

Series: Lifetime Immunity

Review

Impact of Early-Life Exposures on Immune Maturation and Susceptibility to Disease

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Exiting from the largely sterile environment of the womb, the neonatal immune system is not fully mature, and thus neonatal immune cells must simultaneously mount responses against environmental stimuli while maturing. This dynamic process of immune maturation is driven by a variety of cell-intrinsic and extrinsic factors. Recent studies have focused on some of these factors and have shed light on the mechanisms by which they drive immune maturation. We review the interactions and consequences of immune maturation during the pre- and perinatal period. We discuss environmental signals in early life that are needed for healthy immune homeostasis, and highlight detrimental factors that can set an individual on a path towards disease. This early-life period of immune maturation could hold the key to strategies for setting individuals on trajectories towards health and reduced disease susceptibility.

Introduction

Chronic inflammatory disorders, including inflammatory bowel disease (IBD), type 1 diabetes, asthma, and allergy are fundamentally linked with immune dysfunction, and therefore strategies that can shape appropriate immune development have the potential to impact upon disease burden. Epidemiologically it is well established that the pre- and perinatal environment can have profound effects on the development of chronic inflammatory and metabolic diseases [1–3]; however, mechanistic insight into how the early-life environment can impact on the development of the immune system is still emerging. In recent years, early-life events and exposures, particularly mode of delivery [4], diet [5,6], and lifestyle [7–9], have been shown to influence immune cell phenotypes and maturation. One common denominator influenced by all of those factors is the microbiota [10–12], and early-life signals for appropriate immune function have been directly linked with the formation of the microbiota [13–17].

We provide an overview of the leading early-life environmental factors that can shape immune maturation during this key period. We focus on findings examining the impact of pre- and perinatal exposures, and discuss the current understanding of how they shape immune function and the role of the microbiota in this process.

Pre- and Perinatal Immune Development and Function

Fetal Immune Programming

Innate and adaptive immune cells are present in the fetus early during gestation. Most of the immune cell types appear during the first trimester and then expand significantly until birth [6,18]. The first innate cells are monocytes/macrophages and can be found in very low numbers as early as gestational week (GW) 4 in humans [6]. They are followed by granulocytes (especially

Trends

A convergence in the timing of environmental stimuli and maturation of the immune system influences disease susceptibility. Factors that might be innocuous at one stage of development could be detrimental or beneficial at other stages.

The majority of previously described environmental signals that influence disease development are linked to changes in the microbiota. Microbes provide crucial signals for immune maturation.

Early life interventions, particularly by shaping the microbiota, could be the key to disease prevention in the future.

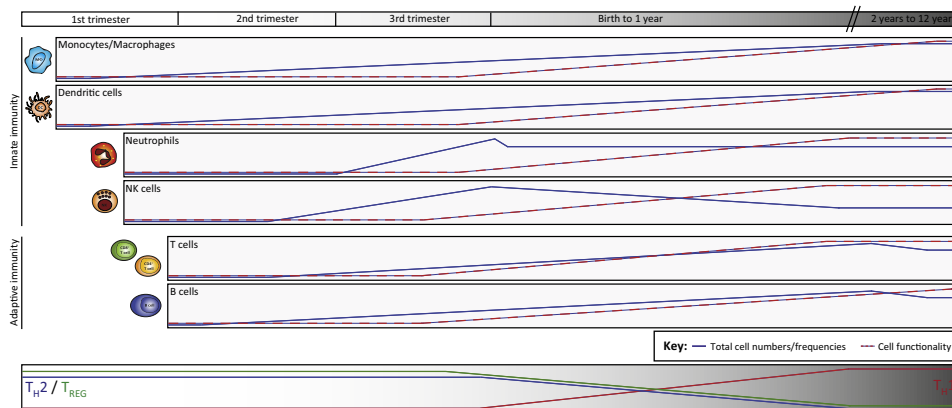
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neutrophils) and natural killer (NK) cells which start to appear by GW8 and then undergo a massive expansion to reach their peak levels around birth [18–21]. B and T cell precursors can also be found from GW8 onwards. Naïve T cells can be detected in low numbers in GW12; however, the fetal T cell pool is predominated by $\gamma\delta$ T cells until approximately GW32, when $\alpha\beta$ T cells become the majority [6]. Immature B cells also appear at GW12, followed by the first mature fetal B1 cells by GW14 [22]. Although innate and adaptive immune cells are already present early during fetal development, their effector functions are considered to be poorly developed during the entire fetal period [6, 18, 23]. One influence is the nature of the antigens the fetus is exposed to during a healthy pregnancy – namely self and maternal antigens. Both types of antigens need to be tolerated [24] to ensure the viability of the fetus during pregnancy, and postnatally to avoid autoimmunity. Hence, the fetal immune system by nature is tolerogenic so as to avoid deleterious inflammatory responses. In line with this, it has been reported that fetal CD4⁺ T cells have a tendency to differentiate into regulatory T (T_{REG}) cells upon stimulation, aiding peripheral tolerance of fetal self-antigens and non-self maternal antigens during development [25]. Furthermore, fetal proinflammatory pathways, such as the induction of type 1 T helper cell (T_H1) immune responses, are thought to be less responsive and weaker in magnitude compared to the same pathways in adults [18] (Figure 1). Whether the tolerogenic bias of fetal T cells is simply due to a lack of strong antigen stimulation, poor costimulation and low or absent microbial stimuli, or at least in part also due to intrinsic cellular immaturity is not entirely clear. It is however important to note that there are very limited data available concerning fetal, as opposed to neonatal, immune maturation profiles. Moreover, it has yet to be studied in great detail how *in utero* exposures influence discrete immune cell activation/maturation pathways, as is starting to be revealed in neonatal studies (discussed below).

Immune Function in the Newborn

The neonatal immune system initially resembles that of the fetus and has therefore been described as being immature and not able to mount strong immune responses [26, 27]. Recent reports have revealed the situation is not as clear-cut as originally thought. In fact, strong stimuli, such as that provided by the Bacillus Calmette–Guérin (BCG) vaccine, can induce efficient T_H1 responses that are comparable to those of adult cells [28, 29]. It has also been shown that, during the first postnatal days, DCs in neonatal mouse lungs exhibit a high expression of a variety of cell



Trends in Immunology

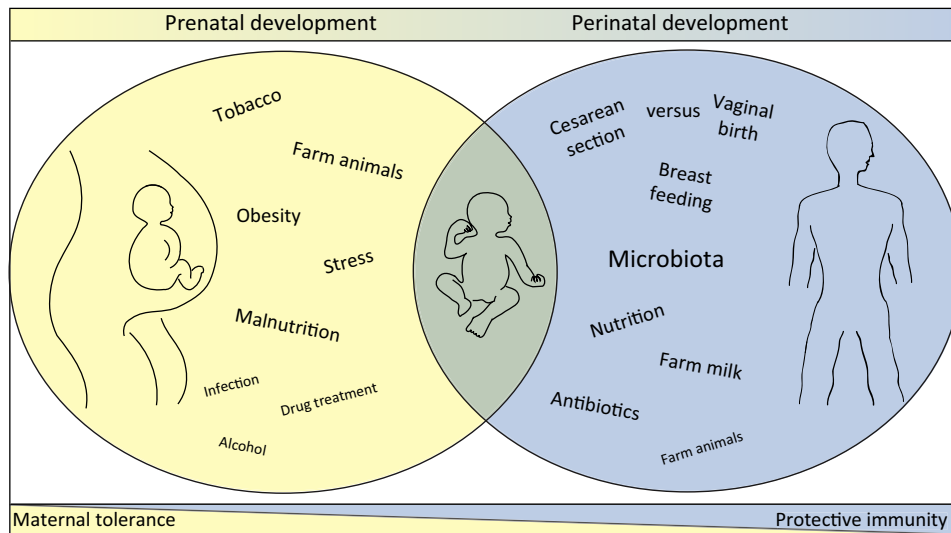
Figure 1. Pre- and Perinatal Development of Immune Cells. Dynamics of innate [monocytes/macrophages, dendritic cells, neutrophils, and natural killer (NK) cells] and adaptive (T and B cells) immune cells during cell maturation from early gestation until adolescence. Shown are changes in total cell numbers/frequencies (blue line) over time and the increase in cell functionality (red broken line). Adult-like cell numbers and functionality for all cell types depicted is reached by the end of the time-line. The switch from anti-inflammatory type 2 T helper cell (T_H2) immunity (green/blue) to T_H1 immunity (red) is illustrated in the lower panel. Full potential to mount T_H1-type immune responses is reached at around 1 year.

activation markers, including ICOSL (inducible T cell co-stimulator ligand) and PD-L1 (programmed death ligand 1), and are very effective at taking up and processing antigens [17]. Studies such as these show that neonatal immune cells cannot globally be referred to as immature. However, some broad conclusions can still be drawn concerning differences between neonatal and adult immune cells, for example, B cell functionality. Mature B cells and the lymphoid structures needed for their interaction with T helper (T_H) cells are largely absent in neonates, significantly impairing antibody production and efficient and long-lasting responses to a variety of vaccines [27,30]. It is noteworthy that a recent study in mice has shown that the propensity towards immunoglobulin (Ig)E production is determined by early-life microbial colonization, with a lack of microbial diversity in early life being linked with hyper-IgE production by B cells in adulthood [13]. Thus, the adaptive immune system, although partly lacking in early life, can be imprinted by early-life exposures. T cells are present in lower numbers than in adults, not reaching adult levels until around 6 years of age in humans [31]. The higher capacity of neonatal $CD4^+$ T cells to differentiate into T_{REG} cells [32] and their bias towards T_H2 immunity [33] are likely remnants of *in utero* mechanisms to avoid inflammatory responses. However, this can leave the neonate susceptible to bacterial or viral infections after birth because neonatal T cell function is linked to a diminished production of the prototypic T_H1 -type cytokine, interferon (IFN)- γ [34] which is required to efficiently fight pathogens. The neonatal T_H2 bias is not solely a result of T cell immaturity but is also due to diminished interleukin (IL)-12 production and the nature of co-stimulation by neonatal antigen-presenting cells (APC) [35,36], especially dendritic cells (DC), indicating that also innate immunity is still altered in the neonate. As already touched upon above, mouse studies have revealed that co-stimulation is not globally impaired in neonatal DCs, as indicated by high surface expression of specific surface markers (e.g., ICOSL and PD-L1) [17]. However, the majority of these markers are linked to T_H2 immunity and tolerance pathways, underlining the point that T_H1 responses are under-represented in the newborn. This is also in line with higher production of the regulatory cytokine, IL-10, in neonatal DCs [37]. Thus the neonatal environment tends to perpetuate an anti-inflammatory environment. Comparatively shortly after birth there is a burst of granulopoiesis, with high levels of circulating neutrophils [18], which is likely to be an innate mechanism that evolved to give immediate protection to the large microbial burden that neonates are suddenly exposed to. However, these neutrophils still show reduced chemotaxis, impaired extracellular net formation, and less phagocytic capacity than in adults [38–40], pointing towards an immature phenotype. NK cells are also present in high numbers at birth, representing another innate cell type involved in infection control and pathogen clearance. Nevertheless, cytotoxic activity of neonatal NK cells is lower than in adults [21], which has been attributed to the cytokine milieu in the newborn (Figure 1).

Overall, fetal and neonatal immune function clearly differs from that of adults. Although it is possible to elicit inflammatory responses and T_H1 immunity [28], potent triggers are needed to overcome the high activation threshold for these responses. The bias towards regulatory and T_H2 responses, that are indispensable for the fetus, is still present in the neonate during the early postnatal period, and gradually changes as external environmental factors provide instructions.

Maternal Factors Influencing Prenatal Immune Development

The environment inside the womb has generally been considered sterile, although recent data suggests that the placenta harbors its own microbiota [41]. As new molecular techniques are developed and used to detect low bacterial biomass samples, in the near future it should be possible to draw firmer conclusions concerning microbial exposures in the womb. Certainly, however, there is constant crosstalk between the mother and fetus and, as such, fetal development with respect to organogenesis and the immune system is influenced by maternal exposures. Some of these exposures have been associated with the development of chronic diseases, including type 1 diabetes [42,43], obesity [44,45], asthma [46], and allergy [47]. What



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Figure 2. Environmental Factors Influencing Pre- and Perinatal Immune Development. *In utero* and early-life environmental exposures that can directly or indirectly shape the immune cell phenotypes and functionality during early-life immune development. Font size indicates the importance during immune maturation attributed to the different types of exposure.

these factors are (Figure 2) and how they can shape the fetal and neonatal immune system and set it on a trajectory towards chronic disease will be discussed in the following section.

Maternal Diet

Maternal malnutrition can have profound effects on fetal growth, cause preterm birth, and also severely compromise the maturation of the fetal immune system [48,49]. Maternal undernutrition leads to deficiencies in a variety of micronutrients, including minerals and vitamins, also in the fetus. Deficiency in minerals such as zinc results in reduced thymus and spleen size, impaired T and B cell activity, and decreased IgG levels in the fetus [6,50]. Vitamins A and D are the strongest immunomodulators of all the vitamins, and deficiencies during pregnancy have been associated with altered immune development in the fetus. Specifically, vitamin A is important for the formation of secondary lymphoid organs by inducing the differentiation of lymphoid-tissue inducer (LTI) cells [51]. Vitamin D is a potent enhancer of the suppressive capacity of T_{REG} cells and can dampen T_H1 immunity [52]. Thus, vitamin D might be involved in maternal–fetal tolerance. Maternal overnutrition and obesity also takes its toll on the fetal immune system and has been associated with several inflammatory and metabolic diseases, including obesity [53] and asthma [54], in the offspring. Mouse models of obesity have shown that pups born to obese mothers have decreased lymphocyte counts and reduced antigen-specific antibody production [55], thus indicating that maternal obesity has detrimental effects on fetal immune maturation (Table 1).

Maternal Stress

Prenatal maternal stress has been shown to have long-lasting effects on immunity of the offspring and has been linked to the development of chronic disorders such as asthma and allergy [46]. Animal models have demonstrated that maternal stress leads to a general immunosuppression in the offspring by modifying lymphocyte effector proliferation and function and by reducing NK cell cytotoxicity [56]. A recent human study has also reported poorly developed adaptive immune responses in infants born to mothers who suffered from anxiety during pregnancy [57]. These infants showed decreased humoral immune responses and a reduced

Table 1. Impact of Maternal Factors on Immune Cell Development in the Fetus

Type of effect	Outcome	Species	Refs
Maternal malnutrition			
Developmental effects	Micronutrient deficiencies in fetus	Human	[48,49]
	Growth retardation	Human	[48,49]
	Preterm birth	Human	[48,49]
	Reduced thymus and spleen size (zinc)	Mouse	[6,50]
Effects on immune cells	Impaired T and B cell activity (zinc)	Mouse	[6,50]
	Decreased IgG levels (zinc)	Mouse	[6,50]
	Impaired secondary lymphoid organ formation (vitamin A)	Mouse	[51]
	Impaired differentiation of LTI cells (vitamin A)	Mouse	[51]
	Reduced suppressive capacity of T _{REG} cells (vitamin D)	Human/mouse	[52]
	Increased T _{H1} -type responses (vitamin D)	Human/mouse	[52]
Maternal obesity			
Effects on immune cells	Decreased lymphocyte counts	Mouse	[55]
	Reduced antigen-specific antibody production	Mouse	[55]
Maternal stress			
Effects on immune cells	Modified lymphocyte effector proliferation and function	Rat	[56]
	Reduced NK cell cytotoxicity	Rat	[56]
	Decreased humoral immunity	Human	[57]
	Decreased capacity to mount T _{H1} -type responses	Human	[57]
Maternal smoking			
Developmental effects	Delayed lung development	Human	[59]
	Decreased pulmonary function	Human	[59]
Effects on immune cells	Decreased T _{REG} cell numbers	Human	[61]
	Altered response towards several TLR ligands	Human	[62]
	Reduced TNF α and IL-6 production by APCs	Human	[62]
Maternal farm exposure			
Effects on immune cells	Increased T _{REG} cell numbers	Human	[8]
	Increased suppressive capacity of T _{REG} cells	Human	[8]
	Decreased T _{H2} -type immune responses	Human	[8]

capacity to elicit T_{H1}-type immune responses. The differences in neonatal immunity could be caused by the transfer of stress hormones (e.g., glucocorticoids) via the placenta. These hormones are known to have immunomodulatory functions [58] and thus could alter the fetal immune maturation process (Table 1).

Maternal Smoking

Maternal smoking can influence lung development in the fetus, leading to decreased pulmonary function and a higher risk for chronic lung diseases [59]. However, fetal tobacco exposure does not only impact on lung structure and function but is also associated with the development of other chronic inflammatory diseases, including congenital heart disease [60] and type 1 diabetes [43]. This in part might be due to the reported reduction in T_{REG} cell populations [61]. It has also been shown that maternal smoking inhibits innate immunity by altering responsiveness to Toll-like receptor (TLR) ligands [62], leading to reduced tumor necrosis factor (TNF)- α and/or IL-6 production by APCs [62] (Table 1).

Maternal Farm Exposures

It has been demonstrated that maternal farm exposures can modulate the immune system of the offspring and protect against the development of asthma and allergy [47]. Cord blood (CB) cells obtained from babies born to farming mothers exhibit higher T_{REG} cell numbers with increased

suppressive capacity as well as decreased T_H2-type immune responses [8]. The amplitude of the immunological changes is directly correlated with the amount of farm animals, particularly cows, to which the mothers were exposed during pregnancy [8] (Table 1).

Environmental Factors Promoting Postnatal Immune Maturation

During and immediately after birth the neonate is exposed to a variety of environmental antigens of both non-pathogenic and pathogenic nature. The immune system of the neonate is challenged to distinguish between ‘the good and the bad’ to avoid overshooting immune responses at the same time as fighting its enemies. Finding the right balance between immune activation and tolerance requires the neonatal immune system to be educated. This immune maturation process is partially driven by cell-intrinsic genetically imprinted events; however, it is also influenced by the environment [63] (Figure 2). Specific environmental factors can skew the development of the neonatal immune system towards a health-promoting balanced phenotype; however, others can also set an individual on a life-long trajectory towards disease susceptibility.

Mode of Delivery and the Importance of Labor

During birth, the future newborn will leave its environment inside the womb and immediately encounter a variety of unknown antigens. The nature of these antigens is partly dependent on the mode of delivery. Vaginally delivered babies pass through the birth canal, where they will be coated with the vaginal microbiota of their mother, whereas those born by Cesarean section (CS) will first encounter maternal as well as non-maternal skin microbes [10]. This influences the early colonization, especially of the intestine [64], which has profound effects on the immune maturation profile of the neonate. More detailed information on how early colonization with a microbiota will influence immune development in the neonate will be discussed in the following section. Another major difference between vaginal birth and CS, especially concerning elective CS without labor, is the level and type of stress the baby encounters during the process [4]. The contractions of the uterus during labor and the hypoxia the fetus is exposed to during its passage through the birth canal lead to the release of stress hormones including catecholamine, dopamine, and cortisol [4]. This has profound effects on the immune phenotype of the newborn. Although the currently available data indicate that adaptive immunity is not grossly affected by labor and the stress response elicited throughout, innate immune cell phenotype and function is clearly influenced. Labor specifically alters neutrophil, monocyte, and NK cell numbers and function [4]. Neutrophil chemotaxis is increased, neutrophil apoptosis is delayed, and responsiveness to lipopolysaccharides (LPS) is elevated [65–67]. This augments their microbicidal capacity and could help to protect the newborn against immediate infection by bacteria. Elevated monocyte counts and expression levels of TLR2 and 4 on these cells after vaginal delivery might further contribute to this protection [68]. NK cell numbers are also increased after labor [69], potentially providing another first-line defense mechanism against neonatal infections. Moreover, inflammatory cytokine production is also altered by the mode of delivery. Elective CS is associated with reduced production of TNF α , IL-12, and IFN- γ by cord blood cells [69,70]. Thus, vaginal delivery and labor seem to have evolved together with immune functionality to prepare the neonate against the immediate risk of infection (Table 2).

Beneficial Effects of Breast-Feeding

Human breast milk contains a variety of beneficial factors that can not only provide immediate protection against infection but also influence immune maturation in the newborn. Breast milk is a rich source of antimicrobial substances, including immunoglobulins, complement proteins, lysozyme, and lactoferrins [71]. These factors are important in the protection against a wide range of pathogens and can support the immature neonatal immune system in the fight against early-life infections. However, human milk constituents can also directly promote the maturation of the neonatal immune system. Breast milk is a means to directly transfer immune cells, including macrophages, neutrophils, and lymphocytes, from the mother to the infant [71]. These

Table 2. Impact of Environmental Factors on Immune Cell Development in the Newborn

Type of effect	Outcome	Species	Timing	Refs
Mode of delivery – vaginal birth				
Effects on immune cells	<ul style="list-style-type: none"> Increased neutrophil chemotaxis and LPS responsiveness Delayed neutrophil apoptosis Increased monocyte numbers and expression of TLR2 and 4 Increased NK cell numbers Increased production of IL-12, TNFα, and IFNγ 	Human	At birth (CB)	[65,67]
		Human	At birth (CB)	[66]
		Human	At birth (CB)	[68]
		Human	1 month	[69]
		Human	1 and 4 days/1 month	[69,70]
Microbiota changes	<ul style="list-style-type: none"> Increased bacterial diversity Increased colonization with <i>Bifidobacteria</i> and <i>Bacteroides</i> 	Human	3 days	[108]
		Human	3 days	[108]
Infant feeding – breast milk				
General effects	<ul style="list-style-type: none"> IL-10 and TGFβ from breast milk induces tolerance towards food and commensal antigens HMOs prevent adhesion of pathogens to the intestinal epithelium 	Human/mouse	–	[74–76]
		Human (<i>in vitro</i>)	–	[83]
Effects on immune cells	<ul style="list-style-type: none"> Altered B and T cell phenotype (due to maternal immune cells) Dampened inflammatory responses (due to lactoferrin/LC-PUFA) Increased production of IFNγ (due to HMO) Dampened T_H2-type responses (due to HMO) Inhibited platelet-neutrophil complex formation (due to HMO) Increased NK cell numbers Decreased CD4:CD8 T cell ratio 	Human	–	[71]
		Human/animal models	Adult	[78,79]
		Human (<i>in vitro</i>)	At birth (CB)	[81]
		Human (<i>in vitro</i>)	At birth (CB)	[81]
		Human (<i>in vitro</i>)	Adult	[82]
		Human	6 months	[88]
Human	6 months	[88]		
Microbiota changes	<ul style="list-style-type: none"> Increased frequencies of <i>Bifidobacteria</i> and <i>Lactobacilli</i> 	Human	6 months	[84]
Farm environment and environmental microbes				
Effects on immune cells	<ul style="list-style-type: none"> Increased mRNA expression of TLR4, TLR5, TLR6, and TLR7 	Human	12 months	[9]
Microbiota changes	<ul style="list-style-type: none"> Lower bacterial diversity Altered bacterial community structure 	Human	5–13 years	[110]
		Human	5–13 years	[110]
Early-life antibiotic treatment				
Effects on immune cells	<ul style="list-style-type: none"> Potential effects on immune function by macrolide antibiotics: Decreased proinflammatory cytokine production 	Human (<i>in vitro</i>)	Adult	[105]

Table 2. (continued)

Type of effect	Outcome	Species	Timing	Refs
	<ul style="list-style-type: none"> • Impaired neutrophil recruitment and activation • Altered DC function 	Human (<i>in vitro</i>)	Adult	[106]
		Human (<i>in vitro</i>)	Adult	[107]
Microbiota changes	<ul style="list-style-type: none"> • Decreased bacterial burden • Decreased bacterial diversity • Altered bacterial community structure (dependent on type of antibiotic used) 	Human	1 and 2 months	[103]
		Human	1 and 2 months	[103]
		Human	1 and 2 months	[103]

cells can not only directly elicit immune responses but have also been proposed to influence the phenotype of neonatal immune cells, especially B and T cells [71]. In addition to live immune cells, a variety of immunologically-active substances are also transferred via breast milk. These compounds include hormones (e.g., estrogen and progesterone), growth factors (e.g., epidermal growth factor and insulin-like growth factor), and a plethora of cytokines [71]. The most prominent cytokine present in human milk is IL-10, which is primarily produced by mammary epithelial cells [72]. IL-10 is an anti-inflammatory cytokine which can dampen immune responses and lead to tolerance [73]. It has been suggested that IL-10 is involved in the induction of tolerance towards dietary [74,75] and microbial antigens. This tolerance induction is further supported by the presence of transforming growth factor (TGF)- β [76]. Although breast milk is also a source of several proinflammatory cytokines, including IL-1 β , IL-6, IL-8, and TNF α [71,77], the amount of IL-10 and TGF β is significantly higher, indicating that the ingestion of breast milk may facilitate a tolerogenic environment in the newborn. This notion is further supported by the presence of lactoferrins, short-chain fatty acids (SCFA), and long-chain polyunsaturated fatty acids, which are known to dampen inflammatory responses [78–80]. Another constituent of breast milk, oligosaccharides, has also been reported to have immunomodulatory functions. It has been shown that human milk oligosaccharides (HMO) can promote the production of IFN- γ in cord-blood T cells and dampen T_H2-type responses [81]. By contrast, they can act as anti-inflammatory molecules by inhibiting the formation of platelet–neutrophil complexes, a highly activated form of neutrophil that is primed for adhesion, phagocytosis, and immediate production of reactive oxygen species (ROS) [82]. An indirect effect on immune cell development has also been attributed to HMOs. It has been shown that HMOs can prevent the adhesion of pathogens to the intestinal epithelium by blocking the interaction partners of a variety of pathogenic bacteria [83]. HMOs are also known to have prebiotic functions, promoting the growth of intestinal *Bifidobacteria* in the breast-fed infant [84]. They can be fermented into SCFA inside the colon of the neonate. These molecules are known immunomodulators [85] which can elicit their functions either directly by acting as histone deacetylase (HDAC) inhibitors or by activating specific G protein-coupled receptors (GPR). The main GPRs activated by SCFA are GPR41 and 43, and it has been shown in mice that this pathway can decrease disease severity in models of allergic asthma and colitis [86,87]. Furthermore, it has also been demonstrated that breast-feeding in general increases the number of NK cells and decreases the CD4:CD8 T cell ratio [88]; however, mechanistic insight into how breast milk is able to induce these changes is still lacking. Moreover, human breast milk has its own microbiota [89], which is also transferred to the infant and is likely to be involved in immune maturation either directly or indirectly by shaping the constituents of the intestinal microbiota (Table 2).

Introduction of Solid Foods and the Diet of the Infant

Only a very limited number of studies provide insight into how the timing of the introduction of solid foods, and the type of solid food ingested, during early infancy can alter immune function in

the neonate [90]. Whether the kind of diet introduced after weaning can directly impact on immune function in later life is still unclear, although epidemiological data show that early introduction of solid food is associated with protection against allergies [91]. In this context, a recent study has demonstrated that early introduction of peanuts (between 4 and 11 month of age) significantly reduces the incidence of peanut allergy at 2.5 years of life. This was also true for infants with preexisting peanut sensitivity, and was associated with an increase in peanut-specific IgG4 and reduced levels of peanut-specific IgE antibodies [92]. Moreover, it is well established that the introduction of solid foods drastically changes the composition of the intestinal microbiota [93,94], which in turn is known to influence immune function (Table 2).

Farm Environment and Environmental Microbes

Numerous epidemiological studies from different centers have shown the beneficial effects of a farm environment and exposure to environmental microbes on the immune development of the neonate and decreased susceptibility to asthma, allergy [95–97], and early-life respiratory infections [98]. Mechanisms by which this is achieved are largely unknown; however, one mechanism is likely to be direct microbial exposure [95]. A recent publication has shown that low-dose endotoxin or farm dust exposure can protect from allergic airway inflammation in mice. This was due to the induction of the A20 protein (TNF- α -induced protein 3/TNFAIP3) in airway epithelial cells [99], which is a downstream molecule of TLR signaling. Thus, constant exposure to environmental microbes during the neonatal period could lead to protection against allergies and asthma by similar mechanisms. Given that, similarly to human breast milk, raw cow milk contains a range of complex short- and long-chain oligosaccharides and lipids, a further mechanism of farm environment-mediated disease protection could be the direct immunomodulatory effects of these substances, their action of shaping the microbiota, or metabolites from their fermentation [86]. It has also been shown that the consumption of farm milk during the first year of life significantly increases mRNA expression of several TLRs, including TLR4, TLR5, TLR6, and TLR7, on immune cells [9] (Table 2).

Immunomodulation Caused by Early-Life Antibiotic Treatment

Despite increasing evidence that early-life antibiotic exposure is associated with the development of chronic disease later in life [100,101], antibiotics are still widely used to treat neonatal infections of unknown origin. It is still unclear whether antibiotics can interfere in the immune maturation process by directly modulating immune cell phenotypes; however, it is well established that they can alter the composition of the microbiota of the infant over the long-term [102,103], which in turn can impact on immune cell frequencies and function. It has been shown that some subclasses of antibiotics – namely macrolides – not only have antimicrobial properties but are also potent immunomodulators [104]. Although not studied in the context of neonatal immune development, these macrolide antibiotics have been demonstrated to provide anti-inflammatory signals by dampening proinflammatory cytokine production [105], impairing neutrophil recruitment and activation [106], and altering DC function [107]. Thus, specific subclasses of antibiotics can have immunomodulatory effects, which, when given repeatedly or over a long period of time, are likely to directly influence immune maturation in the newborn (Table 2).

The Microbiota as Master Regulator of Immune Function

Although all environmental factors discussed above can induce unique immune profiles in the growing infant [10,11,93,102,103], there is one common feature to all of them – they influence the composition of our microbiota. Vaginal birth favors colonization with *Bifidobacteria* and *Bacteroides* and creates a more diverse microbiota than those found in children after elective CS [108]. Breast milk is not sterile and is therefore a direct source of bacteria to colonize the intestine of the infant. The most common bacteria found in human milk are *Bifidobacteria* and *Lactobacilli* [109], and are those typically found at higher frequencies in breast-fed infants [84]. When the infant switches to solid food, the microbial composition of the intestine is also significantly altered

[93]. Depending on the type of food ingested by the infant, microbial profiles will be influenced in distinct manners. Exposure to a farm environment early in life alters both the diversity as well as composition of the intestinal microbiota [110]. The same is true for intensive antibiotic use during the neonatal period, which significantly decreases bacterial burden and diversity and can lead to long-lasting changes in bacterial community structures [103]. Hence, all early-life exposures that have been linked to the development of either appropriate immune function during adulthood or the development of chronic diseases, including IBD, type 1 diabetes, asthma, and allergy [111–115], have been demonstrated to have profound effects on the diversity and composition of the intestinal microbiota of the infant (Table 2). However, does this mean that the microbiota is the major factor educating our immune system during this critical period of immune maturation? Although host–microbiota interactions are clearly not the only immunomodulatory processes during early life, they seem to play an essential and indispensable role. Recently, studies have focused on the importance of early-life microbial colonization on the development of a health-promoting immune phenotype. Mouse studies have demonstrated that early-life microbial colonization is crucial for the induction of T_{REG} cells in the lung and the development of tolerance against aeroallergens later in life [17,116]. Antibiotic treatment during the first 3 weeks of life in mice is associated with decreased microbial diversity during this period and increased allergic airway inflammation after allergen exposure during adulthood [117]. Moreover, it has been shown that mice that lack a microbiota early in life accumulate invariant natural killer T cells in the lungs and intestine, which increases their susceptibility to IBD and asthma [14]. Thus, it has been demonstrated in animal studies that early-life colonization with a microbiota is crucial for the prevention of chronic disease later in life by modulating immune function over the long term. Although a causal relationship between the lack of microbial diversity during the neonatal period and the development of chronic disease in adulthood has not been proven in humans so far, associations between reduced intestinal bacterial diversity during the first month of life, altered immune function, and the prevalence of chronic diseases such as asthma and allergies have been reported [118,119]. Hence, the microbiota colonizing the body after birth has a profound influence on the immune maturation process during the neonatal period, and is one of the major determinants of appropriate immune function throughout life.

Concluding Remarks

In recent years there has been growing support for the concept that early life is a crucial period that influences the development of health and disease throughout life. Studies in mice have recently provided proof that neonatal exposures can modulate immune responses not only in the short-term but also through adulthood, influencing the development of health or disease [13,14,17,117]. These findings have highlighted that microbial exposure during the first 2 weeks of life in mice is crucial, and that exposure to microbes later in life cannot fully restore immune phenotypes [13,14,17]. This goes in line with epidemiological data suggesting that exposure to a farm environment during the first year of life significantly reduces the risk of allergies and asthma: infants older than 1 year at first exposure were not protected [120]. Although adult environmental exposures clearly have been shown to influence the immune system of an individual, it seems that particularly neonatal exposures can alter immune function in the long term. One of the main questions to address in the future will be to determine the exact time-frame of this early-life ‘window of opportunity’. Another important issue to solve is to what extent the mechanisms demonstrated in animal studies can be translated to humans. Given the obvious limitations of human studies, an immediate answer to this question will be hard to achieve. However, the knowledge obtained using animal models is crucial for providing the potential structure of human observational or intervention studies.

Many more questions remain (see Outstanding Questions); however, as evidence mounts, and mechanisms become clear, we will reach a point where rational prevention strategies can be implemented at a population level. Such prevention, as opposed to treatment, will have a

Outstanding Questions

Between which ages is the early-life ‘window of opportunity’ that influences the trajectory of an individual towards health or disease?

How do short-term exposures during this early-life window shape immunity in the long term?

What are the immunological mechanisms by which pre- and perinatal environmental factors shape immunity of the newborn? Is epigenetics a key component?

Which constituents of the microbiota are the main ‘players’ in shaping immune development in the newborn?

fundamental impact upon healthcare and society by helping to curb the incidence and progression of chronic inflammatory diseases.

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