Paneth cells, secretory epithelial cells of the small intestinal crypts, are proposed to contribute to local host defense. Both mouse and human Paneth cells express a collection of antimicrobial proteins, including members of a family of antimicrobial peptides named defensins. In this study, data from an anchored polymerase chain reaction (PCR) strategy suggest that only two defensin mRNA isoforms are expressed in the human small intestine, far fewer than the number expressed in the mouse. The two isoforms detected by this PCR approach were human defensin family members, HD-5 and HD-6. The gene encoding HD-6 was cloned and characterized. HD-6 has a genomic organization similar to HD-5, and the two genes have a striking pattern of similarity localized chiefly in their proximal 5'-flanking regions. Analysis of human fetal RNA by reverse transcriptase-PCR detected enteric defensin HD-5 mRNA at 13.5 weeks of gestation in the small intestine and the colon, but by 17 weeks HD-5 was restricted to the small intestine. HD-6 mRNA was detectable at 13.5-17 weeks of gestation in the small intestine but not in the colon. This pattern of expression coincides with the previously described appearance of Paneth cells as determined by ultrastructural approaches. Northern analysis of total RNA from small intestine revealed quantifiable enteric defensin mRNA in five samples from 19-24 weeks of gestation at levels approximately 40-250-fold less than those observed in the adult, with HD-5 mRNA levels greater than those of HD-6 in all samples. In situ hybridization analysis localized expression of enteric defensin mRNA to Paneth cells at 24 weeks of gestation, as seen in the newborn term infant and the adult. Consistent with earlier morphological studies, the ratio of Paneth cell number per crypt was reduced in samples at 24 weeks of gestation compared with the adult, and this lower cell number partially accounts for the lower defensin mRNA levels as determined by Northern analysis. Low levels of enteric defensin expression in the fetus may be characteristic of an immaturity of local defense, which is thought to predispose infants born prematurely to infection from intestinal microorganisms.

During human fetal organogenesis the intestine undergoes a dramatic transformation, characterized by morphological changes of mucosal epithelial cells and the establishment of a crypt-villus axis (1). Once the epithelium of the small intestine is mature, there is continuous cellular renewal, with evidence of all epithelial cell types arising from common progenitor stem cells (2). This dynamic epithelium has many physiological functions, including a role in host defense. Of the epithelial cell types, Paneth cells, intensely eosinophilic cells located at the bases of intestinal crypts, have ultrastructural hallmarks of secretory cells and are most abundant in the ileum (3-6). Several lines of evidence suggest that an important physiological role of Paneth cells is the synthesis of host defense effector molecules such as lysozyme (7-9), phospholipase A2 (10-12), and antimicrobial peptides (13-19).

Antimicrobial peptides are a prevalent mechanism of host defense utilized by phylogenetically diverse animal species, from insects to humans (for reviews see Refs. 20-22). Defensins are a large family of antimicrobial peptides, identified originally in leucocytes of rabbits and humans (for reviews see Ref. 23). These cationic peptides are 30-35 amino acids in length and are distinguished by a conserved cysteine motif (23). Defensins are membrane active and have microbicidal activity toward a wide range of microorganisms in vitro (23). In leukocytes, these peptides are stored in cytoplasmic granules and are released into phagolysosomes where they contribute to the killing of engulfed microorganisms (24).

More recently, molecular studies have identified a distinct group of enteric defensin genes expressed in Paneth cells of the mouse (13, 25) and humans (18, 19). In the mouse, there is evidence for the expression of 16 or more defensin genes in the small intestine (26), whereas only two human homologues have been identified at this site (18, 19). Mature enteric defensin peptides have been isolated from the murine small bowel, but the homologous human peptides have not yet been isolated (15-17, 26). The murine peptides were found to have antibiotic activity comparable with the previously isolated myeloid counterparts from other species. Activity of two mouse enteric defensins against the intestinal parasite Giardia lamblia was also reported (27).

It has been suggested that immaturity of local intestinal defenses may contribute to the increased susceptibility of neonates to infections from luminal flora and to necrotizing enterocolitis (28, 29). Therefore, we sought to more clearly characterize defensin expression in Paneth cells of the human small intestine.
intestine, with a focus on fetal expression. We observed that defensin expression coincides temporally with Paneth cell detectability and may be a useful marker of these cells. The developmental profile observed suggests that low level defensin expression in fetal development may be characteristic of immaturity enteric mucosal defense. This work also addresses the nature enteric mucosal defense. This work also addresses the expression in fetal development may be characteristic of immaturity enteric mucosal defense. This work also addresses the

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**MATERIALS AND METHODS**

Reagents and general methodology for cloning, sequencing, probe labeling, and PCR amplification were described (18). Human fetal intestinal tissue from second trimester abortuses was obtained and used in accord with guidelines established by the Institutional Review Board at The Children's Hospital of Philadelphia and with permission from the Central Oxford Research Ethics Committee. Sequence data were analyzed using MacVector software (IBI, New Haven, CT).

**Genomic Cloning**—The primary screen of a human genomic library (XFLX phage vector, S44201, Stratagene) was conducted at a phage density of 5000 plaques per 35-mm diameter plate using 24 dishes of a single filter lift (Colonies/Plate Screen, DuPont NEN) were hybridized with 32P-labeled HSIB-309a (TCATCCCTCAGAGGCAGCAGAATCT-TGTTTTATCCATGAGTACG) in 25% formamide, 5 × SSC (1 × SSC = 0.15 M NaCl, 0.15 M sodium citrate, pH 7.0), 1 × Denhardt's, 100 μg/ml yeast RNA, and 1% SDS at 42 °C overnight. The filters were subsequently washed in a sodium wash in 2× SSC/1% SDS at 42 °C for 1 h. Positive clones identified by autoradiography were purified by additional screens at lower density. Phage insert DNA from positive clones was subcloned into the multiple cloning site of pBluescript II SK+ plasmid for further analysis as described (18).

3′ Rapid Amplification of cDNA Ends Analysis—Total RNA from adult human small intestine (full-length) was obtained from a commercial source (Clontech, Palo Alto, CA). Single-strand cDNA synthesis was employed a modified oligo(dT) primer (CCTCTGAGTTCTTCGAGTCAGATGAGGATTGCCAGACGTTTCAATGAAAAGGAGCTGAGGATGATC) and HD-6/405a (TGGATGGCAATGGTCCAGTTTGAAGCAGGAGAATGGCAGCAAG), were at reverse transcription (RT)-PCR was carried out in a modified version of that described previously (34, 35). Briefly, for each reaction, cDNA was synthesized using an antisense primer complementary to the gene fragment of interest, together with an equal concentration of an antisense primer corresponding to the housekeeping gene β-glucocerebrosidase reaction. The antisense primers were allowed to anneal to 1 μg of total RNA from each tissue sample at 65 °C for 10 min, and then reverse transcriptase was used to generate cDNA from each gene during a 60-min incubation at 42 °C. PCR amplification was then performed using the following conditions: 94 °C for 5 min, then 30 cycles of 60 °C for 1 min, 72 °C for 1 min, and 94 °C for 1 min, with a final elongation of 72 °C for 5 min. The following gene-specific primers were used: 1) for HD-5, DEF5a (CCCGCCATGACCGACCGATCC) and DEF5b (TCTATCTAGGAAGCTGAGG), generating a 304-bp product; 2) for HD-6, primers HD-6/402s (CCACTCCAGGGGTAGGATGATC) and HD-6/405a (TGATGCGAATGTTGGAACACAC), generating a 326-bp product; and 3) for β-glucocerebrosidase, GDB/A (CAGATCTTTTGGAATGTCG) and GDM1/DB9 (GACTGTCGACAAAGTGCCG), generating a 572-bp product. The PCR products were resolved by electrophoresis in 1.5% agarose gels. The specific DNA fragments generated by each PCR reaction were verified by direct sequence analysis. RT-PCR primer pairs will amplify a product from genomic DNA, but the product will be substantially larger than from cDNA, because the forward and reverse primers lie in different exons. Controls with no reverse transcriptase in each set of reactions were found not to amplify a product from genomic DNA that might have contaminated the RNA preparations.

Northern analysis was performed as described (18, 19). For HD-6 mRNA detection, HSIB-309a was employed, and for HD-5, HSIA-309a was used. In parallel, nylon membranes spotted with plasmid DNA containing inserts encoding HD-5 and HD-6 were hybridized and washed simultaneously with the Northern bird filters to control for stringency. The control glycolaldehyde-3-phosphate dehydrogenase probe was hybridized using an identical hybridization solution at 42 °C, and the conditions of the final wash were 0.1 × SSC/0.1% SDS at 50 °C for 30 min. The washed filters were exposed to film using an intensifying screen at −70 °C for 2 weeks. The blots were then stripped of probe by soaking in 0.1 × SSC/0.1% SDS at 60 °C for 1 h. Efficient stripping of the probe was documented by autoradiographic exposure for 2 weeks.

**Cloning of Enteric Defensin Gene HD-6**—As a step toward understanding the molecular details crucial to developmental regulation and tissue-specific expression of enteric defensins, the transcribed and flanking regions of the HD-6 gene were cloned. Six genome equivalents of an amplified human genomic library were screened with a HD-6 probe (19). DNA from six isolated clones was digested with several restriction enzymes, and restriction fragment length patterns indicated that each clone was unique, but all of them contained overlapping restriction fragments from the genome. A 6-kilobase EcoRI fragment from one clone (HEG-101) was subcloned and used for sequence analysis (Fig. 1).

**Comparison of the genomic (Fig. 1) and the HD-6 cDNA sequences** (19) indicated that the gene consists of two exons,

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1 The abbreviations used are: PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; RT, reverse transcriptase; bp, base pair(s).
separated by a 914-bp intron. The nucleotide sequence of the putative exons in the genomic clone are in incomplete agreement with those in the cDNA sequence. There are consensus sequences for splice junctions (Fig. 1, bold) and polyadenylation (Fig. 1, boxed). There is a TATA box at nucleotides 1341–1347, beginning 28 nucleotides upstream from the 5'9' terminus of the two most extended cDNAs identified by RACE-PCR (see below, Fig. 1, underlined). A CAAT box is seen at position 1278–1283 (Fig. 1, underlined).

To define the 5' transcription start site of the HD-6 gene, small intestinal cDNA (18) was amplified using the 5'9'RACE-PCR technique (32). The downstream primer (HSIB309a) was chosen from the second exon, and the upstream primer was complementary to the anchor sequence. Amplified products were subcloned and sequenced. The amplified cDNA sequence was identical to the corresponding region of the genomic sequence (data not shown). Four products were found to extend 5' to the putative initiating methionine codon. Three of these terminated 41 nucleotides upstream of the methionine codon. This site of transcription initiation is designated +1.

A dot matrix analysis of nucleotide similarity of HD-5 and HD-6 is shown in Fig. 2A. Various degrees of sequence identity were seen along a diagonal throughout the entire gene sequences. The most striking identity is observed in the proximal 5' region encompassing the first half of exon 1 and nucleotides of the proximal 5'-flanking region (Fig. 2B). Several consensus sequences corresponding to transcription factor binding sites were identified in the flanking region, including two AP2 sites (38) (2784 and 21344) and six nuclear factor interleukin-6 sites (39) (2244, 2305, 2650, 2788, 2863, and 21292). Several of these sites are found in the same location within the HD-5 flanking region, such as AP2 (2781) and nuclear factor interleukin-6 (2651 and 21284), suggesting these sites may prove to be functionally significant.

When HD-6 is compared with the hematopoietic defense gene HNP-1, there is also similarity detected between this 5' region of HD-6 and the 3' region of the first intron of HNP-1 (40) and HNP-4 (A. Tremblay and S. Solomon, GenBank accession number U18745) (data not shown). A similar observation was described previously for a comparison of HD-5 with hematopoietic defensins (40, 42).
3’ RACE Analysis of Small Intestinal cDNA—To investigate the possibility that previously uncharacterized defensin mRNA isoforms are expressed in the human small intestine, an anchored PCR strategy was employed, capitalizing on the highly invariant 5’ nucleotide sequences found in defensin mRNA from all species and tissues studied to date (Fig. 2; Ref. 18). PCR primers, DEF15s and DEF15sI, were selected from this region of high nucleotide similarity (Fig. 3A). RNA from the full length of human small intestine was reverse transcribed using a modified oligo(dT) primer that contained a flanking anchor sequence. The resulting cDNA template was then used in a PCR amplification using either of the two upstream defensin primers and the downstream anchor sequence primer. A product of approximately 450 bp was obtained from each reaction (Fig. 3B), and Southern blot hybridization using an internal probe from the common sequence of defensin cDNA demonstrated strong hybridization (data not shown). The PCR products were subcloned, and 78 individual colonies were isolated.

**Fig. 2. Gene sequence analysis.** A, a Pustell analysis of human enteric defensin genes HD-5 and HD-6. Sequence similarity was analyzed using a window of 14 nucleotides and was scored positive for a match of 10 of 14 (hash value = 1). A nearly identical pattern of nucleotide similarity was obtained when the analysis was scored positive for a match of 9 of 14, although the background was higher (data not shown). The axes indicate the approximate position of the exons (solid boxes) and show the major sites of transcription initiation (arrows). The approximate positions of transcription initiation and the putative translation start codons are shown on the diagonal by arrows. Regions 1, 2, and 3 highlighted by arrows are highly conserved between HD-5 and HD-6, as shown in B. B, nucleotide similarity of 5’-flanking regions of HD-5 (top sequence) and HD-6 (bottom sequence). The sequences were aligned for maximal sequence identity. Regions of particularly striking identity are underlined. The major site of transcription initiation defined by 5’ RACE analysis for HD-6 and HD-5 (18) is indicated with an arrow. The TATA and CAT box sequences are boxed.
Plasmid DNA with inserts of about 450 bp were subjected to further analysis. Hybridization analysis revealed the presence of the previously identified HD-5 and HD-6 clones in a ratio of about 13:1 from PCR using DEF15s (Fig. 3C, 1–44). A relative ratio of approximately 4:1 (Fig. 3C, 45–78), closer to the ratio observed in Northern blot analysis, was observed for the PCR using DEF15si. Two representative clones from each hybridization group were sequenced in their entirety, and the nucleotide sequences corresponded exactly to the previously published sequences of HD-5 (18) and HD-6 (19). Hybridization with two additional probes under high stringency yielded consistent results (data not shown). In addition, several subclones contained inserts smaller than 450 nucleotides, and sequence analysis indicated that these clones contained variously truncated HD-5 or HD-6 clones (Fig. 3C, 53, 58, 66, 67, 69, 70, and 76). The nine remaining subclones were found by direct sequence analysis to be unrelated to defensins (data not shown). Thus, we conclude from these experiments that HD-5 and HD-6 are likely to be the only defensins expressed in the human small intestine. However, we cannot exclude the possibility that additional defensins might also be expressed but either are present at lower levels of expression or are not amenable to detection using this approach.

Developmental Expression—To determine the regional localization of HD-5 and HD-6 mRNA in early development, RNA was isolated from fetal distal small intestine and colon tissue at gestational ages from 13.5–17 weeks. The RNA was reverse transcribed in vitro, and the resulting cDNA was analyzed by PCR using primers that specifically amplify sequences for each corresponding defensin (Fig. 4). At 13.5 weeks, HD-5 mRNA was detectable by RT-PCR in small intestine and colon. Although HD-5 mRNA remains detectable through 17 weeks in the small intestine, the detectable levels in the colon decrease with evidence of only trace amounts at 17 weeks. HD-6 mRNA was detectable by RT-PCR in small intestine at 13.5–17 weeks of development (Fig. 4A). HD-6 mRNA, unlike HD-5, was not detected in the colon within this gestational range (Fig. 4B). As a positive control for all RT-PCR experiments, primers specific for β-glucocerebrosidase were chosen (34) (Fig. 4). As two negative controls, reactions lacking either RNA or reverse transcriptase were included.

Total RNA isolated from fetal specimens of distal small intestine ranging in gestational age from 19 to 24 weeks and from adult small intestine was analyzed by Northern blot hybridization (Fig. 5). The blot was probed sequentially with antisense oligonucleotide probes specific for HD-5 (HSIB-309a) and HD-6 (HsIB-309a). To control for possible cross-hybridization of the defensin probes under the experimental conditions, a slot blot containing HD-5 and HD-6 clones was hybridized and washed under the same conditions as the Northern blot. Specific hybridization was observed with both of the defensin probes. PhosphorImager analysis of the Northern blot indicated that approximately 40 times more HD-5 mRNA is detectable in the adult than in the 24-week fetal sample shown in Fig. 5. 3′-fold less mRNA is detectable in the other specimens on this blot. RNA from a second specimen at 24 weeks of gestation

show hybridization to both HD-5 and HD-6 probes. Direct sequence analysis of the plasmid inserts reveal a tandem ligation of both HD-5 and HD-6 cDNA in each of these clones.

**Fig. 3.** 3′ RACE analysis of defensin cDNA in human small bowel. A, nucleotide sequences of the PCR primers DEF15s and DEF15si aligned with defensin sequences from human, guinea pig, mouse, rat, and rabbit. The vertical lines indicate identity. B, anchored RT-PCR products from human intestinal RNA using either DEF15s (15s) or DEF15si (15si) as an upstream PCR primer were analyzed by agarose gel electrophoresis and ethidium bromide detection. The molecular size standard (M) phiX174 digested with HindIII. C, dot blot of 78 samples of plasmid DNA from recombinant clones containing RACE-PCR products hybridized with a 32P-labeled probe from the common region of defensin mRNA, SIG68a (COMMON PROBE), with a defensin 5-specific oligonucleotide probe, HSIA-309a (HD-5), and with a defensin 6-specific oligonucleotide probe, HSIB-309a (HD-6). Clones 57 and 61
showed lower levels than those in the first sample, comparable with the 21-week sample (data not shown). The relative ratio of HD-5 to HD-6 was estimated to be approximately 3:1, similar to that found in the 3' RACE analysis with DEF15sI. Defensin mRNA from specimens at earlier gestational ages was not detectable by Northern blot analysis under these conditions (data not shown).

In Situ Hybridization—The cellular localization of the defensin mRNA was determined by in situ hybridization. Tissue sections of human distal small intestine from adult, term newborn, and fetus at 24 weeks gestation were probed with sense and antisense 35S-labeled riboprobes. Signal was observed in Paneth cells with the antisense probes of HD-5 and HD-6 in all intestinal specimens (Fig. 6, A–F). No signal was observed in parallel sections if the sense probe was used (Fig. 6 G and H). Phloxine-tartrazine staining of parallel sections of all of these tissues revealed Paneth cells corresponding to those cells expressing HD-5 and HD-6 mRNA (data not shown). Although in situ hybridization is limited in its qualitative ability, examination of numerous sections and fields suggested lower expression for HD-6 compared with HD-5 in all sections, consistent with the Northern blot data. Also, lower signal was detected in fetal than in newborn sections, which was in turn lower than in adult specimens. As previously shown, fewer Paneth cells were observed per crypt in the fetus (44), probably contributing to the lower levels observed with Northern blot analysis.

**DISCUSSION**

**Structure of Enteric Defensin Genes**—The data reported here (Fig. 6), together with previous studies by our group (18, 19), support that expression of defensin genes HD-5 and HD-6 are limited to Paneth cells. As a step toward defining the cis-acting nucleotide sequences that regulate expression of enteric defensin genes, we cloned and characterized the HD-6 gene. HD-6 has two exons (Fig. 1) similar in structure to HD-5, which was
characterized previously (18). This two-exon structure is analogous to that of the mouse enteric defensin genes (25) and contrasts with that for the hematopoietic defensin genes from human (40), guinea pig (45), and rabbit (46), all of which contain three exons, the last two of which encode the prepropeptide. Highly similar respective structures for the enteric and the hematopoietic defensin genes between several mammalian species suggest that corresponding ancestral genes of each type existed in evolution prior to the divergence of these species. A model for a possible evolutionary history of the human defensin gene family has recently been proposed by our group. 

Comparison of the nucleotide sequence of HD-5 and HD-6 shows an unusual and very striking pattern of similarity, with two testable hypotheses emerging from this observation. First, the high similarity across 850 nucleotides of flanking region suggests that cis-elements important in tissue specific and developmentally regulated expression of these genes might be found in this region. Transgenic studies using 6.5 kilobases of 5'-flanking DNA from a mouse enteric defensin gene ligated to a reporter gene showed expression largely restricted to Paneth cells in mature intestinal crypts (47). It is possible that the information necessary for tissue-specific expression is located in the proximal region where we observe high nucleotide identity. Second, the presence of several nuclear factor interleukin-6 recognition sequences (39) throughout the 5'-flanking region offers a rationale to test if constitutive levels of defensin gene expression in the bowel are up-regulated in response to inflammation. Certain members of another group of mammalian antibiotic peptides, the β-defensins, are highly inducible with their expression in differentiated epithelial cells responsive to inflammatory stimuli (48–50).

**Enteric Defensin Expression in Human Small Intestinal Ontogeny—**The appearance of HD-5 and HD-6 mRNA (Fig. 4) coincides approximately with the appearance of morphologically distinguishable Paneth cells, which have been identified at 12 weeks of gestation in humans by electron microscopy (51). Although the physiological significance of low level defensin expression is not yet clear, the data suggest defensin mRNA expression may be an early marker of Paneth cell differentiation. This is consistent with the notion that the human enteric defensins, similar to the hematopoietic defensins, have constitutive levels of expression and are part of a developmental program of the highly differentiated cells in which they are expressed (52).

Northern analysis detected enteric defensin mRNA in the second trimester of gestation at levels approximately 40–250-fold less than those observed in the adult (Fig. 5). Because of the difficulty in establishing gestational ages with precision at this stage of development, we interpret the variability in our Northern blot analysis with some caution. We conclude conservatively that readily quantifiable enteric defensin mRNA accumulates in the latter part of the mid-trimester but at levels much lower than found in adults. In situ hybridization analysis localized the expression of enteric defensin mRNA to Paneth cells (Fig. 6) and suggests that fewer numbers of Paneth cells in the fetal crypts, as compared with the newborn and adult, account for part of the lower level of enteric mRNA observed (Fig. 6, A versus B and C and D versus E and F). Our data are consistent with anatomic data showing lower numbers of Paneth cells in crypts of the mouse at early gestational ages (47, 53).

**Comparison of Enteric Defensin Developmental Expression in Mouse and Human Intestine—**The developmental profile of mouse enteric defensin gene expression is predominantly postnatal (13). This contrasts with the earlier pattern we found in the human, but it also largely parallels the appearance of Paneth cells in mice (53). Morphologically identifiable Paneth cells are not present in mouse intestine until birth or just before (53). The murine defensin mRNA levels then increase gradually, reaching adult levels by the fourth postnatal week (13, 47). Using immunohistochemical methods, Bry et al. (47) have identified cryptin peptide in mouse Paneth cells which coincides with the previously described mRNA expression (13). Enteric defensin peptides also have been isolated from extracts of small bowel mucosa of weaned mice (13, 15–17, 26).

In the mouse, there is evidence for expression of 16 defensin-encoding mRNAs (26). The six characterized murine enteric defensin genes (25) all have very high overall nucleotide similarity (85%), suggesting gene duplication events that occurred relatively recently in evolution. The data reported here (Fig. 3), consistent with previous data from screening of a phage cDNA library (18), suggest that only two defensin genes, HD-5 and HD-6, are expressed in human small intestine. These two genes are not as closely related (Fig. 2A) as those in the mouse (25), consistent with duplication and subsequent divergence much earlier in evolution. The striking species difference in enteric defensin gene numbers remains an enigma and may reflect selective pressures resulting from complex interactions between host and microbial environment.

**Enteric Antibiotic Peptides in Host Defense—**Antimicrobial peptides are host defense effector molecules identified previously in the gastrointestinal tract of insects, amphibians, and mammals (13, 15, 16, 50, 54–56). The finding of antimicrobial peptides of several different gene families expressed in the gastrointestinal tracts of diverse species supports a role in local defense of this mucosal surface. The precise physiological role of these molecules, however, remains an open question. We and others (13, 15–19) have postulated that the epithelial expression of defensins in the intestinal tract serves either to constrain the proliferation of intraluminal flora or to prevent translocation of bacteria across the intestinal mucosa.

An immaturity of specific and nonspecific effectors of the immune response is thought to predispose premature infants to infection. For example, necrotizing enterocolitis is an illness that causes substantial morbidity and mortality among premature infants yet is uncommon in term newborns (28, 41, 43). Histologically, necrotizing enterocolitis is characterized by inflammation and necrosis at affected sites. The etiology appears to be multifactorial, and a likely central feature is clinical infection caused by microbes colonizing the intestinal tract. It has been proposed that characteristics of an immature gastrointestinal tract lead to the development of necrotizing enterocolitis (28, 43). Our results demonstrate very low level expression of defensin by the fetal intestine through 24 weeks of gestation, the lower limit of extrateratine viability. Limited expression of intestinal defensins by the fetus might, therefore, place a preterm infant at risk for bacterial invasion of the intestine and possibly the development of necrotizing enterocolitis. Future studies will be needed to define the biological activities of the human enteric defensins, to determine if levels of defensin expression are altered following premature birth, and to define the possible role of enteric defensins in the pathophysiology of necrotizing enterocolitis.

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