Epithelial cells in fetal intestine produce chemerin to recruit macrophages

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Maheshwari A, Kurundkar AR, Shaik SS, Kelly DR, Hartman Y, Zhang W, Dimmitt R, Saeed S, Randolph DA, Aprahamian C, Datta G, Ohls RK. Epithelial cells in fetal intestine produce chemerin to recruit macrophages. Am J Physiol Gastrointest Liver Physiol 297: G1–G10, 2009. First published May 14, 2009; doi:10.1152/ajpgi.90730.2008.—Macrophages are first seen in the fetal intestine at 11–12 wk and rapidly increase in number during the 12- to 22-wk period of gestation. The development of macrophage populations in the fetal intestine precedes the appearance of lymphocytes and neutrophils and does not require the presence of dietary or microbial antigens. In this study, we investigated the role of chemerin, a recently discovered, relatively selective chemoattractant for macrophages, in the recruitment of macrophage precursors to the fetal intestine. Chemerin mRNA/protein expression was measured in jejun ileal tissue from 10- to 24-wk human fetuses, neonates operated for intestinal obstruction, and adults undergoing bariatric surgery. The expression of chemerin in intestinal epithelial cells (IECs) was confirmed by using cultured primary IECs and IEC-like cell lines in vitro. The regulatory mechanisms involved in chemerin expression were investigated by in silico and immunolocalization techniques, IECs in the fetal, but not mature, intestine express chemerin. Chemerin expression peaked in the fetal intestine at 20–24 wk and then decreased to original low levels by full term. During the 10- to 24-wk period, chemerin accounted for most of the macrophage chemotactic activity of cultured fetal IECs. The maturational changes in chemerin expression correlated with the expression of retinoic acid receptor-β in the intestine. Chemerin is an important mediator of epithelial-macrophage cross talk in the fetal/premature, but not in the mature, intestine. Understanding the regulation of the gut macrophage pool is an important step in development of novel strategies to boost mucosal immunity in premature infants and other patient populations at risk of microbial translocation.

maturity of the local adaptive immune system, and low secretory IgA production (3, 17, 62), intestinal macrophages assume even greater importance as a host defense system because of their ability to eliminate “previously unknown” bacteria through phagocytosis and intracellular killing.

Intestinal macrophages display avid phagocytic and bactericidal activity but are markedly attenuated in their inflammatory responses (55), a unique adaptive mechanism that prevents unnecessary inflammation in the gut mucosa despite the proximity to luminal bacteria. This “inflammatory anergy” of intestinal macrophages is of interest because it may offer opportunities for therapeutic exploitation; strategies aimed at expanding the intestinal macrophage pool might be useful for augmenting innate mucosal immunity and preventing bacterial translocation in at-risk populations, such as premature infants, without incurring an inflammatory response. Elucidation of the mechanisms involved in the recruitment of macrophages to the gut mucosa is an important step in this direction.

Intestinal macrophages are derived from circulating monocytes, which are recruited to the mucosa under the influence of various epithelial and mesenchymal cell-derived chemoattractants (25, 54, 55). Because neither intestinal macrophages nor their precursor monocytes have the ability to undergo clonal expansion (54), the only mechanism available for the development and maintenance of the gut macrophage pool is through the continuous recruitment and differentiation of blood monocytes. In adults, interleukin-8/CXC ligand 8 (IL-8/CXCL8) and transforming growth factor-β (TGF-β) recruit macrophage precursors to the intestinal mucosa (54). However, several lines of evidence indicate that IL-8 and TGF-β may not be important as macrophage chemoattractants in the fetal intestine. In the fetus, IL-8 is comprised mainly of a longer, less-potent 77-amino acid isoform (unlike the shorter 72-amino acid isomer in the adult) (35). Similarly, TGF-β bioactivity is low in the early- and midgestation fetal intestine (A. Maheshwari, N. Ambalavanan, T. Nicola, D. R. Kelly, J. M. Murphy-Ullrich, M. Athar, M. Shimamura, V. Bhandari, C. Aprahamian, R. A. Dimmitt, R. Serra, and R. K. Ohls, unpublished observations). Finally, macrophages appear in the fetal intestine at least a few weeks before lymphocytes or neutrophils (7, 32, 36), suggesting that macrophage precursors are likely to be recruited to the early fetal intestine by chemoattractant(s) more specific for macrophage precursors than IL-8/CXCL8, which recruits both neutrophils and macrophage precursors (14, 54), or TGF-β, which mobilizes macrophage precursors as well as T lymphocytes (1, 54).

Chemerin (previously known as tazarotene-induced gene-2/TIG2 or retinoic acid receptor responder-2/RARRES2) is a...

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THE GASTROINTESTINAL MUCOSA contains the largest reservoir of macrophages in the body (29). Macrophages first appear in the developing intestine at 11–12 wk of gestation, increase rapidly during the 12- to 22-wk period, and then continue to expand at a slower pace through early childhood (7, 20, 32, 36, 50, 57). Intestinal macrophages play a critical host defense role in being the first phagocytic cells of the innate immune system to encounter luminal bacteria that breach the epithelium and gain access to the lamina propria (54, 55). In sick and preterm neonates who are predisposed to bacterial translocation because of an abnormally permeable gut epithelial barrier, im...
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recently discovered chemoattractant with potent activity for macrophages and dendritic cells, which express the cell surface cognate receptor CMKLIR1 (chemokine-like receptor-1, previously ChemR23), but not for neutrophils or lymphocytes (27, 63). In this study, we investigated the role of chemerin as a macrophage chemoattractant in the fetal intestine. Using quantitative and immunolocalization techniques in human intestinal tissues and in vitro models, we show that chemerin is an important mediator of enterocyte-macrophage cross talk in the developing intestine.

METHODS

Human intestinal tissue. Human intestinal tissues were obtained under appropriate oversight by the Institutional Review Boards at University of New Mexico and University of Alabama at Birmingham. Human fetal jejunoileal tissue (11–24 wk, n = 25) was obtained at elective terminations of pregnancy in healthy women. Healthy jejunoileal tissue margins were obtained during surgical resection in preterm neonates (obstruction/repair of ostomy, n = 3; gestational age 29, 30, and 32 wk), term neonates (surgical resection of atresia/repair of enterocutaneous fistula, n = 3), and adults (bariatric surgery; n = 3).

Immunostaining of tissue sections. Paraffin-embedded tissue sections were immunostained for HAM56 (a pan-macrophage marker), chemerin, CMKLIR1 (chemokine-like receptor-1, the cognate receptor for chemerin), and retinoic acid receptor-β (RAR-β) by using our previously reported fluorescence protocol (34). Briefly, tissue sections were deparaffinized with xylene, hydrated in a series of graded alcohols, and rinsed in PBS. Antigen retrieval was achieved by heating the sections in 10 mM sodium citrate buffer, pH 6.0 at 95°C × 10 min. Sections were treated with a serum-free protein-blocking solution (Pierce, Rockford, IL) for 30 min and then stained overnight (4°C) with polyclonal goat anti-human chemerin antibody (R&D Systems, Minneapolis, MN), monoclonal rat anti-human CMKLIR1 antibody (AbD Serotec, Raleigh, NC), polyclonal rabbit anti-human RAR-β IgG (Santa Cruz Biotechnology, Santa Cruz, CA), and/or mouse monoclonal anti-HAM56 IgM antibody (Abcam, Cambridge, MA). Secondary staining was performed at room temperature for 30 min with Alexa 488-conjugated donkey anti-goat IgG, rabbit anti-rat IgG, or Alexa 568-conjugated goat anti-mouse IgM or goat anti-rabbit IgG antibodies (Invitrogen, San Diego, CA). Controls included slides with no primary antibody, with appropriate isotype control, and with competing recombinant chemerin. Cell nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI, Calbiochem, San Diego, CA), diluted 1:1,000 in PBS, applied for 3 min. Imaging was performed with a Zeiss Axiosvert fluorescence microscope.

Real-time PCR. Total RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction protocol (TRizol reagent, Invitrogen). First-strand cDNA was synthesized by use of oligo(dT) primers and Moloney murine leukemia virus reverse transcriptase (Invitrogen). Real-time PCR primers were designed by use of the Beacon Design software (Bio-Rad, Hercules, CA). The primer sequences were as follows: chemerin forward TGAGGAGCACCAGGAGAC, chemerin reverse TTGGAGAAGGCGAACTGTC; Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward AGAACATCATCCTCGCTCTACTG, GAPDH reverse CTCCTGACGCGTCTTCAC. Two-step real-time PCR was performed using a SYBR Green protocol described elsewhere (5, 65). Data were normalized for GAPDH and gene expression was compared between samples by the 2ΔΔCt method.

Intestinal epithelial-conditioned media. We prepared conditioned media from fresh human fetal intestinal tissue by using a previously reported protocol (Maheshwari et al., unpublished observations; 56). Intestinal tissue was first rinsed in Ca2+- and Mg2+-free PBS and then HBSS containing DTT to remove any residual mucus. The tissue was then treated with HBSS containing 0.2 M EDTA and 10 mM 2-mercaptoethanol and placed on an agitator for 30 min to remove the epithelium. The exfoliated IECs were harvested from the media by centrifugation at 200 × g for 5 min, washed twice, and then cultured in serum-free RPMI at a concentration of 1 × 106 cells/ml for 18 h. The conditioned media were clarified by centrifugation at 1,000 g for 5 min and stored in aliquots at −80°C.

Lysates of subepithelial intestinal mucosa. After harvesting the IECs, we homogenized the remaining subepithelial tissue in a commercially available lysis buffer (T-PER, Pierce). Total protein was measured by the BCA (bicinchoninic acid) protein assay (Pierce).

Conditionally immortalized fetal IECs. We used the temperature-sensitive fetal human intestinal (zFHI) cells (48) (kind gift of Dr. Andrea Quaranta, Cornell University) for these studies. These cells were derived from 17- to 19-wk human fetal IECs and were immortalized by use of a temperature-sensitive SV40 T-Ag-bearing pZipSVtsa58 plasmid (45). The zFHI cells were grown at 31°C in DMEM with 4% FBS, 10 ng/ml EGF, 2 mmol/l glutamine, 2 mmol/l Glutamax I, and 10 mmol/l HEPES. Once confluent, cultures can be transferred to 37°C for differentiation, whereupon these cells acquire the functional and phenotypic profile of human fetal IECs. However, in our hands, these cells do not develop tight monolayers. We harvested cell lysates (M-PER lysis buffer, Pierce) to measure chemerin expression under basal conditions and after stimulation with recombinant human TNF-α (10 ng/ml) for 24 h.

Caco-2 IECs. Caco-2 cells (ATCC, Manassas, VA) were cultured in DMEM (UAB media core) with 10% fetal calf serum (Thermo Fisher Scientific, Logan, UT) in six-well plates. These cells were derived from a colonic adenocarcinoma resected from a 72-yr-old patient and undergo spontaneous enterocytic differentiation at confluence to acquire the functional and phenotypic characteristics of adult small intestinal epithelium (23). Cell lysates were harvested (M-PER lysis buffer, Pierce) from basal and TNF-α-stimulated cultures as described for the zFHI cells. We also prepared polarized monolayers by growing Caco-2 cells to confluence in Transwell plates (BioCoat HTS Caco-2 assay system, BD Biosciences, San Jose, CA). Monolayers were used after 72 h if the transepithelial electrical resistance was >250 Ω·cm2·(11) Media were harvested for chemerin measurements from both apical and basolateral compartments.

Measurement of chemerin. Chemerin was immunoprecipitated from epithelial conditioned media (ECMs) or media from Caco-2 monolayers using a polyclonal goat antibody anti-human chemerin antibody (R&D) and Sepharose-immobilized protein A/G per manufacturer’s protocol (Pierce). Chemerin was quantified following immunoprecipitation or in tissue lysates by Western blots (50 μg total protein).

Fig. 1. Chemerin expression in the human small intestine is developmentally regulated and peaks during midgestation. A: mRNA expression. Bar diagram shows the chemerin mRNA expression in the developing intestine, depicted as fold change above 10- to 14-wk fetal tissues (means ± SE). Chemerin expression increased during fetal period to peak at 20–24 wk and then decreased in the later half of gestation. Data represent 3–5 fetuses, neonates, or adults per group. The experiments were repeated twice to exclude experimental errors. B: chemerin immunoreactivity. Photomicrographs (×100) showing developmental changes in chemerin expression (green) in the intestine. Nuclear staining was obtained with 4,6-diamidino-2-phenylindole (DAPI; blue). Each panel includes 2 high-magnification (×1,000) photomicrographs showing the villus tip and crypts to highlight the subcellular (apical/basolateral) pattern of expression. Chemerin immunoreactivity was detected mainly in epithelial cells (arrow) and increased from 10- to 14-wk gestation to become most prominent in the 20- to 24-wk fetus and the 29- to 32-wk premature neonate. In the term neonatal epithelial cells (IECs) did not express chemerin (open arrow). Both basolateral and apical immunoreactivity was noted in IECs. Immunoreactivity was also noted transiently in the muscularis externa in the 13- to 14-wk fetal intestines. Nonspecific staining from red blood cells in the 14-wk and term photomicrographs is marked by asterisks. Data are representative of 3 different fetuses or adults in each group.
protein/lane) on 15% polyacrylamide gels. For detection, we used a murine monoclonal anti-chemerin antibody (R&D), which detects the active form of chemerin, and standard ECL detection systems (Pierce, Rockford, IL). Densitometric analyses on the blots were performed with the NIH/Scion Image software.

Reagents. All-trans retinoic acid (ATRA) and recombinant human TNF-α were purchased from Sigma.

Monocyte-derived macrophages. Cord blood was obtained from full-term neonates delivered by elective cesarean sections prior to the onset of labor (n = 3). In these pregnancies, which were otherwise uncomplicated, the decision for cesarean section by the obstetrician was based primarily on a maternal history of cesarean section in a previous pregnancy. We chose not to include cord blood from pre-mature neonates because most preterm deliveries at our center were associated with maternal hypertension or possible chorioamnionitis and/or neonatal sepsis, conditions that can independently affect leukocyte phenotype and function (38, 44).

Cord blood monocytes were isolated by Ficoll-Hypaque density centrifugation followed by positive immunoselection with anti-CD14-conjugated microbeads (Miltenyi) as described previously (13, 34). Cells were enumerated (Beckman Coulter, Fullerton, CA) and then plated at a concentration of 1 × 10⁶ monocytes/well in 24-well plates in RPMI (UAB media core) with 10% human AB serum, 1% penicillin-streptomycin, and 50 μg/ml gentamicin (Mediatech, Manassas, VA). Monocyte-derived macrophages were harvested by scraping after 5 days. We also prepared macrophages from adult peripheral blood monocytes (n = 3) for positive control for chemerin-induced macrophage chemotaxis (63). The collection of both cord blood and peripheral blood samples from healthy adult volunteers was approved by the local institutional review board.

CMKL1 expression. CMKL1 expression on monocyte-derived macrophages was measured by flow cytometry after immunostaining the cells with a FITC-conjugated rat anti-human CMKL1 antibody (AbD Serotec) via our previously described protocol (54).

Macrophage chemotaxis. Chemotaxis was measured in a fluorescence-based microchemotaxis format as described previously (14). Monocyte-derived macrophages were stained with the fluorescence dye calcine-AM (2 μM; Molecular Probes, Eugene, OR) and suspended in HBSS with 0.1% BSA at a concentration of 1.1 × 10⁶ cells/ml. E-CMs from fetal intestines from 10–14, 15–19, and 20–24 wk were placed in lower wells of microchemotaxis chambers (ChemoTx System, NeuroProbe, Gaithersburg, MD). Recombinant human chemerin (10 and 100 pM) standards were included for positive control. In some wells, we added the E-CMs after preincubation for 30 min with excess antagonists of chemerin receptors. Extracellular calcium was depleted by 10 mM EDTA (Sigma) for 10 min followed by washes in HBSS before addition of E-CMs.

RESULTS

Chemerin expression in the small intestine is developmentally regulated and peaks during midgestation. To determine developmental changes in chemerin expression in the small intestine, we first measured mRNA expression of chemerin in normal human fetal tissue (10–14, 15–19, and 20–24 wk gestation) and in surgically removed jejunoileal tissue from preterm infants (29–32 wk), term neonates, and adults. As shown in Fig. 1A, chemerin expression increased during fetal period to peak at 20–24 wk and then decreased toward term to reach levels similar to those seen in the 10- to 14-wk fetal intestine. Chemerin expression in the term neonate was similar to that in the adult.

We next confirmed our findings by immunohistochemistry. Chemerin immunoreactivity was seen mainly in IECs (Fig. 1B) and increased from 10–14 wk of gestation to become most prominent in the 20- to 24-wk fetus and in 29- to 32-wk premature neonate. In the term neonate, IECs showed only faint immunoreactivity. There was no discernible staining in the adult intestine. Immunoreactivity was noted transiently in the muscularis externa in the 13- to 14-wk fetal intestine.

Chemerin is secreted by fetal IECs. Unlike primary IECs isolated from the adult intestine, fetal IECs remain viable for up to 12–18 h in vitro (47). Therefore, to determine whether fetal IECs secrete chemerin, we used culture media conditioned with exfoliated primary IECs. As shown in Fig. 2, chemerin was detectable in E-CMs prepared from 10- to 24-wk fetal intestines. Chemerin expression in E-CMs increased with gestational maturation, which was consistent with our findings of increasing mRNAs/protein expression noted in Fig. 1.

Cultured IEC-like cells produce chemerin. To investigate the conditions under which IECs produce chemerin, we utilized cultured IEC-like cells. We compared chemerin expression in Caco-2 cells, a widely used cell line model with enterocytic characteristics, with tsFHI cells, which are conditionally immortalized fetal IECs (Fig. 3). Both Caco-2 and the tsFHI cells produced chemerin in vitro. Interestingly, chemerin expression was higher in tsFHI cell cultures, which are of fetal origin, than in Caco-2 cells, which were derived from an adult subject. These findings were consistent with our data on developmental differences in chemerin expression in the intestine.

IECs secrete chemerin into the basolateral membrane compartment. The IEC plasma membrane is divided by the presence of intercellular tight junctions into apical and basolateral membrane compartments. To determine the functional importance of IEC-derived chemerin as a macrophage chemottractant in the intestine, we first needed to ascertain that polarized IECs secrete chemerin into their basolateral membrane compartment (representing the lamina propria vs. the apical compartment that represents the intestinal lumen).
We analyzed the peptide sequence of chemerin in silico to identify any apical-basolateral sorting signals (Fig. 4A). Chemerin is synthesized as a 163-amino acid precursor that contains a 20-amino acid NH2-terminal signal peptide, a 137-amino acid cystatin-like domain in the middle, and a 6-amino acid COOH-terminal prosegment. The precursor molecule undergoes proteolytic processing at both termini to yield a monomeric 16-kDa heparin-binding bioactive molecule. Chemerin does not have known apical sorting signals such as a glycosphingolipid anchor or asparagine-X-threonine/serine motifs (X is any amino acid except proline) (26, 61). Although basolateral sorting signals are not well known for secreted proteins, we screened the chemerin sequence for motifs associated with basolateral localization of transmembrane proteins. Common basolateral motifs are marked by tyrosine-associated β-turns, which are typically formed in COOH-terminal asn-pro-variable amino acid-tyr (NPXY) or tyr-variable amino acid-variable amino acid-hydrophobic amino acid (YXXØ) motifs (59, 66). In some proteins, nontyrosine β-turns and COOH-terminal dileucine motifs may also promote basolateral sorting. We identified a tyr-phe-pro-gly tetrapeptide with a β-turn conformation in chemerin, which is a YXXØ-type basolateral sorting signal. The COOH terminus also showed a nontyrosine β-turn motif, which has also been identified with a higher frequency in basolaterally sorted proteins.

We next compared basolateral and apical production of chemerin in polarized Caco-2 cell monolayers. We used Caco-2 cells in these studies because primary IECs do not form tight monolayers in vitro. As shown in Fig. 4B, chemerin concentrations were significantly higher on the basolateral side than in the apical compartment. To confirm the basolateral secretion of chemerin by primary IECs, we sought the presence of chemerin in the subepithelial fetal intestinal tissue left after removal of IECs. Similar to many CXC-/CC-chemokines and mesenchymal growth factors (12), chemerin is a cationic protein and binds avidly to heparan sulfate and related glycosaminoglycans in the extracellular matrix (ECM) (68). We anticipated that chemerin, following basolateral secretion by IECs, will remain detectable in the subepithelial ECM. Chemerin was detected in these tissues and concentrations increased with gestation, which correlated with changes in epithelial expression of chemerin during this period (data not depicted).

Macrophage chemoattractant activity of 10- to 24-wk fetal epithelial-conditioned media correlates with chemerin concentrations. We first enumerated HAM56+ macrophages in 10- to 24-wk fetal intestinal tissue. As shown in Fig. 5A, the number of macrophages increased with gestational age during this period. Furthermore, intestinal macrophages expressed CMKLR1, the cognate receptor for chemerin (Fig. 5B).

We next investigated whether E-CMs prepared from fetal IECs could recruit macrophages in vitro and whether this chemoattractant activity was due to the presence of chemerin. The expression of CMKLR1 on monocyte-derived macrophages was confirmed by FACS (Fig. 5C, inset a). We then used a previously described microchemotaxis assay (14), in which the migration of macrophages across a polycarbonate filter was measured as a calcein-AM fluorescence signal (inset b). The macrophage-chemotactic activity of 10- to 24-wk fetal intestinal E-CMs increased with gestational age, consistent with changes in epithelial expression of chemerin during this period. This chemoattractant activity of fetal E-CMs was blocked in the presence of neutralizing anti-chemerin antibody, thereby identifying chemerin as the principal macrophage chemokine in these conditioned media.
Chemerin expression in fetal IECs correlates with the expression of retinoic acid receptors. We next sought possible mechanisms underlying the observed developmental changes in chemerin expression. We first analyzed the chemerin promoter sequence in silico to identify important TFBSs. Using two software programs and statistical thresholds that were defined a priori, we identified TFBSs. The sites identified with the greatest statistical confidence in both programs included those for transcription factors GATA-1, PU.1, ABF2, ATBP1, p300, Sp1, glucocorticoid receptors (GR), RAR-β, and MIG1 (Fig. 6 A). We focused on RAR-β because RAR-β binding sites are clustered in the chemerin promoter in close association with the CpG islands, which indicates their importance as regulatory elements (42), and also because RAR-β activation induces chemerin expression in skin cells (10). We first treated tsFHI cells with ATRA, a high-affinity ligand for RAR-β (31), and measured its effect on chemerin expression in vitro. As shown in Fig. 6B, ATRA increased chemerin expression in a dose-dependent fashion, thereby confirming the importance of RAR-β signaling in relevant cells.

We next investigated changes in RAR-β expression in the fetal intestine. As shown in Fig. 6C, RAR-β immunoreactivity was detectable on fetal IECs at 10–14 wk, peaked at 20–24 wk, and then decreased with subsequent maturation. These maturational changes in RAR-β expression correlated with that of chemerin in the fetal intestine, further underscoring the important role of the retinoic acid receptors in the regulation of chemerin expression.

DISCUSSION

We report for the first time that chemerin is expressed during fetal development and that it plays an important role as a macrophage chemoattractant in the developing intestine. Chemerin was previously known to be produced only in adipose tissue and liver (51, 69). Low-level expression of chemerin mRNA has been reported in the small intestine, but the cells of origin were not identified (6). In the fetus, increasing expression of chemerin in the 10- to 24-wk period of gestation provides a mechanism for the initial development of the intestinal macrophage populations. During this period, the bioactivity of both IL-8/CXCL8 and TGF-β, which recruit macrophage precursors in the adult intestine (54), is low and unlikely to support the development of the gut macrophage pool (Maheshwari et al., unpublished observations). The presence of chemerin might explain the recruitment of macrophage precursors to the intestine during early gestation, whereas the emergence of TGF-β and IL-8/CXCL8 beyond midgestation, when chemerin expression is on the wane, might account for the continued expansion of the intestinal macrophage pool.

IECs secrete chemerin preferentially into their basolateral compartment, which is consistent with its role as a macrophage chemoattractant. The endosomal traffic in IECs flows predominantly from the trans-Golgi network toward the basolateral membrane (49), but proteins can be directed specifically toward one of two compartments according to certain COOH-terminal sorting motifs that are recognized by adapter proteins.
These sorting signals were characterized in transmembrane proteins such as receptors and are not yet well known for secreted proteins. Intriguingly, chemerin contains a COOH-terminal YXXΩ and another nontyrosine β-turn motif that are strongly associated with basolateral localization in transmembrane proteins. Chemerin was discovered through reverse genetics, and the molecular events involved in its synthesis and secretion are still unclear. The NH2-terminal signal peptide segment of chemerin has been likened to a transmembrane signal anchor (67), which implies that the cellular “secretion” of chemerin could involve proteolytic release from a type II transmembrane precursor. Such a transmembrane form of chemerin would justify the presence of the basolateral sorting signals. A membrane-bound form of chemerin could function as an attachment and/or...
interaction signal and contribute to enterocyte-macrophage cross talk in the fetal intestine.

The macrophage chemoattractant activity of our fetal IEC-conditioned media was, in large part, due to chemerin. This chemotactic activity of the E-CMs prepared from 10- to 24-wk fetal IECs increased with gestation, correlating with increasing epithelial expression of chemerin and also with the increasing number of intestinal macrophages in sections from the same tissues. Intestinal macrophages are normally downregulated for their inflammatory responses to bacterial products but not for their phagocytic or bactericidal activity (55). Unlike other chemokines, chemerin may contribute to this unique noninflammatory profile of intestinal macrophages. Chemoattractants such as the CXC and CC chemokines activate macrophages and augment their inflammatory responses to bacterial products (39). Although chemerin has a proinflammatory influence on macrophages, proteolytic processing of chemerin by cysteine proteases yields smaller anti-inflammatory peptides that can block the cytokine and chemokine responses of macrophages to bacterial products (9). These observations are interesting because cysteine proteases such as cathepsins are widely expressed in the fetal intestine and in amniotic fluid (ingested in large volumes by the fetus) (2, 18), where these enzymes may release anti-inflammatory fragments from chemerin to downregulate the inflammatory responses of newly recruited macrophages. The inflammatory anergy of intestinal macrophages is established during the second half of gestation (Maheshwari et al., unpublished observations), and the anti-inflammatory effects of these chemerin fragments may be particularly important in the developing intestine.

Chemerin was detectable in our tissue samples from the premature intestine at 29–32 wk of gestation, which contrasted...
with the full-term or adult intestine. This subgroup of premature infants would have been at risk for developing necrotizing enterocolitis (NEC). Macrophase infiltration and activation are commonly encountered histopathological features of NEC (19, 22, 58), and further studies are required to determine whether chemerin expression is increased in diseased tissue and/or blood.

In computational analyses of the chemerin promoter, we identified binding sites for transcription factors GATA-1, PU.1, ABF2, ATBP1, p300, Sp1, GR, RAR-β, and MIG1 with the greatest statistical confidence. GATA-1 and PU.1 are expressed predominantly in the hematopoietic cells (unlike the “endodermal” GATA-4, -5, -6, which are expressed in IECs) (8); p300 plays a key role in crypt-villus differentiation, and mice deficient in p300 have impaired villus formation (53). Sp1 plays an important role in epithelial differentiation (40), but changes in Sp1 expression in IECs do not show a clear gestational pattern and cannot explain the changes we observed in the expression of chemerin (24). Similarly, GR are widely expressed on fetal IECs but do not have a measurable effect on the expression of chemerin (24). Similarly, GR are widely encountered histopathological features of NEC (19, 22, 58), and further studies are required to determine whether chemerin expression is increased in diseased tissue and/or blood.

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