Normal and glucocorticoid-induced development of the human small intestinal xenograft

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The primary function of small intestine is end-stage digestion and absorption of nutrients and is facilitated by the expression of specific hydrolases and transporters on the epithelium (4, 14, 19). Among hydrolases, disaccharidases are responsible for the terminal digestion of carbohydrates (9, 14, 38). With regard to disaccharidases, sucrase, lactase, and trehalase have unique substrate specificity and are synthesized from a single gene into a glycosylated enzyme anchored in differentiated epithelium (9, 14, 38). Expression of sucrase and lactase is specific to small intestine, whereas trehalase is also expressed in the proximal tubular epithelium of the kidney (9, 29, 38). Because of tissue and substrate specificity as well as the ease of biochemical quantitation, these enzymes have been used as classic markers for the last 30 years (11, 14, 19), providing an excellent tool for delineating functional development of the small intestine (12, 14, 17, 25, 29).

Functional development of the mammalian small intestine is well characterized in rats and mice (9, 11, 12, 29, 38) and is expressed in two phases. The first phase of functional development coincides with morphological development just before birth, as the epithelium becomes a monolayer with proliferating and differentiating cells compartmentalized in well-defined crypts and villi, respectively (10, 34, 45). At this time the proteins responsible for digestion and absorption of milk, such as lactase, are expressed. The second phase accomplishes the onset of the adult phenotype of the intestine during the third postnatal week (4, 9, 14, 18, 31). At this time, a number of proteins responsible for digestion and absorption of solid food are expressed. Among them, sucrase and trehalase activities rapidly raise to mature levels (9, 30, 32, 38).

However, in humans, the first phase of functional development occurs during the end of the first trimester (11, 17, 19, 21, 22, 45). Between 9 and 12 wk of gestation, stratified epithelium becomes a monolayer and crypt villus structures become established. By the end of the first trimester, tissue-specific markers such as peptidases, hydrolases, and glucose transporters are expressed in a differentiated villus epithelium. Among disaccharidases, sucrase activity is expressed at a level comparable to the mature intestine, whereas lactase is barely detectable (4, 14, 18, 19, 21). However, lactase activity increases just before birth to its highest level in the newborn infant. It has been debated whether the rise of lactase activity in the human corresponds to the induction of a second phase of functional maturation of the gut and whether the second and third trimesters correspond to the suckling period of rodents (4, 9, 14, 19). A lack of availability of human tissue has precluded testing of this hypothesis as well as identifying other markers for the late gestational development of the fetal human intestine. However, the recent demon-
stratation of disaccharidase expression in the xenograft model (36) has provided an opportunity to test this hypothesis.

In rodents, both endogenous and exogenous glucocorticoids are capable of precociously inducing the second phase of functional development during the first two postnatal weeks (9, 11, 12, 14, 15, 29). If the second and third trimesters of the fetal human intestine correspond to the suckling period of the rodent gut, then the human gut that corresponds to this period should undergo precocious maturation after exposure to exogenous steroids. With the use of lactase as a marker, sensitivity to glucocorticoids had been demonstrated in short-term organ cultures of 12- to 14-wk-old human fetal small intestine (2, 40). However, the absence of a suitable in vivo model system for a dynamic study of the developing human intestine has precluded an understanding of normal development as well as the role of glucocorticoids in functional development.

With the establishment of a xenograft model, fetal human gut subcutaneously transplanted into immunocompromised mice has the capability of regenerating and surviving in these hosts for extended periods of time (36, 37, 48). However, there has been a lack of systematic evaluation of xenografts as a function of time posttransplant to determine whether this model could be used to study the ontogeny of the human intestine in the later stages of gestation. In this report, we have begun to evaluate the potential role of xenografts in recapitulating in utero development and the glucocorticoid effect in modulating the ontogeny of the human intestine by using disaccharidases as developmental markers.

MATERIALS AND METHODS

Chemicals. Ultra-pure D-glucose, sucrose, trehalose, lactose, and bovine serum albumin were obtained from Sigma (St. Louis, MO). Protein concentrations were measured by using a BCL kit (Pierce, Rockford, IL) against BSA standards in a colorimetric assay according to the manufacturer’s protocol. Cell culture media ([DMEM] and Connaught Medical Research Laboratories [CMRL] media), nonessential amino acids, methionine, vitamin A, and penicillin were obtained from Gibco-BRL (Rockville, MD). All other chemicals were either of reagent or molecular grade. Stock solutions of cortisone acetate (Cortone; Merck, Sharp and Dohme, West Point, PA) were purchased from the hospital pharmacy. Glucose was measured by using the Trinder 100 kit from Sigma as described previously (29, 30).

Animals. Four-week-old homozygous scid and nu/nu mice were housed in a specific pathogen-free facility and maintained on rodent laboratory chow 5001 (Ralston Purina, St. Louis, MO) and water ad libitum. The animals were raised in air-conditioned quarters at 21 ± 1°C on a 12:12-h light/dark cycle with lights on at 0600. Sterilized food (rodent laboratory chow 5001) and deionized water were provided ad libitum from the day of arrival until the completion of the experiments. To avoid circadian influences, all animals were killed between 1100 and 1300. Surgery and postsurgical care were carried out according to the approved animal protocol of the research animal care committee of the Massachusetts General Hospital (MGH) as well as the guidelines published by American Physiological Society (1).

Human intestinal tissue. Twenty-week-old human small intestines were obtained from prostaglandin/saline-induced aborted fetuses with informed consent according to the regulations of the Committee for the Protection of Human Subjects from Research Risks at the Brigham and Women’s Hospital and the Human Investigation Committee at MGH. To maintain sterility, tissues were collected only from fetuses in which the abdomen had not been previously opened. These tissues were then transported to the laboratory in ice-cold fresh CMRL 1066 media containing glutamate, nonessential amino acids, penicillin and gentamicin as described before (28). Tissues were processed as described below. Fresh samples of intestine were frozen and assayed for disaccharidase activities along with xenograft tissue.

Small intestinal (duodenal) mucosal biopsies from infants and older children were obtained with informed consent by endoscopy in the Pediatric Endoscopy Suite at MGH when these children were endoscoped for diagnostic purposes. Only biopsies from patients without any histological abnormalities or pathological diagnosis were used in these studies.

Fetal small intestinal xenograft model. Sterile fetal small intestine in 2-cm segments of its mesentery was implanted subcutaneously into homozygous nude or scid mice as described previously (36, 37, 48). Clear and meconium-free jejunal tissues were used for xenotransplantation, because tissue could be easily obtained with minimal handling before surgery. On the day of xenotransplantation mice were anesthetized by avertin at a dose of 200 mg/kg body wt. Avertin consists of 1 g of 3-bromothanol and 0.62 ml of 2-methyl-2-butanol dissolved in 79 ml of deionized-distilled sterile water. Under aseptic conditions, a small incision was made in the proximal and distal end of the dorsal surface of each recipient mouse and an air sac was formed. Then, in each air sac, 2-cm segments of fetal intestine were inserted along the midline and sutured in place at the cranial and caudal ends. The skin was closed with clips, and the animals were returned to their cages. Subsequently, mice were evaluated for the next 3 days and once weekly, thereafter. Earlier experiments were carried out by using nude mice as the host. However, we were able to improve the success rate of xenotransplantation from 45 to 75% when we used scid mice without any difference in the development of the xenografts. Only successful grafts were used for analysis. Animals were killed subsequently and human xenografts were collected and frozen for subsequent biochemical analysis and a section of tissue was processed for histochemistry. Treatment with glucocorticoids was carried out by a single subcutaneous injection of cortisone acetate (CA) suspension at a dose of 50 mg/100 g body wt.

Disaccharidase activities. The region of the small intestine from the stomach to the ligament of treitz was defined as the duodenum, the proximal half of the reminder of the gut was defined as jejunum, and the distal half as ileum for subsequent analyses. The lumen of each section was rinsed with sterile DMEM before freezing for subsequent analysis. Various regions of both human gut and xenograft were used to determine the level of sucrase, lactase and trehalase as described previously (29, 30). Tissues were excised and flushed with ice-cold saline and homogenized in 9 volumes of 0.15 M KCl by using a Potter-Elvehjem Teflon-glass homogenizer. The tissue homogenate was then assayed in duplicate by using ultrapure sucrose, lactose, and trehalose as substrates. The amount of glucose liberated by these disaccharidases was quantitated by using a glucose-oxidase method (Trinder 100 kit from Sigma) as described previously (29, 30). Protein concentrations were measured by using a BCL kit (Pierce) against BSA standards according to the manufac-
tioner’s protocol. Enzyme activity was expressed as micromoles of substrate hydrolyzed per hour per milligram of total protein.

**Processing of tissue for immunoprecipitation.** The xenograft and biopsy samples were biosynthetically labeled as previously described (27). Tissues were labeled continuously with 100 μCi of [35S]methionine (Amersham, Arlington Heights, IL) and 100 μCi of [3H]glucosamine (Amersham) in media for 90 min before being homogenized at 4°C in 250 μl of ice cold saline and stored at -80°C until assayed. The tissue homogenate was then cleared of debris by centrifugation at 1,000 g for 30 min at 4°C and the supernatant was processed for immunoprecipitation as described previously (27). Brieﬂy, 100 μg of protein were precleared twice with 25 μl of protein G-Sepharose beads before being incubated with antibody and then protein G-Sepharose complex for 1 h, respectively, at 4°C with shaking. The beads were then spun down in the cold room and washed three times at 4°C before being denatured in a SDS-sample buffer and fractionated in SDS-PAGE gels. The gels were then incubated in Autoﬂuor (National Diagnostics, Atlanta GA) for 45 min at room temperature before being dried and exposed to Kodak X-Omat ﬁlms. The speciﬁc antibodies for lactase (HBB4/14), sucrase (HIS4/34/4/1), CDX-1 (CPSP) and CDX-2 (CNL) were generous gifts of Drs. Buford Nichols (Houston, TX) and Debra Silberg (Philadelphia, PA) (35, 39). Antibodies for GAPDH were obtained from RDI Research Diagnosis (Flanders, NJ) and all other reagents, including secondary antibodies were obtained either from Santa Cruz Biotech (Santa Cruz, CA) or Jackson ImmunoResearch (West Grove, PA).

**Histology.** Specimens were ﬁxed in 4% paraformaldehyde for 4 h at 4°C and then overnight in PBS containing 40% sucrose at 4°C before being embedded either in parafﬁn or in Tissue Tek optimum cutting temperature (O.C.T.) compound for frozen sections. The parafﬁn and frozen sections were cut at 5- and 7-μm increments, respectively, for staining as described previously (28).

**Statistics.** Results are presented as means ± SE. Effects of age and treatment on enzyme activities were analyzed by a two-way analysis of variance. After the overall signiﬁcance was conﬁrmed, post hoc tests for individual variables were performed by a two-tailed unpaired t-test. Differences with a P value < 0.05 were considered signiﬁcant.

**RESULTS**

**Sucrase, lactase, and trehalase activities in the 20-wk-old human fetal gut.** Various regions of the gut were separated into duodenum, proximal and distal jejunum, and proximal and distal ileum, ﬂushed with ice cold saline and stored at -20°C until assayed. The level of sucrase, lactase, and trehalase activity was determined as described above (Fig. 1). Sucrease activity showed a gradient of expression with the lowest levels in the duodenum and distal ileum and the highest levels of activity in the distal jejunum, whereas trehalase activity showed a decreasing gradient along the length of the small intestine. The distribution of both sucrase and trehalase activities along the anterior-posterior axis of the small intestine was similar to that described for rats and mice (9, 11, 19, 21). The distribution of trehalase in humans has not previously been described but appears to be similar to that of the rodent (14, 30). Overall lactase activity was very low throughout the small intestine, barely being detectable in the duodenum and uniformly expressed at the lower levels in the remaining regions of small intestine. As
reported previously (11, 21), a gradient of lactase activity had not been established by 20 wk of gestation.

Development of the fetal jejunum as a xenograft. Twenty-week-old fetal jejunal sections were xenotransplanted into immunocompromised mice and followed for 24 wk. Successful grafts revascularized and developed into small intestine. A minimum of six xenografts was harvested at 9, 19, 22, and 24 wk after transplantation. Only those tissues that showed an intact gut morphology were used for further biochemical analysis.

A representative morphological section of xenograft is shown in Fig. 2. The jejunal xenograft after 6 (Fig. 2A) and 24 wk (Fig. 2, B–D) was fixed, sectioned, and stained with toluidine blue. Figure 2A shows a developing xenograft 6 wk after implantation. The lumen is covered with an undifferentiated epithelial monolayer, without crypt-villus orientation. Figure 2, B and C clearly depicts the xenograft 6 mo after transplantation with a well developed crypt-villus architecture. A clearly differentiated epithelium with enterocytes, endocrine, and goblet cells can be seen in the villus. A well-differentiated glycocalyx can also be seen on the apical surface of enterocytes. The frequency of the goblet cells is much higher than in normal tissue and as expected, they can be seen in both the villus and crypt compartments. Figure 2D is a representative section through the base of a crypt, where granules within paneth cells can be clearly seen, demonstrating the differentiation of proliferative cells migrating toward the crypt base.

To determine the functional development of these tissues, after taking organ culture samples for metabolic labeling, we homogenized and assayed the remaining tissues for sucrase, lactase, and trehalase activities. These data from jejunal xenografts are shown in Fig. 3. Nine weeks posttransplantation, sucrase, trehalase, and lactase activities in intestinal xenografts were not significantly different from that of the 20-wk-old (Fig. 1) fetus before xenotransplantation (P > 0.3). In addition, the level of sucrase activity was not significantly different between 9 and 24 wk (P > 0.45), reflecting the normal, predicted ontogeny of this enzyme in the jejunum. Trehalase activity in xenograft tissues reflects a developmental profile similar to sucrase. Between 9 and 24 wk, trehalase activity in the jejunal xenograft was not significantly different from the 20-wk-old (Fig. 3) prexenograft fetal jejunum (P > 0.3). In contrast, lactase activity was expressed at very low levels by 9 wk and was comparable to the prexenograft fetal jejunum. However, lactase activity gradually increased between weeks 9 and 24 posttransplantation (P < 0.03) reaching the level of the newborn and thus appears to recapitulate the level of the newborn for the human small intestine in utero.

Fig. 2. Morphological development of human fetal jejunum xenografted subcutaneously into a nude (nu/nu) mouse after a 6-wk and 6-mo period. A: early development of the xenograft in which undifferentiated cells are proliferating and covering the lumen. The tissue lacks a complex villus and crypt structure. Cells also lack brush-border and goblet cells indicating an undifferentiated state. B, C, and D: spatial compartmentalization of proliferative crypts (C) and differentiated villus (V) epithelium with goblet cells (∗) in both compartments separating the lumen (L) from the mucosa, the appearance of brush-border (arrow) and paneth cells (P) containing multiple granules. (A–D: ×63; toluidine blue).
These enzyme activities for sucrase and lactase were confirmed by immunoprecipitation after the tissue was labeled with [35S]methionine (Fig. 4). The lactase protein was absent in 12-wk-old xenograft, but present at low levels in 20 wk and was comparable to the enzyme levels found in 24-wk-old xenografts. However, the level of sucrase protein was unchanged at all stages (Fig. 4A). Because CDX-1 and -2 are two unique transcription factors expressed only in the gut and have been implicated in the expression of these disaccharidases, the level of these proteins along with lactase and sucrase was determined (Fig. 4A). Again, the low lactase expression increases in 14- to 22-wk-old xenografts, but the levels of sucrase and CDX-1 and -2 remained unchanged. The level of sucrase and lactase protein follows the profile of enzyme activity and suggests that the development of the jejunal xenograft follows a predicted ontogeny even in the absence of luminal stimuli. Tissue-specific transcription factors, CDX-1 and -2, appear unlikely to play a role in these developmental changes in human intestinal xenografts.

The effects of cortisone acetate on jejunal xenografts. At 20 wk posttransplantation, half of the animals with jejunal xenografts were either injected with a CA suspension or a saline vehicle. Seven days after treatment, xenografts were harvested and processed for histology and biochemical analysis, as described previously. The data for sucrase, trehalase, and lactase activities from jejunal xenografts are shown in Fig. 5. In a manner similar to that of 20-wk-old fetal intestine (Fig. 3), saline-treated tissue expressed high sucrase and trehalase activities but low lactase activity. Treatment with CA had no significant effect on sucrase (P > 0.47).

Fig. 3. The development of sucrase (A), trehalase (B), and lactase (C) activities in jejunal xenografts. Jejunum was assayed before xenotransplantation (Age: 0) and then subsequently harvested at 9, 19, and 24 wk posttransplantation. Enzyme activities are expressed as micromoles per hour per milligram protein. Results are reported as means ± SE. The number of xenografts for each data point are from 6 specimens from 5 different experiments.

Fig. 4. Developmental expression of sucrase (Suc), lactase (Lac), and GAPDH protein in human xenografts by immunoprecipitation. A: ontogeny of lactase and sucrase in 12-, 20-, and 24-wk-old human xenografts was compared with an infant biopsy (Bp) after metabolic labeling studies. B: expression of CDX-1 and CDX-2 was determined along with lactase and sucrase in 14- and 22-wk-old human xenografts. Unlike lactase, the levels of sucrase, CDX-1, and CDX-2 protein were unchanged. Results of preliminary experiment with reproducible results are shown.

Fig. 5. Effect of cortisone acetate (CA) on the developing intestinal xenograft. Twenty-week posttransplanted jejunal xenografts were injected either with a single injection of CA at 50 mg/100 g body wt or a identical volume of saline vehicle (VEH). Seven days after treatment xenografts were harvested and assayed for sucrase, trehalase, and lactase activities. Enzyme activities were expressed as micromoles per hour per milligram protein. Results are reported as means ± SE. Six xenografts were used to obtain each data point.
Morphological development. This stage in rodents thus the pups are born just after the gut has undergone intestine is largely unknown because of the lack of available tissue and the lack of an appropriate model. Studies Committee at the Massachusetts General Hospital. Table 1 shows the enzyme activity in 2- to 6-mo-old proximal intestine from infants and the level of activity from 24-wk posttransplanted jejunal xenografts. Except for trehalase activity, all other enzyme activities were comparable to xenografts at 24 wk posttransplantation. The overall results again suggest that by 24 wk posttransplantation, xenograft tissue had attained developmental levels that correspond to those of young infants. The discrepancy in trehalase activity could be due to the location of biopsy tissue. The proximal biopsy tissues were obtained before the ligament of treitz (duodenum), whereas tissues from jejunal xenografts were used as 20-wk-old fetal intestinal tissue.

DISCUSSION

In utero development, especially during the second and third trimester of gestation in the human small intestine is largely unknown because of the lack of available tissue and the lack of an appropriate model system that can recapitulate the ontogeny. In rats and mice, the in utero development lasts only 21 days and thus the pups are born just after the gut has undergone morphological development. This stage in rodents corresponds to the 12th wk of human development. For the first time, in this report, we provide preliminary evidence that human xenografts may be used as a simulated in vivo model for the developing human intestine. Using immunocompromised mice as the host for xenotransplantation of 20-wk-old fetal human gut, we have begun to systematically characterize the maturation of jejunal xenografts by using disaccharidases, a classic developmental marker. In this study, development of the xenograft has been shown to recapitulate the predicted in utero development of the human small intestine (9, 11, 14, 21, 22) (Fig. 6). Unlike the short-term organ culture model (2, 23, 24, 40), xenografts provide an attractive long-term approach to delineating complex physiological and genetic signals that may regulate the development of the human intestine. Furthermore, this technique may also provide an appropriate model to study the effect that luminal components, such as amniotic fluid, nutrients, and microbes, may have on the developing human gut.

In these studies, we have used 20-wk-old fetal tissues for xenotransplantation, whereas other studies (36, 37, 48) have used 10- to 14-wk-old fetal tissue. Thus it was not clear whether 20-wk-old tissues were capable of regenerating as a xenograft. The amount of tissue and availability is the primary reason for choosing 20-wk-old fetal gut. Moreover, previous studies used fragmented intestine in which the specific region of the transplanted gut could not be identified, whereas by using 20-wk-old tissue, we could transplant sections of duodenum, jejunum, and ileum separately. Because sterile fetal gut from the intact fetus at 20 wk could be obtained, there is much less chance of infecting the vulnerable scid mouse host. However, in both cases the transplanted tissue undergoes initial degeneration followed by angiogenesis and reepithelialization of the luminal surface of the xenograft. When the xenograft reepithelializes, it does so naturally as pseudostratified epithelium resembling the gut before morphological development (that occurs before 10 wk) of fetal life (data not shown). By 6 wk postxenotransplantation, the epithelium of the xenograft becomes a monolayer without any sign of cytodifferentiation (Fig. 2A). After these changes, crypts and villi are formed with proliferative and differentiated cells compartmentalized at these sites, respectively. Thus regardless of the age of xenograft according to the theoretical model.

Table 1. Specific activity of disaccharidases from biopsies and mature xenografts of proximal small intestine

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Biopsy</th>
<th>Xenograft</th>
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<tbody>
<tr>
<td>Sucrase</td>
<td>4.82 ± 0.56</td>
<td>5.32 ± 0.35</td>
</tr>
<tr>
<td>Lactase</td>
<td>2.21 ± 0.48</td>
<td>2.41 ± 0.23</td>
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<tr>
<td>Trehalase</td>
<td>4.62 ± 0.39</td>
<td>2.6 ± 0.19</td>
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Values are means ± SE of μmol·h⁻¹·mg protein⁻¹.
the fetal tissue used for xenotransplantation, on revascularization, the epithelium regenerates with an appropriate spatiotemporal organization of crypt-villus units.

Week 12 of human in utero development marks the completion of morphological development resulting in a monolayer of epithelial cells. In addition, proliferative and differentiated cells are compartmentalized into crypts and villi, respectively. These major developmental events are accompanied by the expression of digestive enzymes on the brush border of villus enterocytes. This first step in functional development is characterized by the induction of sucrase in humans and lactase in rodents. The second and final stage of functional maturation is characterized by the induction of lactase in the human at the end of the third trimester (Fig. 6) and by sucrase in rodents during the third postnatal week (9, 14, 29, 38). When tissue was collected from the fetus, the anterior-posterior (cephalocaudal) distribution of disaccharidases was determined. Twenty-week-old duodenal tissue was separated and the remaining jejunum and ileum were further subdivided into anterior and posterior regions. Specific disaccharidase activities were then determined. At 20 wk, the level of sucrase in human small intestine had reached almost the level of the newborn (Table 1), whereas, lactase, as reported previously (11, 14, 21), remained at a very low level. Figure 1 illustrates the anterior-posterior gradient of sucrase activity and by 20 wk, it has reached the distribution characteristics of the adult (21). Sucrase activity was low at either end of the small bowel with maximal activity being detected in the middle region of the small intestine. Lactase activity remained at a basal level throughout the entire small intestine, as reported previously (11, 21).

Development and distribution of human trehalase activity in the small intestine heretofore has not been reported. Recently trehalase has been used as a nontoxic cryoprotectant of enzymes, membranes, vaccines, animal and plant cells, and organs for surgical transplant (33). It has been predicted that trehalase can also be used as an ingredient for dried and processed food. With these recent biotechnological applications for the use of trehalase (33), it becomes important to determine the ontogeny of trehalase activity. In rodents, the induction of trehalase parallels sucrase during the third postnatal week (9, 30, 32). In this study, the ontogeny of trehalase activity, like that of sucrase, was accomplished during the first phase of functional maturation of the human gut. The activity was maintained at that level in the newborn that is sufficient to prevent diarrhea if overly exposed to trehalose after birth. These data suggest that trehalase and foods such as mushrooms containing trehalose can be used as a preservative or as a nutrient in infant food without producing a harmful effect. Only the infant with primary trehalose intolerance would not be able to handle this sugar.

Fetal human xenografts also recapitulate the complex heterogeneity of the epithelium. With the use of this approach Fig. 2, B–D, shows fully developed jejunal xenografts 6 mo posttransplantation with a differentiated epithelium displaying all four major cellular lineages including the presence of enterocytes, goblet cells, and endocrine cells in the villi and paneth cells in the crypt base suggesting the establishment of bidirectional epithelial differentiation. This report is consistent with previous studies (36, 37, 48) despite using older tissue for xenotransplantation suggesting that 20-wk-old human fetal gut possesses the same regenerative capacity as the younger tissues. These data on the ontogeny of sucrase and lactase activities in the xenograft suggest that the fetal human intestine reestablished its ontogenic identity after xenotransplantation and then proceeded to mature according to its predisposed genetic program. Because intact gut of the 20-wk-old fetus can be obtained, the xenograft could potentially provide a model to study the development as well as the pathophysiology of disease in the preterm infant gut.

Both endogenous and exogenous glucocorticoids are capable of accelerating the second stage of functional development in the rodent small intestine (4, 5, 7). Due to the lack of a model system, the long-term effect of glucocorticoids in human gut development is not known. However, by using 12- to 14-wk-old fetal tissue for 5 days in organ culture, it has been shown that glucocorticoid is capable of stimulating an increase in lactase activity but not sucrase or trehalase activities (2, 40). A similar effect with EGF was observed for lactase in this same organ culture system (22, 23). The mean level of serum cortisol decreases from 8.4 to 4 ng/ml from the 16th to the 20th week of gestation, but gradually increases ≈20 ng/ml by 35–36 wk and then rapidly reaches 45 ng/ml during the last 4 wk before birth (26). This surge in circulating cortisol in the human fetus precedes a rapid increase of lactase activity in the developing fetal small intestine (11, 22). These changes may explain the effectiveness of cortisone in premature infants and suggests an important protective role for this hormone for the developing gut (3, 6, 7, 13, 41). The increasing circulating levels of cortisol during fetal development complements the biphasic model of functional maturation of the small intestine (Fig. 6) during the third trimester. Only 25 or 50 ng/ml but not 12.5 ng/ml of hydrocortisone is able to induce lactase activity in the organ culture system by using 12- to 14-wk-old fetal human intestine (2). These data are consistent with the levels of circulating cortisol during the late second and early third trimester and capable of precociously inducing lactase activity only when the level was elevated beyond 20 ng/ml to between 25 and 50 ng/ml. The studies presented here also suggest that the xenograft model can be used as an in vivo model system for the developing human intestine and exogenous glucocorticoids are capable of accelerating the maturation of the immature gut. The induction of lactase activity is reflected at the level of its mRNA in organ culture (46). Our preliminary data from the intestinal xenograft also suggest that the induction of lactase activity by glucocorticoids is reflected at the
level of lactase mRNA (Nanthakumar and Walker, unpublished observation). However, for a comprehensive understanding of the functional development of the fetal human intestine, we must include identification of other regional and developmental-specific markers in the xenograft model and compare their expression to that of the biopsies of the infant intestine. Our preliminary studies fail to support a role of tissue-specific transcription factors CDX-1 and -2 in these developmental changes although they have been implicated in the expression of both sucrase and lactase gene expression (8, 20, 22, 44). However, recent studies suggest that the combination of these factors along with other transcription factors may be more significant in the development of sucrase and lactase (8, 20, 44). Those studies are underway to further validate the suitability of the xenograft as an appropriate model for the developing human gut.

Another important aspect of the glucocorticoid effect on the developing small intestine is that the steroid is effective during a brief period. The rat and mouse small intestine responds to glucocorticoids from the time of birth until postnatal day 16 (29). If there is a period of glucocorticoid sensitivity present in the developing human intestine, then glucocorticoids should be effective only during that period. To support this, a meta-analysis of randomized controlled trials of corticosteroid treatment to prevent respiratory distress syndrome in preterm infants (6, 7) suggests that the effectiveness of this steroid is lost by 34 wk of gestation, suggesting the existence of a glucocorticoid sensitive period in the developing human and presumably the human intestine during the third trimester. This is further suggested in studies using corticosteroids to prevent necrotizing enterocolitis, an inflammatory condition in preterm infants. In these studies, protection was considerably greater in mothers given corticosteroids in utero earlier than infants treated postnatally (3, 5, 7). With the use of lactase as marker, studies are presently underway to determine whether the intestinal xenograft exhibits a restrictive period of glucocorticoid sensitivity.

With the use of the xenograft model system, it may be feasible to identify other markers of the gut that display developmental profiles similar to lactase. Furthermore, it will be important to determine the development of other genes responsible for intestinal functions such as the mucosal immune system. Understanding the ontogeny of the epithelium with regard to the mucosal immune system will also be important in delineating the pathophysiology of intestinal infectious diseases in premature infants, such as necrotizing enterocolitis (3) and toxigernic diarrhea (16). Because xenografts form a lumen, it could be an appropriate model to assess the role of luminal signals and/or factors in its ontogeny. During the second and third trimester, the luminal surface is bathed in amniotic fluid that is significantly enriched in various trophic factors (22, 47). In addition, colostrum and mature breast milk contain similar trophic factors (5) and can interact with the premature gut surface. By systematically adding these trophic factors to a defined media and infusing it into the lumen of xenografts, the role of luminal signals in the development of the small intestine can be studied. It is well established that glucocorticoids play a pivotal role in gut development in other systems (42, 43). Thus the xenograft model could be useful in determining the role of steroids and other hormones or trophic factors in the development of the human small intestine in the prevention of age-related diseases.

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