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Intrauterine Growth Restriction Alters Postnatal Colonic Barrier Maturation in Rats

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ABSTRACT: Intrauterine growth restriction (IUGR) is a leading cause of perinatal mortality and morbidity and increases the risk for necrotizing enterocolitis. We hypothesized that colonic barrier disruption could be responsible for intestinal frailty in infants and adults born with IUGR. Mucins and trefoil factor family 3 (TFF3) actively contribute to epithelium protection and healing. Our aim was to determine whether IUGR affects colonic mucosa maturation. IUGR was induced by dietary protein restriction in pregnant dams. Mucins and Tff3 expression and morphologic maturation of the colonic mucosa were followed during postnatal development of the offspring. Before weaning, mucin 2 and Tff3 protein levels were reduced in colonic mucosa of rats with IUGR compared with controls. After weaning, expression of mucin 2 (mRNA and protein) and mucin 4 (mRNA) were lower in colonic mucosa of rats with IUGR. At the same time, IUGR was associated with a reduction of crypt depth and a higher percentage of crypts in fission. We conclude that IUGR impairs mucus barrier development and is associated with long-term alterations of mucin expression. The lack of an efficient colonic barrier induced by IUGR may predispose to colonic injury not only in neonatal life but also in later life. **(*Pediatr Res* 66: 47–52, 2009)**

Intrauterine growth restriction (IUGR) is defined by a birth weight below the 10th percentile for gestational age. IUGR is a leading cause of perinatal morbidity and mortality (1). It is a known risk factor for necrotizing enterocolitis (NEC) (2,3) and epidemiologic data suggest that it predisposes to colorectal cancer in later adult life (4,5). To diminish these risks, it is crucial to understand the mechanisms underlying this increased susceptibility. Our hypothesis is that colonic mucosal barrier disruption could be responsible for intestinal frailty in infants and adults born with IUGR.

Mucosal barrier protects the internal milieu of the human body from potentially noxious luminal content, particularly in the colon, where bacterial proliferation and fermentation are very active (6). The mucosal barrier consists of an intestinal epithelium, covered by a continuous mucus layer, a gel made of mucins, and trefoil peptides that are secreted by epithelial goblet cells. There are two main classes of mucins: secreted gel-forming mucins and membrane-bound mucins (7). In addition to their role as a physical barrier against pathogens, secreted mucins (MUC2) and membrane-bound mucins (MUC1 and 3) have recently been shown to be involved in colonic protection (8–10). Trefoil factor family 3 (TFF3) is a small peptide exerting multiple biologic effects including the modulation of healing, inflammation, and differentiation (11). TFF3 is, thus, considered essential for epithelial restitution (12).

Barrier disruption, involving some of the latter mucosal constituents, has been reported in various diseases. Mucin expression is dramatically altered in inflammatory bowel diseases and colorectal cancer (13). The putative role of intestinal barrier failure in the pathogenesis of NEC, particularly of an impairment of the mucus components, is the focus of intense scrutiny: a lack of expression of TFF3 peptide or MUC2 has been shown in patients with NEC (14,15) and in a model of neonatal mucosal injury (16); moreover, the administration of TFF3 has a therapeutic role in an NEC model (17).

IUGR is associated with a redistribution of cardiac output (18) as an adaptation to chronic placental restriction, which maintains optimal growth and function of key organs (heart and brain) but decreases fetal blood flow to other organs, including the intestine (19). This phenomenon could explain the reduction in intestinal development observed at birth in infants with IUGR (20) and some animal models of IUGR (21). IUGR impairs cell proliferation (22) and absorptive (23) and digestive (24) functions in small intestine. It was found to produce alterations in colonic development, such as a reduction in colonic length or weight, or in the number of goblet cells in animal models of IUGR (20,25). Yet, the specific effect of IUGR on mucin gene expression and secretion has not been addressed.

Evidence from the literature suggests that 1) IUGR is associated with a delay in intestinal development; 2) IUGR is a known risk factor for NEC; and 3) alterations in mucosal constituents are involved in NEC and in other intestinal diseases in the adult. We hypothesized that IUGR may be responsible for a disruption in maturation of the colonic mucosa and we compared the postnatal development of key mucosal constituents (mucins and Tff3) between rats with IUGR and controls.

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Abbreviations: Cdx-2, caudal-related homeobox protein 2; **NEC,** necrotizing enterocolitis; **Tff3,** trefoil factor family 3

METHODS

Animals. Experiments were carried out in accordance with the recommendations of the local Animal Care and Use Committee of Nantes (France). The animal facilities were approved by the government agency in charge of experimental facilities at the French Department of Agriculture and the investigators were accredited by the National Veterinary Agency. Eight-week-old virgin female and male Sprague-Dawley rats (Janvier, Le Genest Saint Isle, France) were caged under standard laboratory conditions with tap water and regular food (16% of protein: A04, Safe, France) provided *ad libitum*, in a 12:12 h light/dark cycle. After 10 d of habituation, female rats were mated overnight with male rats. During the whole gestation, pregnant dams were fed either a normal protein diet (NP, 20% of protein) or an isocaloric low protein diet (LP, 8% of protein) purchased from Arie Block (The Netherlands) (Table 1). At birth, male pups born to restricted (pups with IUGR) and normally fed mothers (control pups) and selected from different litters were adopted by normally fed mothers (NP) until the end of the lactation (eight pups per litter). After weaning (21 d), all pups were fed the NP diet until 40 d of age.

Tissue collection. At day 5 (D5), 12 (D12), 16 (D16), 22 (D22), and 40 (D40), rats were killed using CO_2 asphyxia. Colon length was measured. Proximal and distal colon were excised, opened longitudinally, and cleaned with sterile 0.9% saline. They were frozen in liquid nitrogen and stored at −80°C until mRNA analysis ($n = 8$ per group) or fixed in 4% buffered formaldehyde, dehydrated, and paraffin embedded until morphologic analysis and immunohistochemistry ($n = 4$ per group).

Histologic assessment. Paraffin-embedded sections of colon were dewaxed, rehydrated, and stained with hematoxylin and eosin for histologic examination. Crypt depth, percentage of crypts in fission, and number of goblets cells per crypt were measured using NIS-Elements 2.3 software (Nikon France SA, France). The values obtained from 10 crypts from each colonic segment per animal were averaged.

RNA isolation and real-time RT-PCR. RNA was isolated from snap-frozen proximal or distal colon using the NucleoSpin RNA kit (Macherey-Nagel EURL, France). Total RNA was submitted to DNase digestion following the manufacturer's instructions. cDNA was synthesized from 2 μg of total RNA using random primers and reverse transcriptase AMV (Promega, WI). Real-time PCR was performed starting with 150 ng of cDNA, with 250 nmol/L concentrations of both sense and antisense primers (Table 2) using QuantiTect SYBR Green PCR Kit (Qiagen, France) in the iCycler iQ real-time PCR detection system instrument (Bio-Rad Laboratories, Hercules, CA). mRNA expression was calculated using [Delta][Delta]CT method (26) with β-actin as housekeeping gene and median of control group values at D5 as calibrator.

Immunohistochemistry. Paraffin-embedded sections of proximal and distal colon were dewaxed, rehydrated, and treated as described previously (27) for Muc2 and Tff3 staining: quenching of endogenous peroxidase activity by 20 min incubation in 6% H_2O_2, antigen retrieval by microwaving in 0.01 M citrate buffer (pH 6.0), blocking nonspecific sites by 10% goat serum. Muc2, Muc4, Tff3, and caudal related homeobox protein 2 (Cdx-2) were detected in tissue sections with a polyclonal Muc2 antibody (H-300, sc-15334, 1:250, Santa Cruz CA), a polyclonal anti-Muc4 (28) (1:1000 dilution), a polyclonal Tff3 antibody (29) (1:500 dilution), and with a monoclonal anti-Cdx2 (1:40 dilution, MU392A-UC, Biogenex, CA) and incubated for 60 min at room temperature. An anti-rabbit biotinylated secondary antibody (1:1000 in 1.5% goat serum, Vector Laboratories, Canada) incubated at room temperature for 30 min was used. Binding of secondary antibody was visualized by incubation with avidin-biotin-peroxidase complex (Elite Vectastain ABC kit, Vector Laboratories, CA) at room temperature for 30 min (Muc2) and for 60 min (Tff3) and glucose oxidase-DAB-nickel method (30). The sections were counterstained with hematoxylin. The epithelium was scored blind to treatment for the presence of Muc2 and Tff3 using NIS-Elements 2.3 software: the proportion of stained cells per crypt (0: no/weak staining; 1–3: increasing number of cells stained) and the intensity of staining (0: no/weak staining; 1–3: increasing intensity of staining) were recorded within 10 crypts and four animals per group.

Statistical analysis. Statistical analysis was performed using the Statview 5.0 package (SAS Institute, Cary, NC). Data were first analyzed using two-way ANOVA with age and fetal growth status as the main factors. The expression of data were either determined as means (SEM) or as medians (first quartile Q1, third quartile Q3), and box plot when the number of animals (n) was respectively more and less than 30 per group. Differences among group were thus respectively assessed by Fisher's protected least significance difference test or by Mann-Whitney U test. Differences between means or medians were considered significant at $p \leq 0.05$.

Table 1. *Energy and nutrient composition of experimental diets*

	20% protein (NP) diet	8% protein (LP) diet
Energy (Kcal/100 g)	372.27	367.40
Protein and amino acid*		
Casein	22.00	9.00
Methionine	0.20	0.08
Digestible carbohydrate*		
Dextrose	55.15	68.17
Cornstarch	8.00	8.00
Fat*		
Soya oil	4.30	4.30
Fiber*		
Cellulose	5.00	5.00
Vitamin and mineral mix*	4.95	5.05
Choline*	0.40	0.40

* Values are in grams per 100 g of diet.

RESULTS

Mother and pub growth. Mothers fed a LP diet gained less weight (Table 3) during gestation (G19) compared with mothers fed a NP diet ($p = 0.004$). They ate more food ($p = 0.050$) but protein consumption in the restricted group remained lower (by about 47%) than in the NP group. Protein restriction did not alter the number of pups per litter.

Pups born to mothers fed a LP diet had a significantly lower birth weight (Table 3) compared with control pups ($p < 0.001$). We verified that the median birth weight of pups with IUGR (5.9 g) was lower than the 10th percentile birth weight distribution of the control pups (6.4 g). Rats with IUGR

Table 2. *Accession number of mRNA sequence, sequences of forward (F) and reverse (R) primers and PCR conditions used for real-time PCR*

Gene	Primers sequence	PCR conditions
Muc2 (U07615)*	F: 5′-ACCACCATTACCACCTCA-3′ R: 5′-CGATCACCACCATTGCCACTG-3′	45 cycles (30 s at 95°C, 10 s at 60°C)
Tff3 (NM_013042)*	F: 5′-GTCCTGGTTGCTGGGTCCTC-3′ R: 5′-CCACGGTTGTTACACTGCTCTG-3′	45 cycles (30 s at 95°C, 10 s at 60°C)
β-actin (NM_031144)*	F: 5′-CTATCGGCAATGAGCGGTTCC-3′ R: 5′-GCACTGTGTTGGCATAGAGGTC-3′	45 cycles (30 s at 95°C, 10 s at 60°C)
Muc1(XM_001074423, XM_342281)†	Unknown	40 cycles (15 s at 94°C, 30 s at 55°C, 30 s at 72°C)
Muc4 (XM_221384)†	Unknown	40 cycles (15 s at 94°C, 30 s at 55°C, 30 s at 72°C)
β-actin (NM_031144)†	Unknown	40 cycles (15 s at 94°C, 30 s at 55°C, 30 s at 72°C)

* Designed with Beacon Designer 2.0 software (Premier Biosoft, International, Palo Alto, CA) and synthesized by MWG Biotech (Ebersberg, Germany).
† Designed and synthesized by Qiagen (Courtaboeuf, France).

Table 3. *Effect of dietary protein restriction during gestation on maternal weight gain and food intake, litter size, and pups birth weight*

	Mother fed	
	20% protein (NP) diet	8% protein (LP) diet
Weight gain on the 19th d of gestation* (g)	108.9 (100.2; 121.7)	95.1 (89.2; 110.8)‡
Cumulated food intake through gestation* (g)	365.2 (339.0; 384.8)	381.1 (354.6; 435.2)†
Number of pups per litter*	14.0 (12.0; 14.0)	13.5 (12.0; 15.0)
Birth weight of pups§ (g)	6.95 (0.04)	5.98 (0.05)‖

* Results are shown as median (Q1; Q3) for $n = 26$ mothers per group.
† $p \leq 0.05$, ‡ $p \leq 0.01$. LP *vs.* NP diet (Mann-Whitney *U* test).
§ Results are shown as mean (SEM) for $n = 80$ male pups born to LP diet mothers and $n = 104$ male pups born to NP diet mothers.
‖ $p \leq 0.0001$ LP *vs.* NP diet (Fisher's PLSD test).

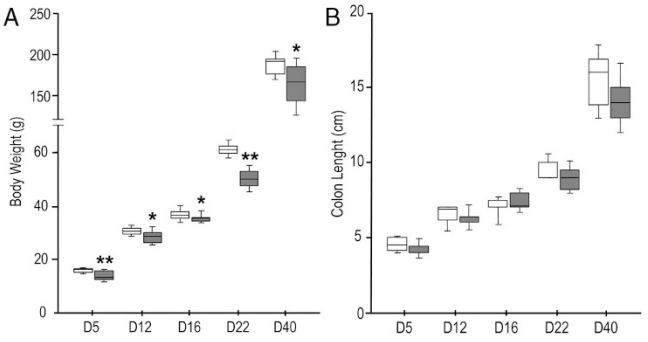

Figure 1. Body weight (*A*) and colon length (*B*) of controls (□) and rats with IUGR (■). Values are shown in box blot of $n = 12$ males per group. *$p \leq 0.05$, **$p \leq 0.01$ *vs.* controls at the same day (Mann-Whitney *U* test).

remained lighter (Fig. 1*A*) than controls throughout the follow-up (until D40).

IUGR altered morphologic postnatal development of the colon. Colon length increased with age in both IUGR and controls (Fig. 1*B*), but the colon length of pups with IUGR was consistently lower than that of controls ($p < 0.001$, ANOVA). Whereas crypt depth (Table 4) increased with age in the proximal and distal colon of controls and rats with IUGR, crypt depth in the proximal colon was lower in rats with IUGR compared with controls ($p = 0.036$, ANOVA); this reduction was particularly significant at D22 and D40 ($p = 0.043$ and 0.017, Mann-Whitney *U* test). The percentage of crypts in fission decreased during postnatal development in the proximal colon of controls. The normal diminution of the percentage of crypts in fission was delayed both in proximal and distal colon of rats with IUGR. The number of goblet cells per crypt increased significantly from D12 to D40 in proximal and distal colon of both controls and rats with IUGR; it was not affected in proximal colon but was decreased by IUGR in distal colon at D22 and D40 ($p = 0.018$ and 0.019).

IUGR disturbed the normal evolution of mucin and Tff3 gene expression in proximal colon. In proximal colon, *Muc2*, *Muc4,* and *Tff3* (Fig. 2*A–C*) expression increased in the aggregate during postnatal development with a steep rise between D16 and D22 ($p < 0.001$). *Muc1* expression showed a peculiar pattern during postnatal development, with two peaks (D12, D22) and a 7-fold drop at D40 (Fig. 2*D*).

IUGR was associated with a delay in the rise of *Muc2* expression, which occurred between D22 and D40 in rats with IUGR compared with D16–D22 in controls (Fig. 2*A*). This delay was responsible for a 3-fold reduction of *Muc2* expression at D22 in proximal colon of rats with IUGR compared with controls ($p = 0.003$). *Muc4* expression was lower in rats with IUGR compared with controls both at D22 and D40 ($p = 0.003$ and 0.004, Fig. 2*C*). *Muc1* expression (Fig. 2*D*) evolved similarly between D5 and D22, in the proximal colon of both groups, although the decline observed in controls at D40 was attenuated in rats with IUGR ($p = 0.028$). Furthermore, at D12, *Tff3* and *Muc1* (Figs. 2*B* and 3*D*) were overexpressed in rats with IUGR compared with controls ($p = 0.007$ and 0.004, respectively).

IUGR induced some changes in mucin and Tff3 gene expression in the distal colon. In the distal colon, *Muc1*, *Muc2*, and *Tff3* expression (Fig. 2*A*, *B,* and *D*) evolved similarly during postnatal development, with a peak of expression

Table 4. *Effect of intrauterine growth restriction on crypt depth and the percentage of crypts in fission in the proximal and distal colon of rats during postnatal development*

		Proximal colon		Distal colon	
	Postnatal day	Controls	IUGR	Controls	IUGR
Crypt depth* (μm)	D5	129 (112; 153)	119 (54; 161)	119 (105; 128)	114 (102; 132)
	D12	135 (127; 141)	126 (109; 129)	129 (128; 133)	132 (125; 142)
	D22	190 (171; 241)†‡	173 (160; 186)†‡§	186 (184; 187)†‡	163 (148; 182)†
	D40	209 (202; 222)†‡	180 (178; 193)†‡§	198 (186; 275)†‡	176 (159; 198)†‡
Percentage of crypts in fission*	D5	15 (14; 17)	20 (12; 21)	ND	ND
	D12	12 (6; 18)†	11 (7; 13)	5 (1; 10)	10 (3; 12)
	D22	7 (5; 13)†	14 (11; 16)	2 (2; 4)	7 (5; 10)§
	D40	5 (1; 10)†‡	4 (4; 5)†‖	4 (3; 5)	2 (0; 5)‖
Number of goblet cells per crypt*	D12	14 (13; 16)	17 (15; 19)	23 (22; 25)	23 (20; 24)
	D22	21 (17; 23)	22 (20; 23)	27 (26; 29)‡	21 (21; 21)§
	D40	23 (22; 26)‡	28 (27; 30)‡‖	36 (35; 37)‡†	26 (25; 26)‡§‖

ND, not determined.
* Results are shown as median (Q1; Q3) of $n = 4$ males per age and per group.
† $p \leq 0.05$ *vs.* D5, ‡ $p \leq 0.05$ *vs.* D12, ‖ $p \leq 0.05$ *vs.* D22 in both the control and the IUGR group (Mann-Whitney *U* test).
§ $p \leq 0.05$ rats which had suffered IUGR *vs.* controls at the same day (Mann-Whitney *U* test).

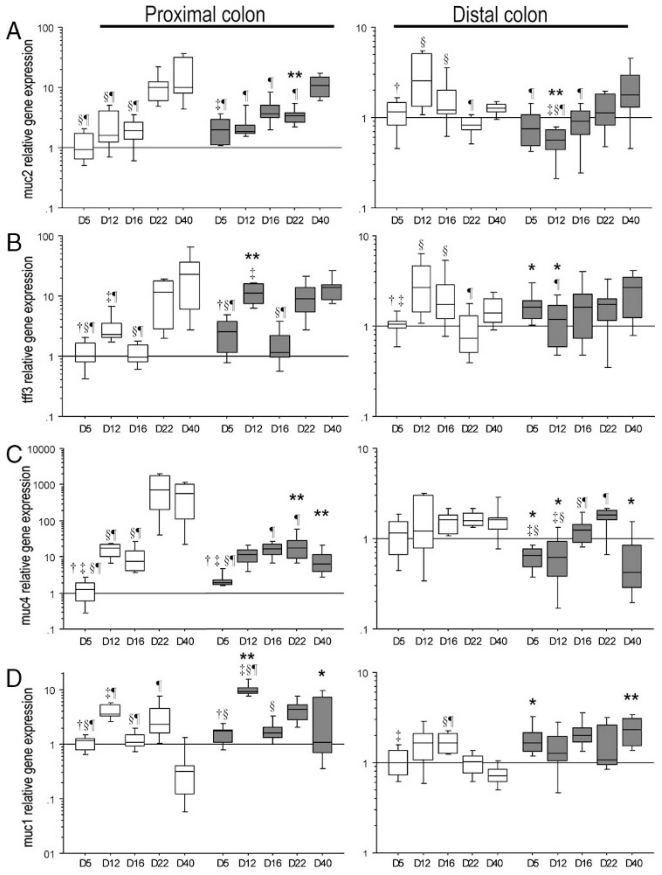

Figure 2. IUGR modulated *Muc2* (*A*), *Tff3* (*B*), *Muc4* (*C*), and *Muc1* (*D*) gene expression in proximal and distal colon during postnatal development. Values are shown in box blot and expressed using $2^{-\Delta\Delta CT}$ method ($n = 6–8$ males per group at each day). †$p \leq 0.05$ *vs*. D12; ‡$p \leq 0.05$ *vs*. D16; §$p \leq 0.05$ *vs*. D22; ¶$p \leq 0.05$ *vs*. D40 in both the control (□) and the IUGR (■) group (Mann-Whitney *U* test). *$p \leq 0.05$, **$p \leq 0.01$ *vs*. controls at the same day (Mann-Whitney *U* test).

at D12. *Muc4* expression in controls did not vary throughout the follow-up period (Fig. 2*C*).

The sharp rise in *Muc2* and *Tff3* expression (Fig. 2*A* and *B*) observed at D12 in controls was not detected in rats with IUGR ($p = 0.001$ and 0.020, respectively). *Muc4* expression was lower in rats with IUGR than in controls at D5 ($p = 0.058$) and D12 ($p = 0.045$). *Muc1* expression in the distal colon of rats with IUGR did not vary during postnatal development but its level of expression was higher than in controls in neonates, *i.e.*, at D5 ($p = 0.013$) and in adulthood, *i.e.*, at D40 ($p = 0.002$).

IUGR modified the expression of Mucin2, Mucin4, and Tff3 proteins but not Cdx-2. In line with gene expression, Muc2 and Tff3 protein expression in the proximal colon of controls and rats with IUGR (Figs. 3*A* and 4*B*) rose with postnatal age. The proportion of positive cells per crypt and the intensity of staining increased significantly during postnatal development in the proximal colon of controls as well as rats with IUGR ($p < 0.01$). IUGR significantly reduced Muc2 immunochemical labeling in the proximal colon of rats at D22 ($p = 0.040$). IUGR did not modify the staining intensity of Tff3 in proximal colon but decreased the proportion of positive cells at D12 ($p = 0.046$).

Regarding the protein expression of Muc4 in proximal colon (Fig. 4*A*), a few secretory granules were detected in both crypts of rats with IUGR and controls at D12. At D22 and D40, the labeling of Muc4 on the cell membrane and also in secretory granules of goblet cells was more pronounced than at D12 in both groups of rats.

The Cdx-2, a key factor regulating *Muc2*, *Muc4*, and *Tff3* transcription in colonic cell lines (28,31–33), was expressed at all stages of development; it was localized in the nucleus and distributed all along the crypt (Fig. 4*B*). Cdx-2 immunoreactivity was stable during development and similar in both groups of rats.

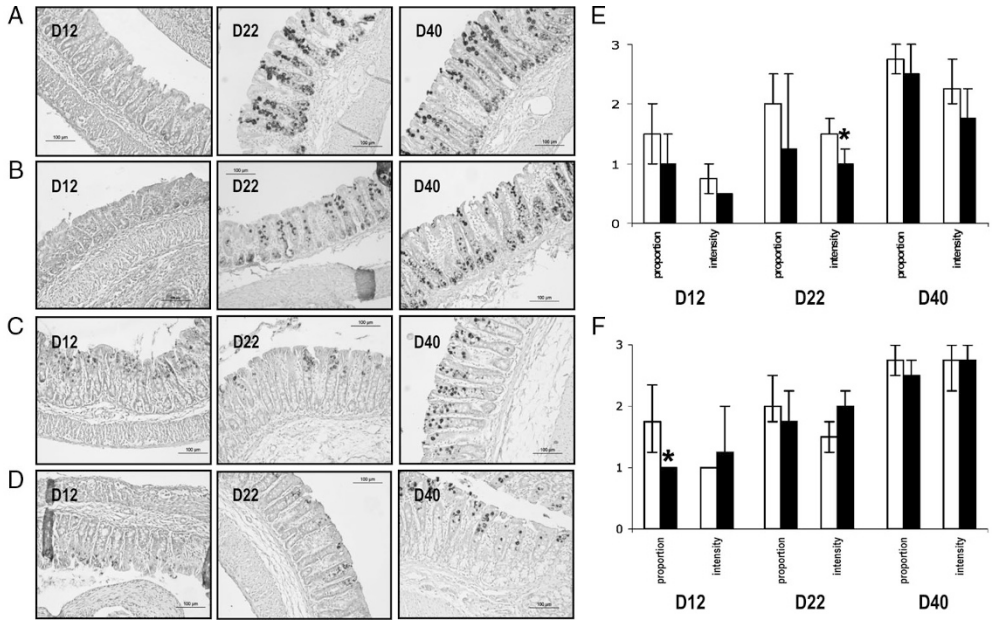

Figure 3. IUGR reduced Muc2 and Tff3 protein expression in proximal colon. Immunohistochemical analysis for Muc2 and Tff3 in proximal colon samples from controls and rats with IUGR at D12, D22, and D40 of life. (*A*) Muc2 staining in control group. (*B*) Muc2 staining in IUGR group. (*C*) Tff3 staining in control group. (*D*) Tff3 staining in IUGR. Staining of tissue sections for Muc2 (*E*) and for Tff3 (*F*) were scored blind to treatment for the proportions of cells stained per crypt (0, 1–3) and for the intensity of staining (0, 1–3) measured on 10 crypts per animal in the control (□) and the IUGR (■) group; values are median (Q1; Q3) of $n = 4$ males per group. *$p \leq 0.05$ *vs*. controls at the same day (Mann-Whitney *U* test).

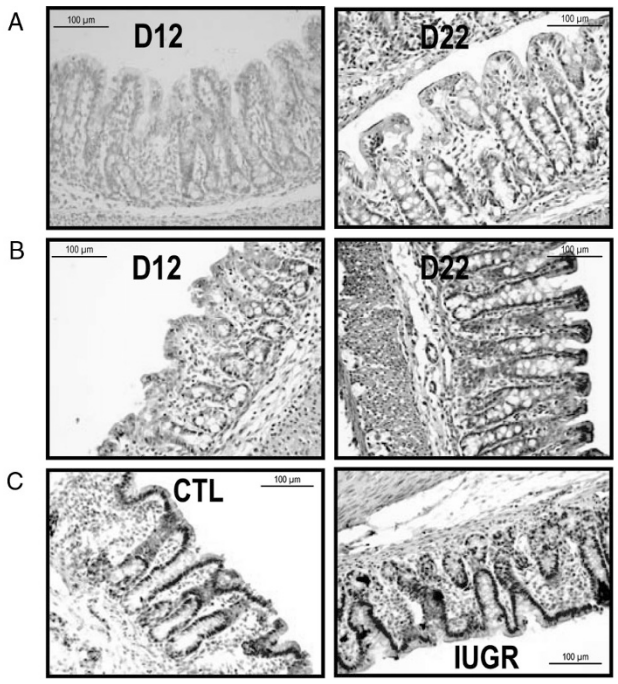

Figure 4. Effect of IUGR on Muc4 and Cdx-2 protein expression in proximal colon. Immunohistochemical analysis for Muc4 in proximal colon samples from controls (*A*) and rats with IUGR (*B*) at D12 and D22. (*C*) Immunohistochemical analysis for Cdx-2 in proximal colon samples from controls (*CTL*) and rats with IUGR at D22.

In distal colon, IUGR was associated with a significant reduction of detection of Muc2 (2.50 *vs*. 1.50, $p = 0.047$) and Tff3 (2.75 *vs*. 1.50, $p = 0.044$) at D12.

DISCUSSION

IUGR is associated with increased neonatal mortality and morbidity and a risk of NEC (2). We hypothesized that disruption of colonic barrier function may be the cause of this higher risk. We therefore investigated the effect of IUGR on the maturation of secreted and membrane-bound mucins and Tff3 in the postnatal development of rat colon.

First, we confirm a morphologic evolution and changes in mucins and Tff3 expression in normal colonic mucosa of rats during postnatal development. Before weaning, the proximal colonic mucosa was not mature as shown by morphologic parameters; furthermore, the goblet cells did not seem completely differentiated considering the low level of Muc2 and Muc4 protein expression. In addition, we provide evidence to suggest that D12 may be a critical period as striking changes occurred with regard to the gene expression of mucins and Tff3 in the proximal and distal colon of normal pups. After weaning, we found a sharp increase in the mRNA and protein expression of Muc2, Muc4, and Tff3 in the proximal colon of normal rat, as previously observed (28,34,35). These changes in the expression of mucus constituents were parallel to morphologic changes of the mucosa (increase in crypt depth and in the number of goblet cells per crypt, reduction in the percentage of crypts in fission) that were consistent with previous studies (36–38) and revealed a maturation of rat colonic mucosa.

Second, we demonstrate that IUGR induced a postnatal delay in the maturation of the colonic mucosa characterized by a reduction of crypt depth and a higher percentage of crypts in fission in proximal and distal colon and a reduction in goblet cells number in distal colon. These morphologic changes were accompanied by a reduction of *Muc2* (mRNA and protein level) and *Muc4* transcript in the proximal colon. Before weaning (D12), IUGR was associated with a low expression of Tff3 and Muc2 at mRNA and protein level in the distal colon and less Tff3 protein in proximal colon. Because *Muc2*, *Muc4*, and *Tff3* mRNA expression are under Cdx-2 regulation in colonic cell lines (31–33), we assessed its protein expression in proximal colon and we observed similar expression of Cdx-2 between controls and rats with IUGR. This finding argues against the role of Cdx-2 as a regulator of mucin and Tff3 synthesis during maturation of rat colon.

The lack of mucin and Tff3 expression in the colon of rats with IUGR we observed in this study could contribute to the frailty of the intestinal barrier associated with IUGR, and the higher risk of NEC and intestinal disease in the long run. The lack of Muc2 and Tff3 protein early after birth may weaken colonic mucosal defense. TFF3 is known to play a critical role in healing by promoting epithelial restitution after mucosal injury (12) and deficient TFF3 expression may be involved in the pathogenesis of NEC. In human infants, TFF3 indeed is downregulated in the colon of patients with NEC compared with healthy infants, and there is a lack of ectopic expression of others TFF peptides (14). In a model of mucosal injury in newborn rat, Lin *et al.* (16) found a downregulation of *Tff3* gene expression. In two different model of hypoxia-induced NEC, TFF3 administration attenuated the tissue damage and inflammatory response (17). Muc2, the major colonic gel-forming mucin, is known to be a critical factor for establishment of goblet cell morphology (10). MUC2 also plays a critical role in mucosal protection by preventing bacterial pathogens from gaining access to the epithelium. Indeed, the protective effect of probiotics on bacterial translocation is associated with MUC2 over expression (39), and the application of mucins to the surface of neonatal epithelial cell line was shown to inhibit bacterial translocation (40). In patients with Hirschsprung's disease, a decrease in the expression of MUC2 has been linked to the development of associated enterocolitis (15). Taken together, these data suggest that Tff3 and Muc2 underexpression in the colon of immature rats with IUGR could be responsible for a default in mucosal protection and therefore may contribute to the development of NEC.

Our study clearly shows that growth restriction during fetal life induced changes in intestinal mucosal barrier in postweaning rat and even in young adult. Recent data have also highlighted the fact that mucins play an essential role in epithelial protection against luminal factors and wound healing. For instance, the expression of the membrane-bound mucin Muc1 increases sharply after oral infection of mice by a bacterial pathogen and Muc1-deficient mice are more susceptible to pathogen colonization and mucosal damage (8). Thus, the inhibition of the expression of the major colonic secreted mucin (Muc2) and of the strongly expressed colonic membrane-bound mucin (Muc4) found in this study may be deleterious when colonic mucosa

is exposed to injury. Moreover, epidemiologic studies found that IUGR predisposes to colorectal cancer in adult life (4,5). The deregulation of mucin production (MUC2, MUC4) has been associated with numerous types of cancers and inflammatory disorders (13). Muc2-deficient mice spontaneously develop colitis and colorectal cancer, and are less resistant to experimentally induced colitis (10). Thus, the alterations of mucin and Tff3 expression due to altered fetal life could predispose the future adult to intestinal pathologies.

In conclusion, we demonstrate that mucin and Tff3 synthesis is regulated during postnatal maturation of colonic barrier. We further demonstrate for the first time that IUGR is associated with a failure in colonic mucus barrier development and long-term alterations in gene expression that persist after weaning. These findings raise the following questions: whether IUGR also affects other intestinal physiologic functions, such as permeability, innate immunity, and inflammatory response and what are the involved mechanisms. In this respect, two hypotheses have to be considered; first, the possible alteration of the gut colonization by bacteria, due to the known capability of the intestinal microbiota to interact with intestinal gene expression; and second, the possible changes in the epigenetic of the colonocytes, particularly in stem cells. All these issues are now under investigation.

Whatever will end up from these studies, the disruption of colonic barrier observed in this study on rat model, if it also occurs in human infants affected with IUGR, may account for the high prevalence of colonic injury and NEC and may contribute to the increased incidence of colon cancer reported in adults born small for gestational age.

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