- 87 Bach J-F (2018) The hygiene hypothesis in autoimmunity: the role of pathogens and commensals. *Nat Rev Immunol* **18**, 105–120.
- 88 Lambrecht BN & Hammad H (2017) The immunology of the allergy epidemic and the hygiene hypothesis. *Nat Immunol* **18**, 1076–1083.
- 89 Blaser MJ & Falkow S (2009) What are the consequences of the disappearing human microbiota? *Nat Rev Microbiol* **7**, 887–894.
- 90 Arnold IC, Lee JY, Amieva MR, Roers A, Flavell RA, Sparwasser T & Müller A (2011) Tolerance rather than immunity protects from *Helicobacter pylori*-induced gastric preneoplasia. *Gastroenterology* **140**, 199–209.e8.
- 91 Altobelli A, Bauer M, Velez K, Cover TL & Müller A (2019) *Helicobacter pylori* VacA targets myeloid cells in the gastric lamina propria to promote peripherally induced regulatory T-Cell differentiation and persistent infection. *MBio* **10**, e00261-19.
- 92 Oertli M, Sundquist M, Hitzler I, Engler DB, Arnold IC, Reuter S, Maxeiner J, Hansson M, Taube C, Quiding-Järbrink M *et al.* (2012) DC-derived IL-18 drives Treg differentiation, murine *Helicobacter pylori*-

- specific immune tolerance, and asthma protection. *J Clin Invest* **122**, 1082–1096.
- 93 de Agüero MG, Ganai-Vonarburg SC, Fuhrer T, Rupp S, Uchimura Y, Li H, Steinert A, Heikenwalder M, Hapfelmeier S, Sauer U *et al.* (2016) The maternal microbiota drives early postnatal innate immune development. *Science* **351**, 1296–1302.
- 94 Kimura I, Miyamoto J, Ohue-Kitano R, Watanabe K, Yamada T, Onuki M, Aoki R, Isobe Y, Kashihara D, Inoue D *et al.* (2020) Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice. *Science* **367**, eaaw8429.
- 95 Nakajima A, Kaga N, Nakanishi Y, Ohno H, Miyamoto J, Kimura I, Hori S, Sasaki T, Hiramatsu K, Okumura K *et al.* (2017) Maternal high fiber diet during pregnancy and lactation influences regulatory T cell differentiation in offspring in mice. *J Immunol* **199**, 3516–3524.
- 96 Thorburn AN, McKenzie CI, Shen S, Stanley D, Macia L, Mason LJ, Roberts LK, Wong CHY, Shim R, Robert R *et al.* (2015) Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. *Nat Commun* **6**, 7320.
- 97 Wilson E & Butcher EC (2004) CCL28 controls immunoglobulin (Ig)A plasma cell accumulation in the lactating mammary gland and IgA antibody transfer to the neonate. *J Exp Med* **200**, 805–809.
- 98 Kramer DR & Cebra JJ (1995) Early appearance of “natural” mucosal IgA responses and germinal centers in suckling mice developing in the absence of maternal antibodies. *J Immunol* **154**, 2051–2062.
- 99 Harris NL, Spoerri I, Schopfer JF, Nembrini C, Merky P, Massacand J, Urban JF, Lamarre A, Burki K, Odermatt B *et al.* (2006) Mechanisms of neonatal mucosal antibody protection. *J Immunol* **177**, 6256–6262.
- 100 Koch MA, Reiner GL, Lugo KA, Kreuk LSM, Stanbery AG, Ansaldo E, Seher TD, Ludington WB & Barton GM (2016) Maternal IgG and IgA antibodies dampen mucosal T helper cell responses in early life. *Cell* **165**, 827–841.
- 101 Kramer DR & Cebra JJ (1995) Role of maternal antibody in the induction of virus specific and bystander IgA responses in Peyer’s patches of suckling mice. *Int Immunol* **7**, 911–918.
- 102 McLean B & Holmes IH (1980) Transfer of antirotaviral antibodies from mothers to their infants. *J Clin Microbiol* **12**, 320–325.
- 103 Zaman S, Carlsson B, Morikawa A, Jeansson S, Narayanan I, Thiringer K, Jalil F & Hanson LA (1993) Poliovirus antibody titres, relative affinity, and neutralising capacity in maternal milk. *Arch Dis Child* **68**, 198–201.
- 104 Van de Perre P (2003) Transfer of antibody via mother’s milk. *Vaccine* **21**, 3374–3376.
- 105 Hanson LA (1998) Breastfeeding provides passive and likely long-lasting active immunity. *Ann Allergy Asthma Immunol* **81**, 523–537.
- 106 Zheng W, Zhao W, Wu M, Song X, Caro F, Sun X, Gazzaniga F, Stefanetti G, Oh S, Mekalanos JJ *et al.* (2020) Microbiota-targeted maternal antibodies protect neonates from enteric infection. *Nature* **577**, 543–548.
- 107 Edwards KM (2015) Maternal antibodies and infant immune responses to vaccines. *Vaccine* **33**, 6469–6472.
- 108 Pyzik M, Sand KMK, Hubbard JJ, Andersen JT, Sandlie I & Blumberg RS (2019) The neonatal Fc receptor (FcRn): a misnomer? *Front Immunol* **10**, 1540.
- 109 Yoshida M, Kobayashi K, Kuo TT, Bry L, Glickman JN, Claypool SM, Kaser A, Nagaishi T, Higgins DE, Mizoguchi E *et al.* (2006) Neonatal Fc receptor for IgG regulates mucosal immune responses to luminal bacteria. *J Clin Invest* **116**, 2142–2151.
- 110 Ohsaki A, Venturelli N, Buccigrosso TM, Osganian SK, Lee J, Blumberg RS & Oyoshi MK (2018) Maternal IgG immune complexes induce food allergen-specific tolerance in offspring. *J Exp Med* **215**, 91–113.
- 111 Vono M, Eberhardt CS, Auderset F, Mastelic-Gavillet B, Lemeille S, Christensen D, Andersen P, Lambert P-H & Siegrist C-A (2019) Maternal antibodies inhibit neonatal and infant responses to vaccination by shaping the early-life B cell repertoire within germinal centers. *Cell Rep* **28**, 1773–1784.e5.
- 112 Ramanan D, Sefik E, Galván-Peña S, Wu M, Yang L, Yang Z, Kostic A, Golovkina TV, Kasper DL, Mathis D *et al.* (2020) An immunologic mode of multigenerational transmission governs a gut treg setpoint. *Cell* **181**, 1276–1290.e13.
- 113 Laouar A (2020) Maternal leukocytes and infant immune programming during breastfeeding. *Trends Immunol* **41**, 225–239.
- 114 Torow N, Yu K, Hassani K, Freitag J, Schulz O, Basic M, Brennecke A, Sparwasser T, Wagner N, Bleich A *et al.* (2015) Active suppression of intestinal CD4 + TCR $\alpha\beta$  + T-lymphocyte maturation during the postnatal period. *Nat Commun* **6**, 1–12.
- 115 Molès J-P, Tuaille E, Kankasa C, Bedin A-S, Nagot N, Marchant A, McDermid JM & de Perre PV (2018) Breastmilk cell trafficking induces microchimerism-mediated immune system maturation in the infant. *Pediatr Allergy Immunol* **29**, 133–143.
- 116 Cabinian A, Sinsimer D, Tang M, Zumba O, Mehta H, Toma A, Sant’Angelo D, Laouar Y & Laouar A (2016) Transfer of maternal immune cells by breastfeeding: maternal cytotoxic T lymphocytes present in breast milk localize in the Peyer’s patches of the nursed infant. *PLoS One* **11**, e0156762.
- 117 Bedin A-S, Molès J-P, Rutagwera D, Nagot N, Kankasa C, Tylleskär T, Valverde-Villegas JM, Durand M, de Perre PV & Tuaille E (2019) MAIT

- cells, TCR  $\gamma\delta$ + cells and ILCs cells in human breast milk and blood from HIV infected and uninfected women. *Pediatr Allergy Immunol* **30**, 479–487.
- 118 Sabbaj S, Ghosh MK, Edwards BH, Leeth R, Decker WD, Goepfert PA & Aldrovandi GM (2005) Breast milk-derived antigen-specific CD8+ T cells: an extralymphoid effector memory cell population in humans. *J Immunol* **174**, 2951–2956.
- 119 Tuaille E, Valea D, Becquart P, Tabaa YA, Meda N, Bolloré K, de Perre PV & Vendrell J-P (2009) Human milk-derived B cells: a highly activated switched memory cell population primed to secrete antibodies. *J Immunol* **182**, 7155–7162.
- 120 Arvola M, Gustafsson E, Svensson L, Jansson L, Holmdahl R, Heyman B, Okabe M & Mattsson R (2000) Immunoglobulin-secreting cells of maternal origin can be detected in B cell-deficient mice. *Biol Reprod* **63**, 1817–1824.
- 121 Bode L (2012) Human milk oligosaccharides: every baby needs a sugar mama. *Glycobiology* **22**, 1147–1162.
- 122 Walsh C, Lane JA, van Sinderen D & Hickey RM (2020) Human milk oligosaccharides: shaping the infant gut microbiota and supporting health. *J Funct Foods* **72**, 104074.
- 123 Marcobal A & Sonnenburg JL (2012) Human milk oligosaccharide consumption by intestinal microbiota. *Clin Microbiol Infect* **18**, 12–15.
- 124 Koning N, Kessen SFM, Van Der Voorn JP, Appelmelk BJ, Jeurink PV, Knippels LMJ, Garssen J & Van Kooyk Y (2015) Human milk blocks DC-SIGN–pathogen interaction via MUC1. *Front Immunol* **6**, 112.
- 125 Noll AJ, Yu Y, Lasanajak Y, Duska-McEwen G, Buck RH, Smith DF & Cummings RD (2016) Human DC-SIGN binds specific human milk glycans. *Biochem J* **473**, 1343–1353.
- 126 Xiao L, Worp WRPH, Stassen R, Maastricht C, Kettelarj N, Stahl B, Blijenberg B, Overbeek SA, Folkerts G, Garssen J *et al.* (2019) Human milk oligosaccharides promote immune tolerance via direct interactions with human dendritic cells. *Eur J Immunol* **49**, 1001–1014.
- 127 Trend S, Strunk T, Hibbert J, Kok CH, Zhang G, Doherty DA, Richmond P, Burgner D, Simmer K, Davidson DJ *et al.* (2015) Antimicrobial protein and peptide concentrations and activity in human breast milk consumed by preterm infants at risk of late-onset neonatal sepsis. *PLoS One* **10**, e0117038.
- 128 Murakami M, Dorschner RA, Stern LJ, Lin KH & Gallo RL (2005) Expression and secretion of cathelicidin antimicrobial peptides in murine mammary glands and human milk. *Pediatr Res* **57**, 10–15.
- 129 Battersby AJ, Khara J, Wright VJ, Levy O & Kampmann B (2016) Antimicrobial proteins and peptides in early life. Ontogeny and translational opportunities. *Front Immunol* **7**, 309.
- 130 Liu B & Newburg DS (2013) Human milk glycoproteins protect infants against human pathogens. *Breastfeed Med* **8**, 354–362.
- 131 Frey EA, Miller DS, Jahr TG, Sundan A, Bazil V, Espevik T, Finlay BB & Wright SD (1992) Soluble CD14 participates in the response of cells to lipopolysaccharide. *J Exp Med* **176**, 1665–1671.
- 132 Labéta MO, Vidal K, Norez JER, Arias M, Vita N, Morgan BP, Guillemot JC, Loyaux D, Ferrara P, Schmid D *et al.* (2000) Innate recognition of bacteria in human milk is mediated by a milk-derived highly expressed pattern recognition receptor, soluble Cd14. *J Exp Med* **191**, 1807–1812.
- 133 LeBouder E, Rey-Norez JE, Raby A-C, Affolter M, Vidal K, Thornton CA & Labéta MO (2006) Modulation of neonatal microbial recognition: TLR-mediated innate immune responses are specifically and differentially modulated by human milk. *J Immunol* **176**, 3742–3752.
- 134 Dawod B & Marshall JS (2019) Cytokines and soluble receptors in breast milk as enhancers of oral tolerance development. *Front Immunol* **10**, 16.
- 135 Dvorak B (2010) Milk epidermal growth factor and gut protection. *J Pediatr* **156**, S31–S35.
- 136 Good M, Sodhi CP, Egan CE, Afrazi A, Jia H, Yamaguchi Y, Lu P, Branca MF, Ma C, Prindle T *et al.* (2015) Breast milk protects against the development of necrotizing enterocolitis through inhibition of Toll-like receptor 4 in the intestinal epithelium via activation of the epidermal growth factor receptor. *Mucosal Immunol* **8**, 1166–1179.
- 137 Knoop KA, Coughlin PE, Floyd AN, Ndao IM, Hall-Moore C, Shaikh N, Gasparrini AJ, Rusconi B, Escobedo M, Good M *et al.* (2020) Maternal activation of the EGFR prevents translocation of gut-residing pathogenic *Escherichia coli* in a model of late-onset neonatal sepsis. *Proc Natl Acad Sci USA* **117**, 7941–7949.
- 138 Nojiri T, Yoshizato T, Fukami T, Obama H, Yagi H, Yotsumoto F & Miyamoto S (2012) Clinical significance of amphiregulin and epidermal growth factor in colostrum. *Arch Gynecol Obstet* **286**, 643–647.
- 139 Sbarbati R (1982) Morphogenesis of the intestinal villi of the mouse embryo: chance and spatial necessity. *J Anat* **135**, 477–499.
- 140 Tourneur E & Chassin C (2013) Neonatal immune adaptation of the gut and its role during infections. *Clin Dev Immunol* **2013**, 1–17.
- 141 Calvert R & Pothier P (1990) Migration of fetal intestinal intervillous cells in neonatal mice. *Anat Rec* **227**, 199–206.
- 142 Bry L, Falk P, Huttner K, Ouellette A, Midtvedt T & Gordon JI (1994) Paneth cell differentiation in the



- developing intestine of normal and transgenic mice. *Proc Natl Acad Sci USA* **91**, 10335–10339.
- 143 Tomas J, Wrzosek L, Bouznad N, Bouet S, Mayeur C, Noordine M-L, Honvo-Houeto E, Langella P, Thomas M & Cherbuy C (2013) Primocolonization is associated with colonic epithelial maturation during conventionalization. *FASEB J* **27**, 645–655.
- 144 Muncan V, Heijmans J, Krasinski SD, Büller NV, Wildenberg ME, Meisner S, Radonjic M, Stapleton KA, Lamers WH, Biemond I *et al.* (2011) Blimp1 regulates the transition of neonatal to adult intestinal epithelium. *Nat Commun* **2**, 452.
- 145 Harper J, Mould A, Andrews RM, Bikoff EK & Robertson EJ (2011) The transcriptional repressor Blimp1/Prdm1 regulates postnatal reprogramming of intestinal enterocytes. *Proc Natl Acad Sci USA* **108**, 10585–10590.
- 146 Birchenough GMH, Dalgakiran F, Witcomb LA, Johansson MEV, McCarthy AJ, Hansson GC & Taylor PW (2017) Postnatal development of the small intestinal mucosa drives age-dependent, regio-selective susceptibility to *Escherichia coli* K1 infection. *Sci Rep* **7**, 1–14.
- 147 Veiga-Fernandes H, Coles MC, Foster KE, Patel A, Williams A, Natarajan D, Barlow A, Pachnis V & Kioussis D (2007) Tyrosine kinase receptor RET is a key regulator of Peyer's Patch organogenesis. *Nature* **446**, 547–551.
- 148 van de Pavert SA, Olivier BJ, Goverse G, Vondenhoff MF, Greuter M, Beke P, Kusser K, Höpken UE, Lipp M, Niederreither K *et al.* (2009) Chemokine CXCL13 is essential for lymph node initiation and is induced by retinoic acid and neuronal stimulation. *Nat Immunol* **10**, 1193–1199.
- 149 van de Pavert SA, Ferreira M, Domingues RG, Ribeiro H, Molenaar R, Moreira-Santos L, Almeida FF, Ibiza S, Barbosa I, Goverse G *et al.* (2014) Maternal retinoids control type 3 innate lymphoid cells and set the offspring immunity. *Nature* **508**, 123–127.
- 150 Eberl G, Marmon S, Sunshine M-J, Rennert PD, Choi Y & Littman DR (2004) An essential function for the nuclear receptor ROR $\gamma$ (t) in the generation of fetal lymphoid tissue inducer cells. *Nat Immunol* **5**, 64–73.
- 151 Yoshida H, Naito A, Inoue J-I, Satoh M, Santee-Cooper SM, Ware CF, Togawa A, Nishikawa S & Nishikawa S-I (2002) Different cytokines induce surface lymphotoxin- $\alpha\beta$  on IL-7 receptor- $\alpha$  cells that differentially engender lymph nodes and Peyer's patches. *Immunity* **17**, 823–833.
- 152 Honda K, Nakano H, Yoshida H, Nishikawa S, Rennert P, Ikuta K, Tamechika M, Yamaguchi K, Fukumoto T, Chiba T *et al.* (2001) Molecular basis for hematopoietic/mesenchymal interaction during initiation of Peyer's patch organogenesis. *J Exp Med* **193**, 621–630.
- 153 Mebius RE (2003) Organogenesis of lymphoid tissues. *Nat Rev Immunol* **3**, 292–303.
- 154 van de Pavert SA & Mebius RE (2010) New insights into the development of lymphoid tissues. *Nat Rev Immunol* **10**, 664–674.
- 155 Yoshida H, Honda K, Shinkura R, Adachi S, Nishikawa S, Maki K, Ikuta K & Nishikawa S-I (1999) IL-7 receptor  $\alpha$ + CD3- cells in the embryonic intestine induces the organizing center of Peyer's patches. *Int Immunol* **11**, 643–655.
- 156 Nakagawa R, Togawa A, Nagasawa T & Nishikawa S (2013) Peyer's Patch inducer cells play a leading role in the formation of B and T cell zone architecture. *J Immunol* **190**, 3309–3318.
- 157 Hays C, Touak G, Bouaboud A, Fouet A, Guignot J, Poyart C & Tazi A (2019) Perinatal hormones favor CC17 group B *Streptococcus* intestinal translocation through M cells and hypervirulence in neonates. *eLife* **8**, e48772.
- 158 Kanaya T, Hase K, Takahashi D, Fukuda S, Hoshino K, Sasaki I, Hemmi H, Knoop KA, Kumar N, Sato M *et al.* (2012) The Ets transcription factor Spi-B is essential for the differentiation of intestinal microfold (M) cells. *Nat Immunol* **13**, 729–736.
- 159 Kimura S, Kobayashi N, Nakamura Y, Kanaya T, Takahashi D, Fujiki R, Mutoh M, Obata Y, Iwanaga T, Nakagawa T *et al.* (2019) Sox8 is essential for M cell maturation to accelerate IgA response at the early stage after weaning in mice. *J Exp Med* **216**, 831–846.
- 160 Kanamori Y, Ishimaru K, Nanno M, Maki K, Ikuta K, Nariuchi H & Ishikawa H (1996) Identification of novel lymphoid tissues in murine intestinal mucosa where clusters of c-kit+ IL-7R+ Thy1+ lymphohemopoietic progenitors develop. *J Exp Med* **184**, 1449–1459.
- 161 Goverse G, Labao-Almeida C, Ferreira M, Molenaar R, Wahlen S, Konijn T, Koning J, Veiga-Fernandes H & Mebius RE (2016) Vitamin A controls the presence of ROR $\gamma$ + innate lymphoid cells and lymphoid tissue in the small intestine. *J Immunol* **196**, 5148–5155.
- 162 Kiss EA, Vonarbourg C, Kopfmann S, Hobeika E, Finke D, Esser C & Diefenbach A (2011) Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. *Science* **334**, 1561–1565.
- 163 Spencer J, MacDonald TT, Finn T & Isaacson PG (1986) The development of gut associated lymphoid tissue in the terminal ileum of fetal human intestine. *Clin Exp Immunol* **64**, 536–543.
- 164 Cornes JS (1965) Number, size, and distribution of Peyer's patches in the human small intestine. *Gut* **6**, 225–229.
- 165 Fenton TM, Jørgensen PB, Niss K, Rubin SJS, Mörbe UM, Riis LB, Da Silva C, Plumb A, Vandamme J,

- Jakobsen HL *et al.* (2020) Immune profiling of human gut-associated lymphoid tissue identifies a role for isolated lymphoid follicles in priming of region-specific immunity. *Immunity* **52**, 557–570.e6.
- 166 Lügering A, Ross M, Sieker M, Heidemann J, Williams IR, Domschke W & Kucharzik T (2010) CCR6 identifies lymphoid tissue inducer cells within cryptopatches. *Clin Exp Immunol* **160**, 440–449.
- 167 Nochi T, Denton PW, Wahl A & Garcia JV (2013) Cryptopatches are essential for the development of human GALT. *Cell Rep* **3**, 1874–1884.
- 168 Ménard S, Förster V, Lotz M, Gütle D, Duerr CU, Gallo RL, Henriques-Normark B, Pütsep K, Andersson M, Glocker EO *et al.* (2008) Developmental switch of intestinal antimicrobial peptide expression. *J Exp Med* **205**, 183–193.
- 169 Sawa S, Lochner M, Satoh-Takayama N, Dulauroy S, Bérard M, Kleinschek M, Cua D, Di Santo JP & Eberl G (2011) ROR $\gamma$ t<sup>+</sup> innate lymphoid cells regulate intestinal homeostasis by integrating negative signals from the symbiotic microbiota. *Nat Immunol* **12**, 320–326.
- 170 Chassin C, Kocur M, Pott J, Duerr CU, Gütle D, Lotz M & Hornef MW (2010) miR-146a mediates protective innate immune tolerance in the neonate intestine. *Cell Host Microbe* **8**, 358–368.
- 171 Lotz M, Gütle D, Walther S, Ménard S, Bogdan C & Hornef MW (2006) Postnatal acquisition of endotoxin tolerance in intestinal epithelial cells. *J Exp Med* **203**, 973–984.
- 172 Nanthakumar N, Meng D, Goldstein AM, Zhu W, Lu L, Uauy R, Llanos A, Claud EC & Walker WA (2011) The mechanism of excessive intestinal inflammation in necrotizing enterocolitis: an immature innate immune response. *PLoS One* **6**, e17776.
- 173 Fulde M, Sommer F, Chassaing B, van Vorst K, Dupont A, Hensel M, Basic M, Klopffleisch R, Rosenstiel P, Bleich A *et al.* (2018) Neonatal selection by Toll-like receptor 5 influences long-term gut microbiota composition. *Nature* **560**, 489–493.
- 174 Price AE, Shamardani K, Lugo KA, Deguine J, Roberts AW, Lee BL & Barton GM (2018) A map of toll-like receptor expression in the intestinal epithelium reveals distinct spatial, cell type-specific, and temporal patterns. *Immunity* **49**, 560–575.e6.
- 175 Lindner C, Wahl B, Föhse L, Suerbaum S, Macpherson AJ, Prinz I & Pabst O (2012) Age, microbiota, and T cells shape diverse individual IgA repertoires in the intestine. *J Exp Med* **209**, 365–377.
- 176 Knoop KA, McDonald KG, McCrate S, McDole JR & Newberry RD (2015) Microbial sensing by goblet cells controls immune surveillance of luminal antigens in the colon. *Mucosal Immunol* **8**, 198–210.
- 177 McDole JR, Wheeler LW, McDonald KG, Wang B, Konjufca V, Knoop KA, Newberry RD & Miller MJ (2012) Goblet cells deliver luminal antigen to CD103<sup>+</sup> dendritic cells in the small intestine. *Nature* **483**, 345–349.
- 178 Coombes JL, Siddiqui KRR, Arancibia-Cárcamo CV, Hall J, Sun C-M, Belkaid Y & Powrie F (2007) A functionally specialized population of mucosal CD103<sup>+</sup> DCs induces Foxp3<sup>+</sup> regulatory T cells via a TGF- $\beta$ - and retinoic acid-dependent mechanism. *J Exp Med* **204**, 1757–1764.
- 179 Sun C-M, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR & Belkaid Y (2007) Small intestine lamina propria dendritic cells promote de novo generation of Foxp3<sup>+</sup> T reg cells via retinoic acid. *J Exp Med* **204**, 1775–1785.
- 180 Kulkarni DH, Gustafsson JK, Knoop KA, McDonald KG, Bidani SS, Davis JE, Floyd AN, Hogan SP, Hsieh C-S & Newberry RD (2020) Goblet cell associated antigen passages support the induction and maintenance of oral tolerance. *Mucosal Immunol* **13**, 271–282.
- 181 Knoop KA, McDonald KG, Hsieh C-S, Tarr PI & Newberry RD (2021) Regulatory T cells developing peri-weaning are continually required to restrain Th2 systemic responses later in life. *Front Immunol* **11**, 3750.
- 182 Turfkruyer M, Rekima A, Macchiaverni P, Bourhis LL, Muncan V, van den Brink GR, Tulic MK & Verhasselt V (2016) Oral tolerance is inefficient in neonatal mice due to a physiological vitamin A deficiency. *Mucosal Immunol* **9**, 479–491.
- 183 He Y-M, Li X, Peregó M, Nefedova Y, Kossenkova AV, Jensen EA, Kagan V, Liu Y-F, Fu S-Y, Ye Q-J *et al.* (2018) Transitory presence of myeloid-derived suppressor cells in neonates is critical for control of inflammation. *Nat Med* **24**, 224–231.
- 184 Rudd BD (2020) Neonatal T cells: a reinterpretation. *Annu Rev Immunol* **38**, 229–247.
- 185 Li N, van Unen V, Abdelaal T, Guo N, Kasatskaya SA, Ladell K, McLaren JE, Egorov ES, Izraelson M, de Sousa C *et al.* (2019) Memory CD4<sup>+</sup> T cells are generated in the human fetal intestine. *Nat Immunol* **20**, 301–312.
- 186 Schreurs RRCE, Baumdick ME, Sagebiel AF, Kaufmann M, Mokry M, Klarenbeek PL, Schaltenberg N, Steinert FL, van Rijn JM, Drewniak A *et al.* (2019) Human fetal TNF- $\alpha$ -cytokine-producing CD4<sup>+</sup> effector memory T cells promote intestinal development and mediate inflammation early in life. *Immunity* **50**, 462–476.e8.
- 187 Pratama A, Schnell A, Mathis D & Benoist C (2019) Developmental and cellular age direct conversion of CD4<sup>+</sup> T cells into ROR $\gamma$ t<sup>+</sup> or Helios<sup>+</sup> colon Treg cells. *J Exp Med* **217**, e20190428.
- 188 Li L, Lee H-H, Bell JJ, Gregg RK, Ellis JS, Gessner A & Zaghoulani H (2004) IL-4 utilizes an alternative

- receptor to drive apoptosis of Th1 cells and skews neonatal immunity toward Th2. *Immunity* **20**, 429–440.
- 189 Lee H-H, Hoeman CM, Hardaway JC, Guloglu FB, Ellis JS, Jain R, Divekar R, Tartar DM, Haymaker CL & Zaghoulani H (2008) Delayed maturation of an IL-12-producing dendritic cell subset explains the early Th2 bias in neonatal immunity. *J Exp Med* **205**, 2269–2280.
- 190 Torres D, Köhler A, Delbaue S, Caminschi I, Lahoud MH, Shortman K & Flamand V (2016) IL-12p40/IL-10 producing preCD8 $\alpha$ /Clec9A+ dendritic cells are induced in neonates upon *Listeria monocytogenes* infection. *PLoS Pathog* **12**, e1005561.
- 191 Martinez X, Brandt C, Saddallah F, Tougne C, Barrios C, Wild F, Dougan G, Lambert P-H & Siegrist C-A (1997) DNA immunization circumvents deficient induction of T helper type 1 and cytotoxic T lymphocyte responses in neonates and during early life. *Proc Natl Acad Sci USA* **94**, 8726–8731.
- 192 Köhler A, Delbaue S, Smout J, Torres D & Flamand V (2020) Very early-life exposure to microbiota-induced TNF drives the maturation of neonatal pre-cDC1. *Gut*, **70**, 511–521.
- 193 El Aidy S, van Baarlen P, Derrien M, Lindenbergh-Kortleve DJ, Hooiveld G, Levenez F, Doré J, Dekker J, Samsom JN, Nieuwenhuis EES *et al.* (2012) Temporal and spatial interplay of microbiota and intestinal mucosa drive establishment of immune homeostasis in conventionalized mice. *Mucosal Immunol* **5**, 567–579.
- 194 Olszak T, An D, Zeissig S, Vera MP, Richter J, Franke A, Glickman JN, Siebert R, Baron RM, Kasper DL *et al.* (2012) Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* **336**, 489–493.
- 195 An D, Oh SF, Olszak T, Neves JF, Avci FY, Erturk-Hasdemir D, Lu X, Zeissig S, Blumberg RS & Kasper DL (2014) Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer T cells. *Cell* **156**, 123–133.
- 196 Cahenzli J, Köhler Y, Wyss M, Geuking MB & McCoy KD (2013) Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. *Cell Host Microbe* **14**, 559–570.
- 197 Scharschmidt TC, Vasquez KS, Truong H-A, Gearty SV, Pauli ML, Nosbaum A, Gratz IK, Otto M, Moon JJ, Liese J *et al.* (2015) A wave of regulatory T cells into neonatal skin mediates tolerance to commensal microbes. *Immunity* **43**, 1011–1021.
- 198 Constantinides MG, Link VM, Tamoutounour S, Wong AC, Perez-Chaparro PJ, Han S-J, Chen YE, Li K, Farhat S, Weckel A *et al.* (2019) MAIT cells are imprinted by the microbiota in early life and promote tissue repair. *Science* **366**, eaax6624.
- 199 Pié S, Lallès JP, Blazy F, Laffitte J, Sève B & Oswald IP (2004) Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. *J Nutr* **134**, 641–647.
- 200 Bonnardel J, Da Silva C, Wagner C, Bonifay R, Chasson L, Masse M, Pollet E, Dalod M, Gorvel J-P & Lelouard H (2017) Distribution, location, and transcriptional profile of Peyer's patch conventional DC subsets at steady state and under TLR7 ligand stimulation. *Mucosal Immunol* **10**, 1412–1430.
- 201 Yrlid U, Milling SWF, Miller JL, Cartland S, Jenkins CD & MacPherson GG (2006) Regulation of intestinal dendritic cell migration and activation by plasmacytoid dendritic cells, TNF- $\alpha$  and Type 1 IFNs after feeding a TLR7/8 ligand. *J Immunol* **176**, 5205–5212.
- 202 Vatanen T, Kostic AD, d'Hennezel E, Siljander H, Franzosa EA, Yassour M, Kolde R, Vlamakis H, Arthur TD, Hämäläinen A-M *et al.* (2016) Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans. *Cell* **165**, 842–853.
- 203 Bouskra D, Brézillon C, Bérard M, Werts C, Varona R, Boneca IG & Eberl G (2008) Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* **456**, 507–510.
- 204 Rios D, Wood MB, Li J, Chassaing B, Gewirtz AT & Williams IR (2016) Antigen sampling by intestinal M cells is the principal pathway initiating mucosal IgA production to commensal enteric bacteria. *Mucosal Immunol* **9**, 907–916.
- 205 Bonnardel J, Da Silva C, Henri S, Tamoutounour S, Chasson L, Montañana-Sanchis F, Gorvel J-P & Lelouard H (2015) Innate and adaptive immune functions of Peyer's patch monocyte-derived cells. *Cell Rep* **11**, 770–784.
- 206 Lelouard H, Henri S, De Bovis B, Mugnier B, Chollat-Namy A, Malissen B, Méresse S & Gorvel J (2010) Pathogenic bacteria and dead cells are internalized by a unique subset of Peyer's patch dendritic cells that express lysozyme. *Gastroenterology* **138**, 173–184.e3.
- 207 Lindner C, Thomsen I, Wahl B, Ugur M, Sethi MK, Friedrichsen M, Smoczek A, Ott S, Baumann U, Suerbaum S *et al.* (2015) Diversification of memory B cells drives the continuous adaptation of secretory antibodies to gut microbiota. *Nat Immunol* **16**, 880–888.
- 208 Lopez MC & Roux ME (1989) Impaired differentiation of Ig A-B cell precursors in the Peyer's patches of protein depleted rats. *Dev Comp Immunol* **13**, 253–262.
- 209 Bain CC, Bravo-Blas A, Scott CL, Perdiguero EG, Geissmann F, Henri S, Malissen B, Osborne LC, Artis D & Mowat AM (2014) Constant replenishment from circulating monocytes maintains the macrophage pool

- in the intestine of adult mice. *Nat Immunol* **15**, 929–937.
- 210 Shaw TN, Houston SA, Wemyss K, Bridgeman HM, Barbera TA, Zangerle-Murray T, Strangward P, Ridley AJL, Wang P, Tamoutounour S *et al.* (2018) Tissue-resident macrophages in the intestine are long lived and defined by Tim-4 and CD4 expression. *J Exp Med* **215**, 1507–1518.
- 211 De Schepper S, Verheijden S, Aguilera-Lizarraga J, Viola MF, Boesmans W, Stakenborg N, Voytyuk I, Schmidt I, Boeckx B, Dierckx de Casterlé I *et al.* (2018) Self-maintaining gut macrophages are essential for intestinal homeostasis. *Cell* **175**, 400–415.e13.
- 212 Shouval DS, Biswas A, Goettel JA, McCann K, Conaway E, Redhu NS, Mascanfroni ID, Al Adham Z, Lavoie S, Ibourk M *et al.* (2014) Interleukin-10 receptor signaling in innate immune cells regulates mucosal immune tolerance and anti-inflammatory macrophage function. *Immunity* **40**, 706–719.
- 213 Zigmond E, Bernshtein B, Friedlander G, Walker CR, Yona S, Kim K-W, Brenner O, Krauthgamer R, Varol C, Müller W *et al.* (2014) Macrophage-restricted interleukin-10 receptor deficiency, but not IL-10 deficiency, causes severe spontaneous colitis. *Immunity* **40**, 720–733.
- 214 Bernshtein B, Curato C, Ioannou M, Thaiss CA, Gross-Vered M, Kolesnikov M, Wang Q, David E, Chappell-Maor L, Harmelin A *et al.* (2019) IL-23-producing IL-10R $\alpha$ -deficient gut macrophages elicit an IL-22-driven proinflammatory epithelial cell response. *Sci Immunol* **4**, eaau6571.
- 215 Ochi T, Feng Y, Kitamoto S, Nagao-Kitamoto H, Kuffa P, Atarashi K, Honda K, Teitelbaum DH & Kamada N (2016) Diet-dependent, microbiota-independent regulation of IL-10-producing lamina propria macrophages in the small intestine. *Sci Rep* **6**, 27634.
- 216 Kim KS, Hong S-W, Han D, Yi J, Jung J, Yang B-G, Lee JY, Lee M & Surh CD (2016) Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. *Science* **351**, 858–863.
- 217 Herzenberg LA & Herzenberg LA (1989) Toward a layered immune system. *Cell* **59**, 953–954.
- 218 Zens KD, Chen JK, Guyer RS, Wu FL, Cvetkovski F, Miron M & Farber DL (2017) Reduced generation of lung tissue-resident memory T cells during infancy. *J Exp Med* **214**, 2915–2932.
- 219 Davis CP & Savage DC (1974) Habitat, succession, attachment, and morphology of segmented, filamentous microbes indigenous to the murine gastrointestinal tract. *Infect Immun* **10**, 948–956.
- 220 Jepson MA, Clark MA, Simmons NL & Hirst BH (1993) Actin accumulation at sites of attachment of indigenous apathogenic segmented filamentous bacteria to mouse ileal epithelial cells. *Infect Immun* **61**, 4001–4004.
- 221 Chen B, Chen H, Shu X, Yin Y, Li J, Qin J, Chen L, Peng K, Xu F, Gu W *et al.* (2018) Presence of segmented filamentous bacteria in human children and its potential role in the modulation of human gut immunity. *Front Microbiol* **9**, 1403.
- 222 Chen H, Wang L, Wang X, Wang X, Liu H & Yin Y (2020) Distribution and Strain Diversity of Immunoregulating Segmented Filamentous Bacteria in Human Intestinal Lavage Samples. *Microb Ecol* **79**, 1021–1033.
- 223 Gaboriau-Routhiau V, Rakotobe S, Lécuyer E, Mulder I, Lan A, Bridonneau C, Rochet V, Pisi A, De Paepe M, Brandt G *et al.* (2009) The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* **31**, 677–689.
- 224 Lécuyer E, Rakotobe S, Lengliné-Garnier H, Lebreton C, Picard M, Juste C, Fritzen R, Eberl G, McCoy KD, Macpherson AJ *et al.* (2014) Segmented filamentous bacterium uses secondary and tertiary lymphoid tissues to induce gut IgA and specific T helper 17 cell responses. *Immunity* **40**, 608–620.
- 225 Talham GL, Jiang H-Q, Bos NA & Cebra JJ (1999) Segmented filamentous bacteria are potent stimuli of a physiologically normal state of the murine gut mucosal immune system. *Infect Immun* **67**, 1992–2000.
- 226 Cho H, Jaime H, de Oliveira RP, Kang B, Spolski R, Vaziri T, Myers TG, Thovarai V, Shen Z, Fox JG *et al.* (2019) Defective IgA response to atypical intestinal commensals in IL-21 receptor deficiency reshapes immune cell homeostasis and mucosal immunity. *Mucosal Immunol* **12**, 85–96.
- 227 Fung TC, Bessman NJ, Hepworth MR, Kumar N, Shibata N, Kobuley D, Wang K, Ziegler CGK, Goc J, Shima T *et al.* (2016) Lymphoid-tissue-resident commensal bacteria promote members of the IL-10 cytokine family to establish mutualism. *Immunity* **44**, 634–646.
- 228 Obata T, Goto Y, Kunisawa J, Sato S, Sakamoto M, Setoyama H, Matsuki T, Nonaka K, Shibata N, Gohda M *et al.* (2010) Indigenous opportunistic bacteria inhabit mammalian gut-associated lymphoid tissues and share a mucosal antibody-mediated symbiosis. *Proc Natl Acad Sci USA* **107**, 7419–7424.
- 229 Million M, Tomas J, Wagner C, Lelouard H, Raoult D & Gorvel J-P (2018) New insights in gut microbiota and mucosal immunity of the small intestine. *Hum Microbiome J* **7–8**, 23–32.