

The development of mucosal immunity

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The development of the intestinal immune system is a complex sequence of events that begins *in utero* under various genetic influences, but continues after birth, being modified by factors such as bacteria, hormones and feeds. This review discusses what is known about the ontogeny of each aspect of the mucosal immune system so as to provide a better understanding of how aberrations in the system might lead to systemic disease. *Eur J Gastroenterol Hepatol* 17:1273–1278 © 2005 Lippincott Williams & Wilkins.

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Introduction

The development of mucosal immunity begins *in utero* as the framework is laid for what will be a complex set of interacting structural and immunological components of the gut in order to establish the largest immune organ of the body (Fig. 1) [1]. After birth intestinal maturation is regulated by three factors: an intrinsic genetic timing mechanism, circulating hormones including corticosteroids, and extrinsic factors such as luminal bacteria [2]. Insight into the development of this system was initially provided by morphological and histological observations. The most recent advances in our understanding of the ontogeny of mucosal immunity come from studies of germ-free animals, cytokine analysis, and immunohistology.

Structural components of the mucosal immune system

The epithelial cell

Part of mucosal immunity involves the creation of a physical barrier by intestinal epithelial cells that separates the 'external' and 'internal' environments. At 9–10 weeks' gestation villi with simple columnar epithelium of differentiated absorptive cells are present in the small intestine. Early stages of tight junction assembly have been observed in the colons of 10-week fetuses [3], and more mature forms are seen during the second trimester [4,5]. However, the functional property of transepithelial resistance during the second and third trimesters is not known. Major proteins in the junctional complex, occludins and claudins, have been identified; however, their developmental regulation is unknown.

Despite the apparent normal structure of these tight junctions, the barrier function does not appear to be fully functional at birth because intestinal permeability is higher in the newborn than in the adult [6,7]. Other

factors are probably responsible for this difference including quantitative and qualitative differences in mucus [8], lower gastric acid and pancreatic enzyme secretion [9,10], and slower gut motility [11]. Mucus may be particularly relevant as competition between mucus glycoproteins and epithelial receptors can influence interactions with bacteria [12,13]. Some authors have hypothesized that incompletely developed tight junctions in the newborn period allow for the translocation of maternal antibodies and trophic factors in mother's milk to contribute to the initial mucosal defence [14]. Within weeks, closure occurs and the gut becomes less permeable (in rodents this occurs just before weaning) [5,15]. Exogenous glucocorticoids can accelerate closure [14].

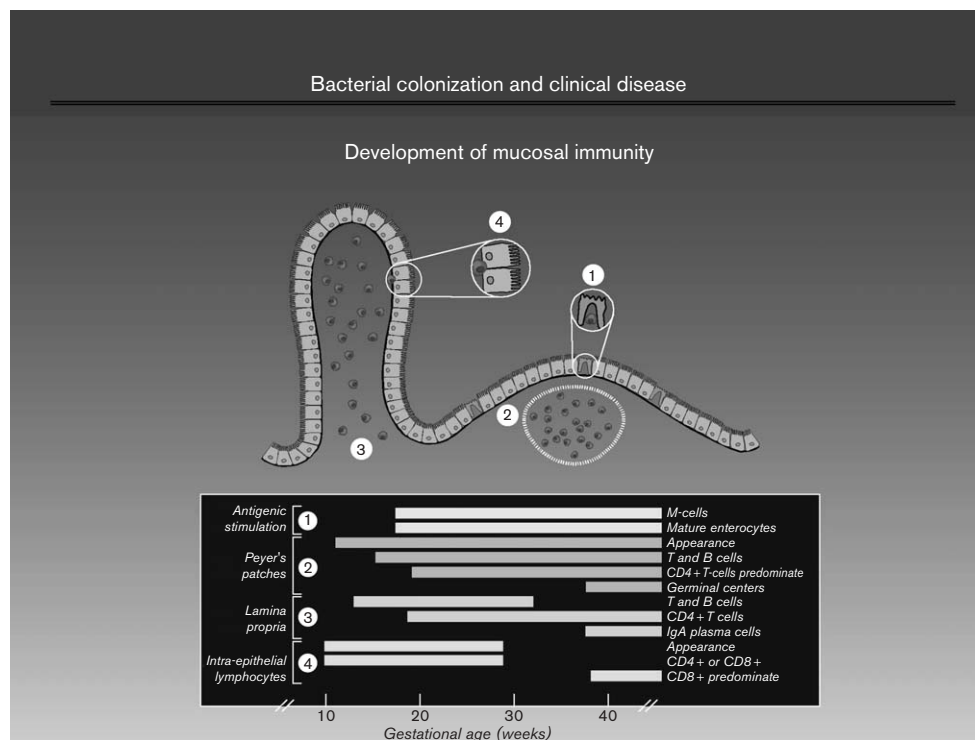
The epithelial cell surface is covered with oligosaccharides attached to membrane proteins and lipids. The patterns of glycosylation appear to change with time. In the rodent, most surface oligosaccharides on intestinal epithelial cells contain terminal sialylation before weaning and terminal fucosylation after weaning [16,17]. This correlates with changes in sialyltransferases and fucosyltransferases [18]. This same pattern is seen in human ontogeny, with fetal sialylation being followed by adult fucosylation and galactosylation [19].

These changes in glycosylation patterns may affect colonization patterns within the intestine. For example, the attachment of *Helicobacter pylori* to fucosylated versus sialylated glycans influences pathogenicity, and colonization with *Bacteroides thetaiotaomicron* is facilitated by fucosylation because it uses L-fucose as a nutrient source [20].

Peyer's patches

Peyer's patches are organized lymphoid tissues in the wall of the small intestine. They contain B lymphoid follicles

Fig. 1



Ontogeny of the human mucosal immune system. The major components of the mucosal immune system are shown. The respective developmental time points for key components are highlighted. Note germinal centers in Peyer's patches. IgA plasma cells in the lamina propria and predominant CD8+ intraepithelial lymphocytes do not appear until the perinatal and neonatal periods. From Insoft *et al.* [1], with permission.

and interfollicular populations of CD4 and CD8 T cells. These T cells have the propensity to recirculate to and selectively populate the mucosal tissue [21]. The development of gut-associated lymphoid tissue requires an intact thymus and a gut that is exposed to environmental antigens. The emergence of Peyer's patches parallels the development of the spleen and peripheral lymph nodes. T cells are not observed in the intestine until the thymus is mature [22].

Rudimentary elements of Peyer's patches are seen in the 11-week fetus. Initially, the clusters of cells are CD4 cells, which will go on to become MHC class II restricted T cells. By 14 weeks' gestation CD4 and CD8 T cells make up the follicles within the Peyer's patches [23]. At 16–18 weeks both T and B cells are identified, with CD4 T cells predominating over CD8 T cells. At this time B cells with IgM and IgD are admixed among the T cells. The relatively mature B cells in the intestine at 16 weeks probably derive from pre-B cells in the bone marrow or liver. The discreet localization of T and B-cell populations is evident at 19 weeks' gestation. Also at 19 weeks, differentiation of macrophages and dendritic cells can be seen in the follicles [24]. T cells in the fetus are not activated, and probably represent early thymic emigrants. Cornes's microscopic studies revealed that the number of

Peyer's patches with at least five primary lymphoid follicles was 45 at 24 weeks, 60 at 29 weeks' gestation, and 80–120 at birth. This number increases to 250 in the midteens, but declines to 100 in the ninth decade [25].

The normally activated state of B-cell follicles in Peyer's patches with continuous germinal center reactivity is dependent on chronic and novel gut mucosal stimulation by enteric antigens [21]. Neonatal mice show delayed increases in antigen-specific B cells compared with the normal adult. These delays are thought to be preprogrammed genetic recombination events, and progress along specific bacterial determinants such as α 1–3 dextran, α 1–6 dextran β 2–1 inulin, phosphocholine, and β galactosyl [26]. This may be a developmental modulation based on the natural stimulation of the immune system by typical bacterial colonizers [27].

Lamina propria

The lamina propria is a meshwork of connective tissue beneath the gut epithelium, and contains a broad range of myeloid and lymphoid cells, in particular IgA plasma blasts, CD4 T cells, dendritic cells and mast cells [21]. The main cell in the fetal lamina propria is an HLA-DR-positive cell with dendritic morphology [24]. They are also CD4 positive and have CD45 (leukocyte common

antigen). These cells are probably there at 11 weeks' gestation. T cells are present in the lamina propria from 12 weeks [28], and are abundant by 20 weeks. These cells are mostly CD4 positive [29]. Eosinophils and granulocytes are present beginning at 19 weeks' gestation [30]. As there is no stimulation of Peyer's patches in fetal life, the lamina propria has no IgA secreting cells [23]. At birth, antigenic exposure results in mucosal immune system activation and by 12 days after birth there are IgA and IgM plasma cells in the lamina propria [31]. Initially, IgM cells are more common than IgA, but IgA plasma cells predominate after 6 months.

Mast cells are seen in significant numbers in the lamina propria. They are the effector cells in IgE-mediated allergic responses, as well as playing a role in the defence against intestinal parasites and enteric bacterial pathogens. These cells appear in equal numbers and distribution in the fetal and adult stages of humans and rodents [32].

Intraepithelial lymphocytes

Intraepithelial lymphocytes (IEL) have been visualized with electron microscopy at 11 weeks' gestation [33]. These IEL are located in the basal portion of the epithelial layer, as they are in the adult. CD3 + IEL appear in the fetal gut at 14 weeks. Prenatally, only 50% of IEL express CD4 or CD8, whereas the other half are CD3-positive, CD4-negative, CD8-negative. Postnatally, IEL are almost completely CD8-positive [34].

Cellular components of the mucosal immune system

Microfold cells

Microfold cells are morphologically distinct specialized enterocytes that can breach the barrier formed by the epithelial layer in order to sample luminal contents, and allow for luminal antigens to tolerize or activate the cells of the adaptive immune system. Microfold cells are formed at week 17 of gestation, shortly after the appearance of intestinal lymphoid aggregates [35]. This temporal relationship in their development is probably significant. Luminal microbial attachments and paracrine action by B cells can result in microfold cell differentiation. This effect can be demonstrated when luminal bacteria and lamina propria lymphocytes are grown with the human cancer cell line Caco-2, and one observes that differentiated enterocytes can transdifferentiate into microfold cells [36]. This is further supported by the observation that B-cell-deficient mice have impaired development of Peyer's patches, follicle-associated epithelium, and microfold cells [37]. Work with recombinase-activating gene knockout mice that lack T and B cells indicates that B cells are required to form full-size follicle-associated epithelium, but non-lymphocytes can support microfold cell differentiation [38].

Macrophages

Macrophages are the major resident phagocytic cells in the gut. They are found throughout the lamina propria, mainly in a subepithelial distribution [39], as well as clustered in Peyer's patches. The human fetal intestine becomes populated with macrophages from about week 12 of gestation, although specific subpopulations differ with gestational age and location. Lamina propria macrophages specifically lack CD14, an essential coreceptor for Toll-like receptor (TLR) 4, and are therefore lipopolysaccharide hyporesponsive [40]. This may prevent the inappropriate activation of inflammatory signals in response to lipopolysaccharide from commensal bacteria. It is not known whether the lack of CD14 is developmentally regulated or is secondary to the recruitment of a specific subset of macrophages into the gut.

Dendritic cells

Dendritic cells are antigen-processing cells. Immature dendritic cells are phagocytic and endocytic and thus take up and process antigens. Intestinal lamina propria dendritic cells can extend cytoplasmic processes between enterocytes without disrupting the integrity of the epithelial barrier. Pathogenic bacteria such as *Salmonella* spp. activate the dendritic cells to leave the intestine and probably migrate to regional lymph nodes. Alternatively, commensal bacteria do not induce migration [41]. The ontogeny of the dendritic cell has not been examined in detail. They appear at the same time as macrophages, with a similar distribution. Evidence from dendritic cells derived from human umbilical cord monocytes demonstrates the impaired production of lipopolysaccharide-induced IL-12 [42], thus suggesting the existence of an immature state in neonates.

Paneth cells

Paneth cells are derived from the same stem cells that give rise to enterocytes. Clusters of paneth cells can be found at the bottom of each crypt. These specialized secretory cells release broad-spectrum antimicrobial peptides, defensins, as part of a non-specific innate immune response to bacteria. These cells are first seen in the human fetus at 17 weeks of gestation [43].

The adaptive immune system

Adaptive immune responses take longer to develop than innate responses. Furthermore, they are antigen specific and involve the development of immunological memory. It is the lymphocyte that mediates adaptive immunity. B and T-cell populations change significantly postnatally on the basis of responses to feeding and commensal flora.

The fetus appears to have the ability to mount an adaptive immune response against foreign antigens. In an area of malaria endemicity, the cord blood of infants demonstrated T and B-cell immunoreactivity to

Plasmodium [44]. However, there are probably differences in immune responsiveness between neonatal and adult lymphocytes, suggesting maturational stages.

T cells from newborns are also more difficult to stimulate *in vitro* than their more mature counterparts, perhaps suggesting greater requirements for co-stimulatory signals. The microenvironment of the intestine favors the suppression of local immune responses. This has been attributed to the local production of IL-10, transforming growth factor beta, and other immunomodulatory molecules by T cells, macrophages, dendritic cells, and epithelial cells.

In vivo the gut appears to rely on a surge of glucocorticoids to coordinate the induction of functional and inflammatory maturation. Glucocorticoids can initiate the differentiation of proliferative cells into more mature villus epithelium [1]. After an ill-defined responsive period between 20 and 30 weeks' gestation the sensitivity to steroids appears to be lost [45].

Production of inflammatory cytokines

Luminal microorganisms can induce the production of cytokines, chemokines, and other inflammatory mediators from enterocytes. Only pathogenic bacteria can do so, either because of peculiarities in the enterocyte interaction via surface molecules or because of the bacterial expression of virulence factors [46].

Animal studies have suggested that the ability to produce cytokines in response to inflammatory stimuli may be developmentally regulated. A human fetal intestinal cell line produces relatively more IL-8 in response to inflammatory stimuli (e.g. IL-1 β , and TNF- α) [47,48] than mature intestinal epithelial cells [47]. Similarly, increasing levels of the inhibitor of nuclear factor kappa B, the regulator of the nuclear factor kappa B-dependent inflammatory pathway, are found with maturation of the intestinal epithelial cells. Data have suggested that this increase in the expression of the inhibitor of nuclear factor kappa B leads to a decrease in flagellin-induced IL-8 expression [49].

Additional differences between cytokine production and function in newborns versus adults have been documented for IFN- γ , IL-4 and IL-6 [50].

Receptors for bacterial products

Bacterial toxins have been shown to initiate cascades that can lead to derangements in enterocyte function. The effects of some of these toxins appear to be developmentally regulated. Newborn animals are more susceptible than older animals to cholera toxin and *Escherichia coli* heat-stable toxin. The former is caused by a post-receptor mechanism [51], whereas the latter is caused by a post-

weaning decrease in receptor numbers. Conversely, newborns are less susceptible to *Clostridium difficile* toxin and Shiga toxin because fewer high affinity receptors are expressed in the newborn compared with the adult [52].

TLR have been implicated in the recognition of and the triggering of an inflammatory response to bacterial lipopolysaccharides and other microbial molecules. TLR2 and TLR4 messenger RNA and protein have been found in fetal intestinal epithelial cell lines and in primary fetal enterocytes, suggesting that the intestinal epithelium can respond to lipopolysaccharide [53].

Intracellular and cytosolic proteins also have the ability to interact with bacterial lipopolysaccharide. These intracellular proteins, NOD1 and NOD2, respond to lipopolysaccharide with the activation of nuclear factor kappa B, a transcription factor crucial for turning on the genetic programme involved in inflammation. Information on the developmental regulation of NOD1 or NOD2 expression or function is currently lacking.

Expression of stress-associated molecules

Enterocytes can respond to changes in the local environment by expressing specific surface molecules that act as danger signals. MACA and MACB are examples of such molecules, and are non-classic relatives of the MHC class I proteins. MACA and MACB are expressed in a patchy distribution on the intestinal epithelium, and their expression can be upregulated by stressful conditions such as heat shock [54] and infection [52]. These proteins function as ligands for the NKG2D receptor expressed on natural killer cells and various lymphocyte populations. The developmental regulation of these molecules has not been determined.

Commensal bacteria

Commensal bacteria can have an anti-inflammatory effect on the developing immune system. For example, *in utero* a T helper type 2 response is predominant [55]. With gut colonization a balance between T helper types 1 and 2 is established to prevent the development of allergic food reactions, and to establish a T helper type 3 response that provides tolerance to oral protein antigens [52]. In addition, commensal bacteria have global effects on gene expression, and can thus influence multiple intestinal functions, including barrier function, and maturation of the immune system [56] (see Table 1).

Feeding allows for antigenic stimulation and bacterial colonization of the gut. This is required for the development of IgM and IgA-producing plasma cells in the intestinal lamina propria [66]. Breast milk provides passive protection with antibacterial components such as IgA, lysozyme, and lactoferrin, promotes the development of commensal flora rich in bifidobacteria, and decreases

Table 1 The effect of commensal bacteria on the development of the immune system

Increases epithelial cell expression of matrilysin, an enzyme involved in processing pro-defensins [57]
Inhibits epithelial NF- κ B activation and inflammatory gene expression [58]
Activates CD4 cells in Peyer's patches [59]
Activates CD8 or natural killer cells in intraepithelial leukocyte spaces [21]
Increases numbers of T and B cells, including CD86-positive cells [60]
Organizes the special relationships between T, B, and dendritic cells in the Peyer's patches [61]
Increases the numbers of microfold cells [61]
Increases IgA producing B cells [52]
Hypertrophies Peyer's patches and the development of germinal centers [62]
Increases the number and cytolytic activity of $\gamma\delta$ IEL [63,64]
Changes in epithelial cell glycosylation patterns [65]

IEL, Intraepithelial lymphocytes; NF- κ B, nuclear factor kappa B.

colonization with potential pathogens [67,68]. Human milk during the colostral phase of lactation supplies a disproportionate amount of polymeric IgA relative to total human milk content. This can be modified in prematurely born infants, as IgA levels in premature infants' mothers' milk are significantly higher than those of full-term infants' mothers [69]. Maternal milk is also rich in IgA antibodies specific to her microflora and other antigens in the maternal gut. The immunization of mothers against pneumococcal meningitis has been shown to provide passive protection via the secretion of specific antibodies to the breastfeeding infant [70].

Breast milk also provides epidermal growth factor, insulin-like growth factor, and transforming growth factor beta, which affect enterocyte growth, migration, and maturation, and thus strengthen the epithelial barrier. Colostrum deprivation delays closure of the mucosal membranes [71]. Breast milk also has immunomodulatory molecules that can influence the development and function of the mucosal immune system and modulate inflammation [72].

Alterations in the normal development of the immune system can lead to chronic disease states. Antibiotic use in the neonatal period and infancy can interfere with the development of a healthy commensal flora and may result in subsequent allergic disease [73]. In young mice, antibiotic exposure resulted in increased IgE and a T helper type 2-skewed response [74]. The use of probiotics as mediators of the mucosal immune system thus appears promising.

Conflict of interest

None declared.

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