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Ontogeny of intestinal epithelium immune functions: developmental and environmental regulation

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Abstract. Intestinal mucosa integrates primary digestive functions with immune functions such as pathogen surveillance, antigen transport and induction of mucosal immunity and tolerance. Intestinal adaptive immunity is elicited in organized mucosa-associated lymphoid tissue (O-MALT) that is composed of antigen-presenting cells and lymphocytes and achieved by effector cells widely distributed in mucosa (diffuse MALT or D-MALT). Interaction between the intestinal epithelium, the O-MALT and the diffuse MALT plays a critical role in establishing an adequate immune response. In regions associated to O-MALT, lympho-epithelial cross-talks lead to acquisition of a specific epithelial phenotype that contributes to O-MALT organization and functionality. Beyond the expression of several innate immune functions, the intestinal epithelium may directly take up and present antigens due to the expression of major histocompatibility complex (MHC) and MHC-related molecules. A complex genetic program that will be outlined in the present review controls the development of immune functions of the intestinal epithelium. The effect of environmental signals on the modulation of this ontogenetic program during development and neonatal life, from bioactive components of amniotic fluid to lactation and bacterial colonization, will be discussed.

Key words. Human intestine; innate immunity; epithelium; development.

Introduction

The gastrointestinal tract (GIT) is composed of an endoderm-derived epithelium immersed in mesoderm-derived tissues. Four major steps have been proposed for the development of endoderm-derived organs [1]: (i) endoderm formation during gastrulation (gestational week 3 in humans), (ii) morphogenesis of the gut tube (week 4), (iii) budding of different organs from the initial tube (week 4) and (iv) differentiation of organ-specific cell types (week 12). GIT morphogenesis is controlled by a precise sequence of gene activation [2]. Although development results from the unfolding of an endogenous genetic program, multiple environmental signals contribute to the regulation of gene expression and GIT homeostasis.
Epithelial cell differentiation is the best-studied aspect of the ontogeny of the fetal GIT structure and function. The patterning along the cranial-caudal axis during organogenesis of GIT and associated organs is dominated by complex signaling mechanisms that involve endoderm-mesoderm interactions that are common to most vertebrates [3]. Recent advances in our understanding of such mechanisms have been reviewed elsewhere [4, 5]. The role that signals from the epithelium apical side, such as amniotic fluid, play at early developmental stages has not been extensively explored.
The intestinal epithelium participates in the local immune response through a diversity of constitutive and inducible innate immune functions. In addition, intestinal epithelial cells (IECs) can participate in the initial events of adaptive immune response, such as antigen presentation [6]. IECs modulate mucosal innate and adaptive immune responses through secretory products that promote activation or inhibition of professional immune cells. In this review, we will discuss how these different activities are developmentally acquired and how they are modulated by environmental changes from gestation until weaning and introduction of a complete diet with acquisition of normal microbiota.

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Intestinal epithelium development and onset of innate immune function

In humans, small intestine anatomical features appear for the first time when epithelial projections form the primitive villi. This process proceeds in a cranial-caudal manner starting around week 9 in the proximal (duodenal) region. Almost simultaneously, the transition from stratified to simple epithelium takes place starting on primitive villi, and nuclear polarity in the duodenum and proximal jejunum becomes apparent. At about the same time, mitotic activity is concentrated in the stratified epithelium of the intervillous regions, leading to crypt formation around week 12 [2]. The polarization of primitive enterocytes implies not only basal positioning of the nuclei but also the appearance of apical hydrolases [7, 8], transporters [9] and basolateral expression of integrins [10]. From about week 10, epithelial cells are associated in a tight cellular monolayer by the establishment of tight junctions (TJs) [11] that are the anatomical basis for an epithelial barrier. TJs contribute to establish cell-to-cell junctions, columnar morphogenesis and polarization [12], and constitute a fence to prevent mixing of apical and basolateral membrane proteins and lipids [13]. A selective epithelial barrier is thus established that regulates molecule diffusion and ion passage across the para-cellular route.

At this point, the overall intestinal epithelium organization with spatially defined patterns of proliferation, differentiation, gene expression, cell migration and cell senescence is achieved [14]. All these features will be maintained throughout adult life, as the gut epithelium is continuously self-renewing. Experiments with transgenic and knockout mice have provided insight on the programs that direct the patterns of gene transcription along the crypt-villus and cranial-caudal axes of the intestinal tract. Many molecules and signaling pathways, such as Hedgehog, Notch, transforming growth factor β (TGF-β)/bone morphogenetic protein (BMP), Cdx-1, Cdx-2 and Wnt, are involved. The current knowledge on these topics is beyond the scope of the present article and can be found in recent reviews [15, 16].

The intestinal epithelium participates in mucosal defense via several mechanisms. Most notably, as the external boundary of the organism, it performs a surveillance function on the luminal environment, expressing constitutive and inducible defense systems. Most epithelial cell types participating in immune functions in the adult GIT can be found early in development. At week 8, primitive enterocytes, goblet cells and enterochromaffin cells are observed [17]. Enterocytes constitute the most abundant cell type, and beyond their contribution to nutrient absorption, they express several immune functions that will be discussed here. Mucus production is a major constitutive defense mechanism that arises from *muc2* gene expression by goblet cells by week 12 [18, 19]. At this time, Paneth cells appear at the base of the crypts [17]. These cells contribute to innate immunity by producing an array of antimicrobial molecules [20] (see review by Peschel et al., this issue). α-Defensin production by Paneth cells is first detected at week 13 [21] . Paneth cells also contribute to the innate defense of epithelium by the production of lysozyme, starting around week 20, and they are the main source of tumor necrosis factor α (TNF-α). This production of TNF-α remains at low levels before birth but can be increased by intrauterine infections [22].

Fetal enterocytes express different receptors and signaling molecules that are involved in the innate immune response [23]. Although the precise timing of expression of these molecules is unknown, it has been shown that neonatal enterocytes produce interleukin 8 (IL-8 or CXCL8) after exposure to lipopolysaccharide (LPS) or interleukin 1 (IL-1), in a manner similar to the innate response of adult IECs [24]. These findings suggest that at birth (i) the LPS-detecting receptor, Toll-like receptor 4 (TLR4), is expressed in enterocytes and that (ii) the signaling machinery that links receptors (TLRs, IL-1R) to nuclear factor kappa B (NF-κB) signaling molecules is functional. Moreover, a stronger reactivity of fetal enterocytes to TLR ligands has been observed when compared with that of adult IECs [24, 25]. Lower reactivity of adult IECs was shown to be caused by regulated expression of MD-2 and TLR4 [26]; furthermore, a plausible molecular explanation for neonatal hyper-reactivity has been recently formulated [27]. Lower levels of regulatory molecules of NF-κB – IκBα, IκBβ and IκBε – have been observed in fetal compared with adult human IEC lines and in preweaned rats compared with the postweaning intestine. Thus, the downregulated expression of these regulatory components results in enhanced activation and nuclear translocation of NF-κB and proinflammatory gene transcription.

Immunoglobulin (Ig) translocation across the epithelial barrier is another important immune-related function of IECs. The intestinal epithelium has the capacity to secrete polymeric IgA and IgM due to the expression of polymeric immunoglobulin receptor (pIgR). pIgR binds to the joining (J) chain of polymeric Ig at the basolateral side of the epithelium and is transported to the lumen, where pIgR is cleaved, leaving a piece called the secretory component (SC) within the secretory Ig and J chain (see review by Kunisawa and Kiyono, this issue). The SC and J chain are detected during development at about week 4 with a widespread distribution, including the gastrointestinal, respiratory and urogenital tracts [28]. Although massive secretion of IgA arises in the neonatal period [29], the early expression of SC accounts for the low amount of secretory Ig in amniotic fluid [30]. On the other hand, IECs express the neonatal Fc receptor (FcRn) on the brush border of the proximal small intestine that participates in IgG transfer through the epithelial barrier.

Recently it was established that FcRn functions as a dual shuttle, facilitating the passage of IgG to the lumen and also the uptake of immune complexes from the lumen into subepithelial antigen-presenting cells, thus taking part in luminal antigen sampling and modulation of mucosal immune responses [31]. Intestinal expression of FcRn is already detected at week 18 of gestation [32] and is maintained through childhood and adult life. Since the presence of IgG in amniotic fluid is detected from week 12 [30], it is possible that these transfer mechanisms operate during fetal life. After birth, enterocytes are important in the transfer of passive immunity from mother's milk to offspring. Significant amounts of breast milk IgG are transferred to the suckling neonate through the epithelium using the FcRn system, which remains functional through adulthood and probably exerts an antigen sampling role.

Ontogeny of organized mucosal lymphoid tissue and epithelial-lymphoid interactions

Specialized primary and secondary organs and different cell populations, either resident or circulating through the whole organism, compose the immune system. The GIT harbors the largest immune cell populations that constitute the mucosa-associated lymphoid tissue (MALT). MALT is composed of different immune cells scattered along the GIT, constituting the diffuse MALT (D-MALT) and specialized histological structures, mainly Peyer's patches (PPs) in the small intestine and lymphoid follicles in the large intestine, that act as secondary lymphoid organs (organized or O-MALT). Due to strategic cellular distribution and the presence of specialized epithelium with antigen-sampling capacity, these sites act as inductive sites of mucosal immune responses [33].

Lymphoid populations can be observed in the developing human intestine as soon as week 12 [34, 35]. Clusters of lymphoid cells are distributed according to a regional pattern, and they constitute the precursors of the adult O-MALT. In addition, scattered lymphoid cells are observed at the same time in the lamina propria and the intraepithelial compartment, which together originate D-MALT.

O-MALT formation results from the molecular cross-talk between hematopoietic lymphoid cells and stromal cells, which through a sequence of intercellular activations causes production of chemokines that induce sequential cell recruitment and, thus, generation of the secondary lymphoid organs [36]. The molecular mechanisms controlling this process have been recapitulated in recent reviews [36, 37]. In this section, we will summarize major events and outline similarities and differences between mouse and human. At embryonic day 15.5 (E15.5) of mouse development, stromal cells expressing vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1) group together, forming clusters on the antemesenteric side of the small intestinal wall. These clusters are the first indicators of PP location [38] and are formed before the arrival of hematopoietic cells to the PP anlage. In humans, clusters of VCAM-1^+ ICAM-1^+ stromal cells are found as soon as gestational week 11 [39] and indicate the position of future PPs as well. In mice, lymphoid cells first colonize the PP anlage on E15.5, clustering together with VCAM-1^+ ICAM-1^+ stromal cells and starting lymphoid-stromal cell-to-cell cross-talk in which lymphotoxin β (also named $LT\alpha_2\beta_1$) and lymphotoxin β receptor ($LT\beta R$) interaction plays a critical role [36]. The hematopoietic population that clusters with VCAM-1, ICAM-1 stromal cells has the characteristic phenotype $CD4^+CD3^-LT\alpha_2\beta_1^+IL7R^+$ and triggers CXCL13 expression on stromal cells that, in turn, promotes further recruitment of $CXCR5^+$ hematopoietic cells [40]. Compartmentalization in B and T cell zones and the appearance of follicular dendritic cells (DCs) are established only after birth, upon arrival of mature B and T cells to the developing PP [41].

In humans, human leukocyte antigen (HLA)-DR^+CD4^+ cells were reported as early as developmental week 11, and the presence of $CD3^+$ T and $CD19^+$ B lymphocytes were detected after week 16 [41, 42]. Whether the $CD4^+$ population is functionally equivalent to the $CD4^+CD3^-$ precursor cells in mice has not been established. In contrast to mice, the arrival of mature lymphocytes and the establishment of PP compartments in humans are achieved in the early stages of fetal life. By week 19 of gestation, B cell follicles containing follicular DCs are distinctively established, and T cells are located mainly in the interfollicular regions [35]. Secondary follicles with germinal centers denoting B cell activation form only after birth, indicating a dependence on exogenous stimulation.

The progressive development of lymphoid compartments in PPs has an influence on epithelial and stromal cell differentiation. The arrival of lymphoid cells induces specific changes on stromal cells, such as impaired differentiation to myofibroblasts, which are missing in the PP area, and alterations in the production of extracellular matrix molecules [42]. Epithelial differentiation in PPs is also under the influence of the underlying follicular and interfollicular immune cells, and the specific features of follicle-associates epithelium (FAE) are established early during development. Thus, lower goblet cell density, lower proliferation rate, differential gene expression and M cell differentiation take place in FAE in contrast to villi epithelium. M cells are epithelial cells dedicated to antigen sampling by means of a facilitated transcytosis of material from the apical to the basolateral side (see [43]). In humans, M cells are detected on FAE by microscopical features at week 17 [17]. Downregulation of digestive enzymes and pIgR, as well as weak mucus production

due to a low number of goblet cells, improves antigen access to FAE. Information on FAE development in humans is lacking. It is possible that sampling of amniotic fluid through M cells occurs in the prenatal period, but the relevance of such a process has not been established.

Production of the chemokine CCL20 is a hallmark of FAE. This chemokine is expressed constitutively in the FAE of different species, including mice and humans [44]. CCL20 is responsible for recruitment of immature DCs to the subepithelial dome region, strategically placing the antigen-presenting machinery close to the site of antigen sampling [45]. In mice, FAE-specific *ccl20* expression has been observed as soon as E17.5 [46]. It has been suggested that epithelial signaling through basolateral LTβR triggers the onset of *ccl20* expression. In mice, the hematopoietic cells present at E17.5 in the PP anlage provide the LTβ signal. There is no definitive evidence that humans share this mechanism. However, the fact that human intestinal epithelial cell lines respond to LTβR stimulation by upregulating *ccl20* expression [46] supports this hypothesis. The chemokine CCL23 is also specifically produced by human FAE [M. Rumbo, unpublished data], although precisely when it appears is unknown. Prenatal FAE chemokine production would attract immature DCs to the subepithelial dome, which together with early development of the antigen-sampling ability of FAE would ensure the capacity for immune response to the exogenous antigens that arrive in the luminal compartment soon after birth.

Environmental regulators of gut epithelium biology: from amniotic fluid to lactation and weaning

The inherited program of IEC development is under the epigenetic influence of the gut lumen environment during both the prenatal and the postnatal periods. Three major sources of gene-expression regulatory signals can be considered: amniotic fluid, breast milk and neonatal intestinal microbiota.

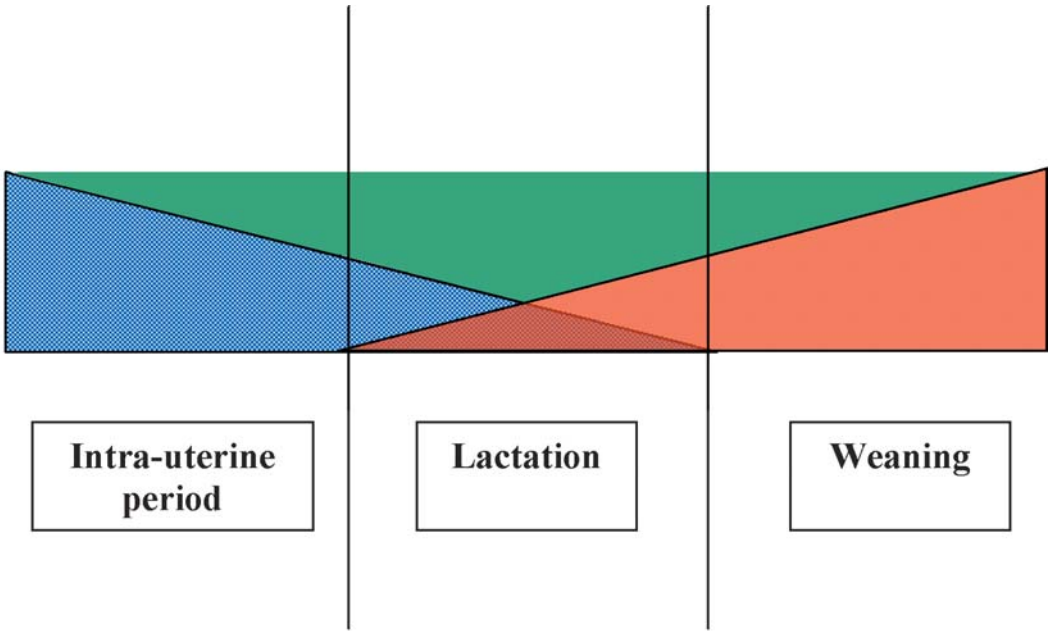
Amniotic- and milk-mediated regulation of intestinal immunity

In humans, fetal swallowing activity is detected at week 16 [2]. During development, the intestinal epithelium is thus exposed to amniotic fluid that contains a broad diversity of bioactive molecules with different functions and changing concentrations along gestation.

Intestinal epithelial morphogenesis and terminal differentiation depend in part on the modulatory activity of a range of peptides, cytokines and growth factors [47, 48]. The secretory and absorptive functions are only partially developed by week 26, and functional maturation proceeds until delivery. Both aspects, mucosal morphogenesis and functional development, are regulated by factors present in the amniotic fluid [49]. In addition, the mature intestinal epithelium expresses surface molecules and cytokines that orchestrate the immune reactivity of the intestinal mucosa [50]. In contrast, fetal enterocytes show hyperreactivity when confronted with microbial challenges; thus, they are unable to initiate an adequate defense response [25]. Factors present in the amniotic fluid and breast milk can compensate for immaturity by modulating exaggerated reactivity on the one hand and promoting immune-maturation on the other, thereby helping to support immunocompetence at early age [51]. A similar molecular asset has been found in the amniotic fluid and colostrum/mature milk, suggesting that they act as modulators during the transition from intrauterine to extrauterine life, particularly upon the onslaught of microbial communities during neonatal intestinal colonization [52] (fig. 1). Concentrations of growth-modulating factors in the amniotic fluid, such as insulin-like growth factors I and II and granulocyte colony-stimulating factor (G-CSF), correlate positively with gestational age [53]. The TGF-β pathway is responsible for different functions and is of central importance in epithelial morphogenesis as well as modulation of immune responses [48].

The presence of various cytokines has been detected in the amniotic fluid. Among others, IL-6, IL-8/CXCL8, IL-10, IL-11, IL-12, IL-15 and TNF-α can be detected at mid-trimester and until the end of pregnancy [54]. Some studies indicate that TNF, acting on its receptor 1 (TNFR1), induces an apoptotic detachment of the enterocytes from the tip of the villi, whereas in the enterocytes from the crypts TNF, via either TNFR1 or TNFR2, increases the expression of p53 without inducing apoptosis, thus contributing to cellular renovation and tissue organization in the developing intestinal mucosa [55]. IEC expression of IL-8 receptors CXCR1 and CXCR2 has been observed from week 16 [56], and it has been shown that IL-8, acting through CXCR2, can modulate the cytotoxic effect of TNF-α on IECs [57]. CXCL8 and CXCL8-related chemokines are supplied after birth in breast milk [58], an example of how amniotic fluid bioactive molecules are mirrored in breast milk. The IL-6 receptor is also expressed by fetal enterocytes [56], making this tissue a putative target of IL-6, which is produced in the case of perinatal microbial infection [59].

IECs are not fully developed at birth, and because of their immunological immaturity, they cannot afford protection against colonizing microorganisms. Breast milk, then, plays a dual role: (i) it provides the neonatal intestinal environment with molecules that will undertake defensive antimicrobial functions and (ii) it continues to provide, similar to amniotic fluid, growth factors and immunomodulatory and anti-inflammatory molecules that promote development and give neonatal immune protection and



Trophic and immunomodulatory factors: amniotic fluid is rich in several trophic and immunomodulatory factors that are also found in breast milk (see text). Along lactation and suckling/weaning transition, the composition and levels of these factors change. After weaning luminal stimulation is maintained only by autocrine and paracrine mechanisms. Examples: transforming growth factor β and interleukin 10.

Passive immunity/humoral immunity: passage of immunoglobulins from maternal to fetal circulation is the main source of antigen-specific antibodies along the intrauterine period. Immunoglobulins (mainly of maternal origin) are also present in the amniotic fluid. These molecules may have a protective effect and may also condition the fetal naive antibody repertoire through anti-idiotype antibodies. During lactation, cells, innate immune molecules and immunoglobulins are delivered in milk and subsequently transferred to the suckling infant. After weaning, secretory IgA is produced by the offspring, which will contribute to immune exclusion of luminal antigens.

Environmental antigenic challenge: Along intrauterine life there could be passage of antigens from maternal to fetal circulation following a poorly characterized mechanism. This may also contribute to shape the reactivity of the naive immune system. After birth, neonates are exposed through the gut to a wide variety of microbes and dietary antigens. The complexity of the intestinal environment increases considerably from exclusive breastfeeding to full diet after weaning. This antigenic challenge is necessary for full development of MALT.

Figure 1. Distribution of factors that influence development of intestinal epithelial cells and their immune functions along the perinatal period.

instruction (fig. 1). Lactoferrin and the derived peptide lactoferricin protect against neonatal infections [60] by depleting the intestinal environment of iron, which is essential for bacterial growth. Lysozyme, secretory IgA, oligosaccharides and milk mucins such as MUC-1 play anti-infectious roles through bactericidal activity or inhibition of pathogen binding to the epithelial cells. Breast milk provides several molecules with trophic activity on IECs [61], such as epithelial growth factor (EGF) [62], cortisol [63] and polyamines [64, 65]. Stimulation of IEC maturation on its own potentiates the barrier function of epithelium and prevents bacterial translocation. Moreover, various hormones and growth factors present in breast milk can prevent pathogen colonization and invasion [66].

More recently, soluble pattern recognition receptors (PRRs) such as soluble CD14 (sCD14) and soluble Toll-like receptor 2 (sTLR2) have been identified in human milk [67, 68]. The functional relevance of these molecules in the neonatal gut is not completely understood; they could play a sentinel role, warning the immature epithelium of the presence of bacteria and promoting an innate response, or they could prevent epithelial reaction to bacterial products through a scavenging capacity of microbe-

associated molecular patterns (MAMPs) such as endotoxins and peptidoglycans.

The intestinal surface is an interface with a rather stable environment in the prenatal period but with dramatic changes after birth, when bacterial cells and MAMPs become dominant in the gut lumen. The interaction between the intestinal content and the mucosal surface, i.e. IECs, at both the prenatal and the neonatal stages needs to ensure a homeostatic balance to achieve a successful pregnancy and preserve the integrity of the intestinal mucosa. The neonatal period is a critical moment in terms of mucosal defense and abnormal antigenic priming because the epithelial barrier and the immunoregulatory network are incompletely developed [69]. IL-10 and TGF-β are central players of immunological tolerance in the adult, where a robust adaptive mechanism is in place, and they are both detected in amniotic fluid and breast milk at variable levels during pregnancy and lactation [70, 71]. TGF-β in amniotic fluid could play a role in maternal immunity to retain the fetal allograft [51].

Immune immaturity may play a role in antigen priming for allergic diseases [69]. Prenatal allergic sensitization has been suggested. The finding of antigen/allergen-specific IgE in cord blood (of fetal origin since IgE does not cross the placental barrier) is considered a marker of later atopy development [72]. If this is the case, prenatal antigen/allergen sensitization should occur. In utero sensitization to environmental antigens has been suggested, specifically to house dust mite, cow's milk and hen's egg. Such reactivity supports the contention that fetal exposure occurs to both dietary and inhalant allergens [73, 74]. Although different routes of sensitization are possible during fetal life, IECs remain an important port of entry for antigens present in the amniotic fluid. This pathway accounts for 70% of the daily protein turnover in the amniotic fluid. The presence of immunosuppressive factors such as TGF-β or IL-10 in amniotic fluid may contribute to minimize the effect of prenatal antigen exposure. In addition, sCD14 is also present in amniotic fluid [75], and low levels of sCD14 in amniotic fluid or breast milk have been found in atopic infants [76]. This suggests that early exposure to sCD14 could be a means of influencing immune development in infants and preventing immune-mediated diseases later in life.

The microbial challenge in extrauterine life

After delivery, microbial colonization of the neonate GIT begins. The kinetics of colonization and the nature of microorganisms is complex and is influenced by the route of delivery, exposure to microbial sources and onset of nutrient ingestion [77]. Intestinal microbiota have a major influence on gene expression in the gut. Recent work has shown that the association of germ-free rodents – the gnotobiotic model– with commensal bacteria, *Bacteroides thetaiotaomicron*, results in major changes of GIT gene expression and physiology [78]. IEC gene expression is particularly influenced by bacteria and involves genes contributing to enhancement of the barrier function, immunoglobulin secretion, digestive function [78, 79] and antimicrobial activity [80]. Commensal bacteria are not ignored by the host but are recognized initially by PRRs expressed on IECs and other immune cells such as lamina propria macrophages. The way in which this mutual recognition takes place may set the basis for tolerance to commensal bacteria or, in contrast, cause a conflictive relationship with commensals that can result in inflammatory disorders. Using adult animal models, a recent study has shown that under normal conditions commensals will interact mainly with IEC-expressed PRRs, contributing to a cytoprotective epithelial response and promoting barrier integrity and repair, thus preventing bacterial translocation [81]. It is not known to what extent this mechanism operates in newborns.

The neonatal period is crucial for the acquisition of the endogenous microbiota. At the same time, it is associated with high sensitivity to infectious agents and exaggerated innate immune responsiveness. Neonatal necrotizing enterocolitis (NEC) is an example of how disruption of the fragile equilibrium in the neonatal intestine may lead to overt pathology. NEC is characterized by an exacerbated intestinal inflammatory response associated with an altered microbial flora and has a high incidence of infant morbidity and mortality [82]. Although its etiology and pathogenesis are not completely understood, NEC is observed almost exclusively in preterm infants. The intestine in preterm infants, especially in the absence of breast-feeding [83], is a vulnerable system owing to epithelial and digestive tract immaturity and transient immunodeficiency. Early introduction of oral feeding and abnormal bacterial colonization of the gut play a central role in NEC development [25]. Indeed, a particular microbiota establishes in the gut of neonates suffering from NEC, with a predominance of unusual anaerobes and enterobacteria detrimental to protective *Bifidobacterium* [84, 85]. Neonatal GIT colonization is a complex, dynamic process influenced by multiple factors [77], and a better understanding of the mutual interactions between the microbial gut ecosystem and the innate immune response is just emerging. It has been proposed that some bacteria species, including *Bacteroides,* downregulate the overall proinflammatory effect of enterobacteria [86]. Since neonatal gut colonization with symbiotic 'anti-inflammatory' microorganisms depends on environment and nutrients, it is tightly controlled by early breastfeeding, which is not always operative in preterm neonates. Changes in the microbiota that disturb colonization of the gut by 'anti-inflammatory' bacteria, together with epithelial hyper-reactivity, may act synergistically to promote the onset of NEC.

Perspectives

In recent years, intestinal epithelium biology has switched from a classical view of an absorptive cell type and a cellular shield that separates the host from the luminal environment toward a dynamic interface that is able to sense environmental changes and initiate integrated responses. Epithelial cells can 'judge' whether signals from the gut lumen are infectious nonself, dangerous or harmless. A major functional feature of intestinal epithelial cells is the orchestration of homeostatic defense responses without severe tissue damage. This feature depends on different underlying mechanisms such as tolerance to LPS and other microbial products, immunological tolerance and development of secretory immunity. These functions depend on the developmental status of epithelial cells. The amniotic fluid during pregnancy, and breast milk during the neonatal period, compensate for developmental delays of the epithelial function and, in addition, assure the normal completion of maturation. The growing understanding of this process is not only of theoretical interest but is also beginning to influence clinical practice in the treatment of prematurity. The use of EGF, G-CSF and other growth factors that target mainly the intestinal epithelium have expanded the neonatal clinician therapeutic options.

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