Regulation of Functional Development of the Small Intestine

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The gastrointestinal tract is responsible for acquisition of energy and nutrients to sustain life. The small intestine, an important tissue of this organ, is responsible for the terminal stages of digestion and absorption (1–3). Diseases that disrupt the function of this tissue are rare and usually incompatible with life. However, any disease that directly affects the gut presents a significant challenge to the physician to prevent disruption of digestion and absorption (4). If specific nutrients are not digested because of malabsorption, diarrhea ensues. Diagnosing the cause of symptoms requires a complete understanding of small intestinal function and its development.

Understanding development is important, especially for physicians caring for immature infants and adults with short bowel syndrome (4,5). Medical advances have resulted in increased survival of premature infants with an underdeveloped gastrointestinal tract (7). To ensure the survival and growth of a premature infant, special attention must be paid to nutritional intake (8). Thus, understanding the functional development of the small intestine is critical (9). The immature gut responds inappropriately when exposed to nutrients and microbes in diseased conditions such as necrotizing enterocolitis (NEC) (10). However, the use of prenatal cortisone in the prevention of hyaline membrane disease resulting from lack of lung development has also fortuitously produced significant reductions in the incidence of NEC (11,12). These observations suggest that steroids can stimulate development of the human gut. Glucocorticoids have a profound effect on all aspects of the developing gastrointestinal tract in rodent models, thereby facilitating the transition from milk to a solid food (13). Apart from precociously inducing digestive and absorptive functions, glucocorticoids are responsible for the important process of gut closure that results in the transient increase in permeability of the gut to large molecules such as antibodies and growth factors that are found in colostrum and milk (4,14,15). This is the way in which the infant acquires passive immunity from the mother. Whether steroid treatment in premature infants alters the ontogeny of this process is not completely understood at present. Thus, it is important to understand the role of glucocorticoids...
in normal and precocious development of the gut. Understanding the cellular and molecular mechanisms by which glucocorticoids alter functional development of the small intestine will help gastroenterologists to devise strategies to provide appropriate supplements to mother’s milk or formula that will enhance the growth of the infant but will also prevent the onset of NEC. This will also help physicians care for mucosal dysfunction in trauma patients and after extensive bowel resection.

**ORGANIZATION OF THE SMALL INTESTINE**

Most of the final digestive and absorptive functions are carried out by the differentiated enterocytes of the small intestine (16,17). In the mature epithelium, the absorptive enterocytes make up 93% to 95% of cells (18,19). The remaining differentiated epithelia consist of mucus-secreting goblet cells (20–22), gastrointestinal hormone–producing enteroendocrine cells (18,23,24), and defensin-producing Paneth’s cells. With the exception of Paneth’s cells (21,22,25,26), these differentiated cells migrate on to tonguelike projections called villi. Each villus is surrounded by a number of pitlike structures called crypts of Lieberkühn. The proliferative and undifferentiated cells reside in the crypts and they continuously supply enterocytes, goblet cells, and enteroendocrine cells to the villi (18,20,27). As cells leave the proliferative phase, they undergo cytodifferentiation in a lineage-specific manner. Thus, a continuous state of cellular proliferation, differentiation, and migration occurs along the crypt–villus axis, discussed extensively in Chapter 1 (18,20). During development, the ontogenic changes are superimposed on a constant proliferation and differentiation within this epithelium.

In adults, a single stem cell (18,21), which is located at the lower half of each crypt, produces all differentiated cells after a strict program of hierarchical proliferation (27–30). Most of the cells differentiate as enterocytes as they leave the crypts (20). The vertical migration of these cells from crypts to villi takes 2 to 3 days in adult rats and mice (31,32) and 5 to 6 days in humans (32,33). As enterocytes leave the crypts, a burst of transcriptional activation of differentiation markers occurs. However, accumulation of differentiated markers decreases as the cells migrate toward the villus tip where they undergo apoptosis (20). The nature of the stem cell and the mechanism of lineage differentiation are still a poorly understood area in intestinal epithelial biology.

Enterocytes increase their apical surface by the presence of microvilli, also known as the brush border (20,34,35). The digestive enzymes and absorptive transporters, which are anchored in the microvilli, provide an excellent tool for the study of cellular differentiation and intestinal development. Among these, the disaccharidases are widely used markers because of ease of assay and tissue specificity. The disaccharidases are lactase, sucrase, maltase, and trehalase, which are responsible for terminal digestion of carbohydrates (34,36); enzyme complexes occur, which include lactase-phlorizin hydrolase, sucrase-isomaltase, and maltase-glucoamylase (34). These enzymes are synthesized as a single polypeptide, glycosylated, anchored on the apical membrane, and cleaved by pancreatic proteases into two subunits, which remain together noncovalently as a dimeric enzyme complex (34). The disaccharidases are synthesized from a single copy gene through a single species of mRNA.
The expression of sucrase–isomaltase and lactase–phlorizin hydrolase is intestinal specific, whereas the other two enzymes are also expressed in the brush border of proximal tubules in the kidney (34).

The small intestinal mucosa displays a gradient of morphologic and functional characteristics along the duodenal–ileal (longitudinal) axis. The villus height decreases from the duodenum to ileum (20) and, consequently, the time spent by each cell on the villus before being shed decreases (31). The proliferative rate of the crypt does not change (each crypt produces on average 13–16 new cells/h) (27,28,31), but the average number of cells in each crypt decreases along the gut (18,28). Also, the number of crypts supplying each villus decreases along the longitudinal axis (28). Therefore, the surface area for absorption is maximal in the proximal gut. Morphologic differences in enterocytes along the longitudinal axis include changes in microvillus density (37) and fluidity, because of changes in the protein to lipid ratio (38). The expression of disaccharidases and other intestinal markers displays distinct gradients along the duodenal–ileal axis. Goblet cells are found with increasing frequency (39) and the Paneth’s cells increase in number (21) from the duodenum to ileum. Also, various subsets of enteroendocrine cells producing peptide hormones have a distinct distribution along the length of the gut (18).

The polarized epithelium is separated by a thin continuous basement membrane from the underlying lamina propria (35,40). Both epithelial cells and the underlying fibroblasts (35,41) contribute components of the extracellular matrix. The sheet of fibroblasts present beneath the crypt epithelium proliferates and migrates along the villi in the matrix, along with the epithelium (40,42). It has been suggested that migrating subepithelial fibroblasts deposit different matrix components along the proliferative compartment and then along the differentiation compartment, and that these different matrices signal the differentiating epithelium to switch from proliferation to differentiation (43). The significance of the epithelial–mesenchymal interaction during cellular differentiation along the crypt–villus axis has been well established (35,41) and will be discussed in the next chapter.

DEVELOPMENT OF THE SMALL INTESTINE

Both in rodents and humans, the functional development of the small intestine is carried out in two phases. The first phase of maturation follows organogenesis, where the initial enterocyte-specific differentiation is established (Fig. 1). In humans, the first phase of functional development coincides with the beginning of the second trimester and in rodents just before birth. However, our knowledge of intestinal development is incomplete for the period after 22 weeks of fetal human development until birth because of a lack of access to human tissue (9,32). Fortunately, the development of the rat and mouse small intestine is well characterized, and comparisons can be made of the similarities between rodent and human gut development to illustrate salient developmental features (1,20,33). Several characteristics are functionally conserved from Drosophila to mice to humans (43), which should not be surprising as the genetic control of gastrointestinal development is conserved from worms to Drosophila to mice. However, a few differences are seen between these two mammalian species, which will be noted as the ontogeny of the gut is discussed.
In rodents (rats and mice), gestation is 21 days (1,20), whereas in humans it is 40 weeks (9,32). During this period, embryonic and fetal development are completed and at birth the small intestine is ready to digest both milk and solid food in humans, but only milk (1) in rodents. Functional maturation coincides with the end of organogenesis as the epithelium becomes a polarized monolayer. As the columnar cells appear, they begin to express the first set of developmental markers (e.g., alkaline phosphatase and a few disaccharidases), initiating the first phase of functional development of the small intestine (1,16,17,44). Just before birth, in rodents and by 12 weeks of gestation in humans, well-developed crypts are formed (27,45). By the terminal stage of functional development, the crypt-villus architecture is established and the enterocytes begin to express the second set of developmental markers and the adult markers (13,16). This phase of development is initiated during the third trimester of human gestation and during the third week of postnatal development in rodents. These two phases of functional development can be defined using differentiation-specific enterocyte markers.
Disaccharidases are classical developmental markers. These enzymes provide excellent tools for delineating functional development because they can be quantified by a reproducible, sensitive assay. They are first detected by initial cytodifferentiation of the enterocytes, but the levels of disaccharidases vary according to species (29,36,40). In humans, lactase remains very low in utero but sucrase is very high, equivalent to levels found in infants, whereas in rodents sucrase is undetectable and lactase is raised to maximal activity. The level of maltase is low during this phase of development and reaches adult level by the terminal stage of development. However, trehalase is expressed in a similar manner to sucrase. At the end of the first phase of functional development, rodents are ready to digest and absorb only milk carbohydrates, whereas humans can digest solid food as well.

The final phase of functional development, often referred to as “terminal maturation,” is initiated during the third trimester in humans and during weaning (third postnatal week) in rats and mice. In rodents, the expression of sucrase, trehalase, glucoamylase, and maltase activities increases to adult levels (13,16). At the same time, adult markers begin to establish their gradients along the duodenal–ileal axis (16,18,46). During this period, lactase activity rapidly declines to the lower levels seen in adult rodents. Terminal maturation of the small intestine temporarily coincides with weaning, and the enzymatic changes reflect the adaptive processes necessary for survival of rat and mouse pups in the absence of maternal milk (16). It is apparent from this brief discussion of development that rodent animal models have been important in our understanding of late gestational changes in humans and early neonatal small intestinal development.

In mice, during the first phase of development, more than one stem cell is present in each crypt (i.e., the crypt is pluripotent). Just before the next phase of development, crypts become monoclonal (i.e., each crypt contains a single stem cell) (27,28,45,47). However the timing of clonality and the nature of stem cells are not known in humans and may have an important significance on age viability of preterm infants in the extrauterine environment.

REGULATION OF INTESTINAL DEVELOPMENT

The transition from the first to the terminal phase of functional development is regulated by various factors. To unravel the complex mechanisms of development, extensive studies have been done in the rodent model. Owing to the inaccessibility of human tissues and an adequate model system, few data are available on human gut development. Therefore, studies from rodents are summarized in this section as a guide to presumed human gut development. The regulators of intestinal development can be either extrinsic (luminal) factors (e.g., amniotic fluid, diet, and microbial flora), or intrinsic factors (e.g., circulating growth factors such as glucocorticoids, thyroids, insulin, epidermal growth factor [EGF], and so on), intrinsic timing mechanisms (a biological clock), and epithelial–mesenchyme interactions. The roles of these possible regulators are briefly discussed below.
Extrinsic (Luminal) Factors

Mother’s milk is a complex biological fluid that contains many substances including proteins such as casein, micelles, membranes, membrane bound globules, and viable cells. A complete citation of the macro- and micronutrients in milk is published in a recent review (48). However, detailed discussion of the role of the factors present in breast milk is beyond the scope of this chapter. Numerous growth factors and hormones are present in physiologic quantities (Table 1) and their roles are being defined in the context of intestinal development (4,49,50). Other factors in human milk such as lactoferrin are also helpful in modulating luminal protein digestion and maintaining an appropriate luminal pH, both processes being poorly developed in the newborn human gastrointestinal tract (51). As “knockout mice” models become available, the role of growth factors alone or in combination is being defined in intestinal development.

In rodents, terminal maturation of the small intestine coincides with dietary changes associated with spontaneous weaning of the pup. During the third week of life, the diet of the pup changes from high fat, low carbohydrate milk to low fat, high carbohydrate solid food. Therefore, the temporal relationship of the dietary changes could be a causal factor for the maturation of the small intestine. However,

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cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate.

experimental evidence is contrary to this view. When pups are prevented from weaning, the ontogenic changes that occur during the third postnatal week still occur (1,17). The conflicting data from early weaning experiments are difficult to interpret. In these experiments, pups were stressed by early weaning and as a result endogenous glucocorticoid secretion increased, which may be a potent modulator of the ontogenic changes of the gut (see below). This hypothesis was later supported by studies in which early weaning induced the maturational changes that normally occur during the third week in the intact pup but not in adrenalectomized rats (16,52). Therefore, despite the temporal relationship, it is unlikely that a primary causal relationship exists between the dietary changes of weaning and the final phase of the developing small intestine.

Other studies support the conclusion that a change in diet does not play a direct role in the terminal maturation of the rat small intestine. When sucrose was fed to artificially reared rat pups, precocious appearance of sucrase activity and increased cell proliferation was observed, but not in adrenalectomized pups (53). These results indicate that precocious induction of the artificially reared pups was caused by a stress-induced increase in glucocorticoid secretion resulting from the altered diet rather than as a direct dietary or weaning response. Studies using intestinal loop diversion from the luminal stream showed that direct changes in the luminal contact with food had no role in developmental induction of sucrase and maltase activities (52,54). Furthermore, in vitro studies with intestinal explants from the small intestine of suckling pups have shown no influence of nutrients in the media on the initiation of adult enzyme markers (52).

At the time of weaning, a major change occurs in the composition of microbial flora in the intestine. Thus, is it possible that the changes in microbial flora and their products could influence functional development during this period. To answer this question, experiments were carried out in germ-free rats (55,56). This study showed no difference in ontogenic changes in the small intestine when compared with conventional animals. However, developmental changes in the proliferation rate of crypt cells and migration in the adult germ-free animals were very similar to those of suckling animals. These studies suggest that changing microbial flora at the time of weaning can have a causal role in crypt cell proliferation and migration. When the germ-free animals were placed in a normal environment, gradual microbial colonization occurred in the small intestine, which was accompanied by a change in the epithelial transit time from 4 to 2 days, similar to the ontogenic changes that are known to occur during the third postnatal week (16,55–57). Therefore, maturation of the small intestine at weaning, an event similar to changes in the human at birth, is a complex process involving secondary hormonal changes and alteration in microbial flora.

**Intrinsic Factors**

Several intrinsic factors have been implicated as potential regulators in the functional development of the small intestine. Among these are circulating growth factors, a putative intrinsic timing mechanism, and epithelial–mesenchymal interaction. These three intrinsic factors are discussed in detail below.
Circulating Growth Factors

To establish a circulating growth factor as a candidate regulator of the developing small intestine (13) four important criteria must be satisfied: (a) early administration should initiate the developmental changes precociously; (b) the circulating level of this factor should increase just before the ontogenic changes; (c) this should directly induce the same maturational events in vitro; and (d) removal of the organ secreting this before its rise in the circulation should prevent the functional development of the gut.

Several factors have thus far been implicated as potential growth factors regulating the development of the rodent small intestine (1,13,17). Among these, only glucocorticoids meet all the above criteria and, thus, are discussed in detail below. The other factors that have been studied are thyroxine, insulin, gastrin, EGF, prostaglandin, transforming growth factor α (TGF-α), TGF-β, and cholecystokinin.

The pioneering work by Moog (58) initially demonstrated the role of the pituitary–adrenal axis in postnatal development of the mouse small intestine using alkaline phosphatase as a marker. Later, Doell and Kretchmer (59) identified glucocorticoids as potential regulators of developmental induction of sucrase activity in the developing rat small intestine in vivo and in vitro. Both exogenous and endogenous glucocorticoids could induce a precocious rise in various digestive enzymes and absorptive transporters in the small intestine (1,60,61). To meet the second criterion for a candidate circulatory factor modulating the ontogeny of the gut, circulating levels of free and total glucocorticoids were measured, along with jejunal lactase and sucrase activities in the same animal during the first 4 weeks of postnatal life (62). This study showed a developmental surge of circulating levels of corticosterone at the end of the second week, just 2 days before the ontogenic changes of both enzyme activities. Thus, glucocorticoids could play a role in regulating the development of the terminal maturation. In vitro studies, either with suckling or fetal intestinal explant cultures, confirmed that the glucocorticoids act directly on the immature intestine to elicit precocious maturation (1,16,52,61). These experiments strongly suggest that the glucocorticoids regulate the functional maturation of the small intestine. To establish the instructive role of endogenous glucocorticoids, adrenalectomy was performed in suckling animals before circulating levels of corticosterone rose (63,64). Adrenalectomy did not abolish the developmental changes that characterize the terminal maturation of the rat and mouse small intestine. However, it did retard the rate of increase of adult markers and delay the decline of lactase. This observation was in agreement with a study in which glucocorticoid antagonists did not totally abolish the changing pattern of enzyme activities during the third week (65). Therefore, glucocorticoids are not absolutely necessary for the final stage of functional development, but do play a critical role in regulating the rate of transition from one phase to another.

An attempt was made to determine the site of glucocorticoid action along the crypt–villus axis (66,67). Expression of differentiated villus enterocytes at the time of treatment was unaffected by steroids. After 24 hours, markers such as sucrase activity and mRNA were detected at the crypt–villus junction, and then, as time...
progressed, the activity was extended along the villi at a rate consistent with that of cellular migration. As the undifferentiated cells, in turn, differentiate and replace the villus epithelium, they begin to express adult markers such as sucrase and trehalase activity, immunoreactive protein, and mRNA (68). These results suggest that glucocorticoids act on newly divided crypt cells. However, direct action of this hormone in undifferentiated crypt epithelium has not been demonstrated. Furthermore, elegant co-culture experiments have proved the role of mesenchyme in normal and precocious glucocorticoid-induced developmental changes (see below) (35,41). Therefore, the targets of glucocorticoids could be mesenchymal cells as well (40).

The effect of glucocorticoids in suckling rodents is not confined to the small intestine, but also influences the terminal maturation of the entire gastrointestinal tract (1). Either exogenous glucocorticoids or endogenous glucocorticoids increased by corticotropin before day 10 have been shown to cause precocious induction of salivary amylase, gastrin receptor, pancreatic amylase, ileal bile salt transport, hepatic pyruvate kinase, hepatic tryptophan oxygenase, and hepatic ornithine aminotransferase, as well as to accelerate the ontogenic decline of ileal lysosomal enzymes, jejunal and ileal pinocytosis, and hepatic production of α-fetoprotein (1,16,52). Thus, glucocorticoids may act on a pathway widely used by these circulating hormones.

Glucocorticoids also play a role in changing epithelial proliferation and the migration of intestinal epithelium during the terminal phase of functional development. Crypt cell proliferation and migration, as measured by the transit time of labeled cells from the crypt to the villus tip, were dramatically increased, along with changing epithelial markers of the developing small intestine (27,31,68–70). However, these changes could be precociously induced by treatment with glucocorticoids in suckling rodents (67,68). Adrenalectomy before the rise in circulating corticosteroids prevents the developmental changes in the proliferation rate (64). Therefore, glucocorticoids play an important role not only in the terminal maturation of the digestive and absorptive function of the gut, but also in epithelial proliferation and migration. However, the mechanism by which steroids affect the cell cycle, differentiation, and migration is not known.

Noncirculating autocrine or paracrine factors may also affect the ontogeny of the intestine (13,26). New factors have been identified (71) that may play a role in human epithelial differentiation and intestinal development; however, their roles in autocrine or paracrine control of the intestinal epithelia have yet to be established (71). Direct evidence for their role in the maturation of the gut is not available because of the lack of a suitable in vitro system to study the developing small intestine (72,73). As in vitro models and human intestinal cell lines are established, the additive role of growth factors in conjunction with glucocorticoids can be determined. This information may provide a better understanding of gut development and also be used in gut repair after resection or vascular insufficiency.

An Intrinsic Timing Mechanism (a Biological Clock)

Several investigators have sought to determine the intrinsic factors controlling the terminal maturation of the rodent small intestine (74,75). Removal of endogenous
glucocorticoids (in adrenalectomized animals) delays the ontogenic changes, but does not abolish them (52,63), suggesting that the timing of transition to the terminal phase of development is not dependent on hormones. To study the ontogenic changes \textit{in vitro} was not possible, because of lack of availability of a model system (76). Explant culture is an alternative method, but such a model maintains reliable mucosal morphology only for a short period, depending on the age of the gut (74,77–80). Ménard used this model extensively to study developing gastric function and these studies will be discussed in a later chapter. As an alternative approach to determining the intrinsic capabilities of maturation in the small intestine, isograft and bypassed intestinal loops provide useful \textit{in vivo} model systems. The studies performed using these techniques with fetal and suckling small intestine suggest that an intrinsic timing mechanism or a biological clock initiates the functional development of the gut. The existence of a “hot-wired local trigger” mechanism in the small intestine has been suggested (54) and a similar hypothesis for other intrinsic timing mechanisms has been proposed in other systems (30). However, the cellular basis and the molecular nature of this clock are unknown and remain to be elucidated.

\textit{Epithelial–Mesenchyme Interactions}

The importance of epithelial–mesenchymal interactions during development and crypt–villus differentiation has been noted using various inter- and intraspecies hybrids as \textit{in vivo} grafts or \textit{in vitro} co-cultures (74,81). In all of these studies, functional development, as well as differentiation in response to mesenchyme, was assessed by species- and region-specific markers. The formation of the three-dimensional structure of crypts and villi was determined by scanning electron microscopy, and the timing and establishment of all cellular lineages were determined by enzyme markers.

The pioneering work by Le Douarin (82) has established the role of epithelial–mesenchymal interaction in the developing gut. Using interspecies recombinants composed of chick–rat and chick–human intestinal anlagans, a specific role for mesenchyme in epithelial differentiation and cytodifferentiation was established. Both epithelium and mesenchyme are necessary for proper development; however, the epithelium has an instructive role and the mesenchyme has a permissive role in epithelial cytodifferentiation of the small intestine. Furthermore, species-specific epithelial expression of disaccharidases indicates that mesenchyme cannot switch epithelial differentiation toward the species from which it was derived but can stimulate epithelial differentiation inherent to the epithelial species. A few exceptions, however, have been reported (81), indicating the inductive properties of small intestinal mesenchyme on heterologous endoderm derived from other parts of the digestive tract.

The proliferative sheath of subepithelial fibroblasts and their migration along with epithelium may contribute to epithelial differentiation (40). To establish a role for fibroblast cells in epithelial differentiation, experiments were carried out using an IEC-17 cell line, an immortalized, undifferentiated rat crypt epithelial cell (76). The
IEC-17 cells were then associated either with cultured submucosal fibroblast cells (35) in intact mesenchymal sheath (83), or with skin fibroblast cells previously cultured with primary epithelial cells from the intestine (43). The results clearly show that IEC-17 cells could complete cytodifferentiation with all four major epithelial cell types with enterocytes expressing differentiated markers. Furthermore, the inductive capacity of skin fibroblasts to differentiate IEC-17 cells only when previously cultured with primary enterocytes clearly establishes that the primary instructive signal originates from the epithelium. Similarly, differentiating primary crypt cells could induce fibroblasts to develop into muscle layers (83–85). However, the IEC-17 cells do not differentiate when cultured alone or with untreated skin fibroblasts, suggesting that these cells have the capability to differentiate only if they are co-cultured in a proper environment and with mesenchymal cells.

As stated, glucocorticoids are well established as a factor in the development of the gut. When intact or heterologous epithelial and mesenchymal recombinants were grafted in a poor hormonal environment (chicken embryo), development was arrested at the first phase (86), whereas complete maturation was observed when they were grafted into the strong hormonal environment of the adult rat (87). Furthermore, hybrid intestine in co-culture experiments showed similar results when the in vitro culture medium was supplemented with glucocorticoids (86), suggesting that their action may be mediated through epithelial–mesenchymal interactions. However, no direct evidence exists for the site of action of glucocorticoids in the functional maturation of small intestine.

On the basis of these studies, it has been suggested that the mesenchymal cells are the actual mediators of glucocorticoid action. As endodermal cells are not capable of differentiating in the absence of corresponding mesenchymal cells, it is difficult to delineate the actual mechanism. It has been postulated that glucocorticoids may affect cell-to-cell communication through the extracellular matrix components (43) or through soluble factors that activate surface receptors (88,89). When intestinal cells were cultured appropriately with mesenchymal cells of different species, the expression of enterocyte-specific markers and hormonal responsiveness appeared to be independent of the mesenchymal origin (74), suggesting that the dominant signal has to be intrinsic to the epithelium.

The mechanism by which the mesenchyme is able to transmit signals to the epithelium remains to be determined. Changes in receptor expression for the extracellular matrix components on epithelial cells are another possible explanation for the regulating mesenchymal influence. Currently, several investigators have begun to show evidence for a crucial change in the extracellular matrix during postnatal development (76,90–94). The mesenchymal interaction could also be mediated by (a) an "epithelial signal" inducing the formation of receptors in the adjacent mesenchyme, as demonstrated in the mammary gland (95); (b) production of one or more factors which, in turn, trigger a response in the epithelial cells, as in the fetal lung (96) or fetal liver (97); or (c) a combination of both types of signaling mechanism. Even a putative biological clock, responsible for the precise events leading to final maturation, could be one of these signals, rather than being a cellular component of specific tissue.
Various recent studies may have shed light on mediators of epithelial–mesenchymal interaction. The soluble protein "sonic hedgehog" (shh) is expressed by the endodermal cells (88) and determines positional information along the anteroposterior (AP) axis of the developing embryonic gut (89,98), except for the region that is destined to be pancreas. These secreted proteins induce BMP-4 (a member of the TGF-β family), which is implicated in the proper development of visceral mesoderm (89), and members of the Abd-B class of Hox genes (known regulators of body pattern) in the surrounding mesoderm of the mid- and hindgut (99). BMP-4 is another soluble factor, and its inappropriate expression in the foregut mesenchyme may convert foregut into midgut development (89). However, the role of these factors in glucocorticoid-mediated intestinal development is not known. From the information presently available, the initiation of these markers or of hormonal action could either be manifested directly by factors such as shh at the epithelial cells (which appear to have an instructive property), or when cultured with appropriate mesenchyme or factors such as BMP-4 at the mesenchymal cells (which seem to play a permissive role), or by a combination of both in the development of the gut. Elucidation of molecular action of shh, BMP-4, and various Hox genes provides a better understanding of the role of mesenchymal–epithelial interaction in developing small intestine. SMADs are cytosolic proteins responsible for the signal transduction of BMP-4 and other factors involved in TGF-β signaling (72,100), and the Smad gene has been shown to be a tumor suppressor gene in colon and pancreatic cancer (73,100). Therefore, understanding the mechanism of epithelial–mesenchymal interaction in gut development may lead to prevention of the premalignant state or to the identification of early cancer markers for gut malignancy.

In the investigations of intestinal differentiation or development, the lack of a suitable in vitro system has severely hampered any further understanding of the role of mesenchyme at the molecular level. However, new techniques such as immortalized, nonmalignant human cell lines may help answer this question.

SUMMARY AND CONCLUSIONS

The current understanding of morphologic appearance and cytodifferentiation of the intestinal epithelium and the development of the small intestine have been summarized in this chapter. The development of the human gastrointestinal tract may be different from the extensively studied rodent model. However, emphasis has been on the similarity between rodents and humans, so that studies in animal models could be correlated with the developing human small intestine, or at least provide a starting point to begin in vitro studies in human intestinal models (cell lines, organ culture, and so on). This review was purposefully devoted only to the functional development of the gut. Another important aspect of the gut ontogeny is the development of mucosal immunity, which is extensively reviewed elsewhere (10,14,101,102), and in a subsequent chapter by Brandtzaeg in this text.

The morphologic and functional development of the small intestine is divided into two distinct phases, and some of the factors important for these developmental
changes are currently being elucidated in gene knockout mice models. As these ontogenic programs are conserved from worms to insects to mice (103), it is likely that some of the fundamental components will be the same in human development as well. Knowledge of the function of these important genes will be critical for the treatment of early preterm infants with digestive disorders and adults after extensive bowel resection. As the survival of humans depends on the proper function of the gut, the care of disrupted function in the intestine of a child or an adult will depend on the developing knowledge of the gastrointestinal tract. A better understanding of development will also help in our approach to identifying the premalignant state and malignant degeneration.

ACKNOWLEDGMENTS

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FUNCTIONAL DEVELOPMENT OF THE SMALL INTESTINE


DISCUSSION

*Dr. Zoppi:* Two lactases are found in the human intestinal tract. One is a specific β-galactosidase and the second is a nonspecific esterase. Were you talking about the first or the second?

*Dr. Nanthakumar:* The first. We use a specific inhibitor that will inhibit lysosomal lactase and we can then assay for the specific activity of the β-galactosidase.

*Dr. Bäller:* In looking at the grafted intestine for 10 to 12 months, what happened to the lactase? It should have come down if it is hardwired. You have a lovely experiment to show that.

*Dr. Menard:* Have you had the opportunity to look at the response of the human xenograft to glucocorticoids or growth factors?

*Dr. Nairn:* Did you look at the biosynthesis and structural features of lactase in these human xenografts, as compared with the normal situation?

*Dr. Nanthakumar:* We are getting started on this, but mice are small, so only a very small amount of tissue is in the graft. At the moment, our success rate is approximately 50% to 70%. We are putting all our efforts in maximizing the success rate so we can do better experiments. We are also trying to use immunocompromised rats, which can accommodate much larger amounts of tissue and which withstand the surgery better. At the moment, we are concentrating on the method, although your question is one of the things we would eventually like to explore.

*Dr. Zoppi:* Only small explants are needed for these experiments—not very much material.

*Dr. Menard:* Have you had the opportunity to look at the response of the human xenograft to glucocorticoids or growth factors?

*Dr. Nanthakumar:* We are doing these experiments at the moment. We are not yet in a
position to report on this, but it looks as though glucocorticoids may have a primary effect on lactase expression.

*Dr. Lentze:* In xenografts, you have a model where the pieces of gut are completely deprived of luminal factors. So you have a model with zero luminal influence, but the clock of development is still ticking. Did you try to manipulate the mice in any way, such as fasting or overfeeding, to trigger other events in these xenografts?

*Dr. Nanthakumar:* We are at the moment trying to see if specific bacterial toxins or nutrients placed in the xenograft lumen have an effect on any of the markers we have been following. We are certainly thinking along the lines you suggest.

*Dr. Marini:* In the human preterm baby, a difference is seen if glucocorticoids are given before birth or a few days after birth. Before birth, a clear reduction is seen in enterocolitis; after birth, there may be risk of harm. It is clear that even in normal mice or rats, steroid use accelerates maturation. Is the effect the same if they are given before birth or after birth? Have you any experience with this in your model?

*Dr. Nanthakumar:* Our data suggest that if they are given at a time corresponding to prenatal administration, they will accelerate gut maturation, but we are only looking at a very limited number of markers. We need to look at a number of different transporters, immune functions, and other factors before making any statement about what is likely to be happening in the human gut.

*Dr. Marini:* Another problem is one of nutrition. These animals are fed with natural milk, which contains many factors that can affect the gut, not only nutrients but stimulating factors.

*Dr. Nanthakumar:* Indeed, growth factors found in milk have been implicated in the maturation of the gut. We are in the process of analyzing some of these at present.

*Dr. N. Wright:* You told us that 5'-bromodeoxyuridine (BrdU) inhibited the precocious action of glucocorticoids but stimulated normal development. What is the mechanism of that? Is it related to the mutagenic action of BrdU?

*Dr. Nanthakumar:* Developmental change in organs such as liver or pancreas is superimposed on differentiation in the small intestine. For example, pancreatic amylase is turned on in the differentiated cell itself, whereas in the small intestine, even in the suckling animal, the epithelial differentiation program results in the suckling animal turning on lactase and not sucrase. As the development progresses, however, the differentiation program itself is switched on and it now expresses high sucrase and low lactase. When glucocorticoids are given, it takes 24 hours to see an effect, because the hormone seems to act somewhere in the proliferated cells (1,2). However, other studies (3-5) have clearly shown that the hormone effect requires both mesenchyme and epithelium. And, depending on which species and which region of the gut and mesenchyme are being examined, essential primary signals come from the epithelium, but secondary signals, which are also important, come from the mesenchyme. As to the molecular details, we have no idea as yet.

*Dr. N. Wright:* But why does BrdU inhibit the precocious stabilization of glucocorticoids. What is the mechanism of that?

*Dr. Nanthakumar:* During pancreatic development, or hematopoietic development, myogenesis, and so on, if when the nucleosome opens up repressor protein is bound in those regions, incorporation of BrdU affects the rate of dissociation and association of transcription factors at those developmental sites. It was shown long ago that only one gene nick is required for myogenesis (6-9).

*Dr. Milla:* CDX2 and other similar transcription factors seem to be the key to how the epithelium starts to differentiate. Have you any insight into what the downstream targets of CDX2 might be?
Dr. Nanthakumar: The situation is very complicated. Two CDX forms, CDX1 and CDX2, are expressed in different compartments. CDX forms are expressed from the time of the first pre-endodermal cell from the initial state of endodermal development. However, it has clearly been shown that CDX is important in activating marker genes such as sucrase or carbonic anhydrase in the colon. However, CDXs are expressed way ahead, whereas sucrase is expressed at the right time during the postnatal development. This suggests that the elements that CDX bind are usually repressed or inaccessible to CDX during development. At the proper developmental time, however, they open up and CDX can bind and then they activate the transcription factor. However, we have no idea what the mechanism is and how it happens.

Dr. Lé: Can you tell us about the different processes that are triggered by CDX?

Dr. Nanthakumar: At the moment we know that CDX2 knockout mice do not develop, because CDX2 is necessary for preimplantation extra-embryonic endoderm. So, the fetus never implants and does not develop. Stage-specific induction of CDX2 is being developed in Trabers’ laboratory to show whether CDX2 is required for gut development, and if so at what point.

Dr. Maki: Could CDX2 be involved in TGF-β function? We have shown that TGF-β is very necessary in epithelial cell differentiation.

Dr. Nanthakumar: Drusilla Roberts has shown, using the chick gut development model, that a member of the TGF-β family called “BMP4” protein is clearly important in epithelium-mesenchyme communication and it is likely that this is the mechanism whereby the TGF-β family is important for development and maintenance. TGF-β and several other factors are extremely important in the way in which epithelium and lymphocytes in the mesenchyme respond during specific inflammatory processes.

Dr. Delvin: I have a question about CDX and the homeobox genes. I thought that homeobox genes were active very early on during embryogenesis. So, in a model such as yours, do you think you can fully appreciate the role of those genes? Has most of the cell programming not already been established in your model, so the cells are already dedicated to a particular role, even though the clock has not yet been started?

Dr. Nanthakumar: In the fly, the homeotic genes have a much simpler developmental program than in mammals. In the mouse are found four families of Hox genes that regulate anterior-posterior development, whereas in Drosophila only one family is seen. In the mammal, functional redundancy is clearly shown and these genes talk to each other. A gradient of expression of the Hox genes is necessary for proper development. However, in certain tissues such as CDX and PDX2, different homeotic genes called “nonclustered homeotic genes” are found, which are important in specific functions of specific tissues. When those genes are inactivated, they affect only certain parts of the program. To answer your question: various homeotic genes exist in epithelia and various homeotic genes in the mesenchyme, and when a proper combination of these is achieved, normal developmental progression occurs at the right time.

Dr. Ghoos: What is the physiologic meaning of having sucrase activity in the colon?

Dr. Nanthakumar: During the second trimester in the human fetus, the epithelium from the small intestine and from the colon look almost exactly the same and the colon functions like the small intestine. During the third trimester, colonic mucosa loses the villi and assumes the normal colonic appearance. Thus, at 20 weeks, the colon expresses intestine-specific markers, because it looks and functions like the small intestine. A hypothesis related to colon cancer is that the colon regresses in development and if cells are immortalized at this stage, they may express intestine-specific markers.
REFERENCES