Developmentally regulated IκB expression in intestinal epithelium and susceptibility to flagellin-induced inflammation

Erika C. Claud*†, Lei Lu*, Pauline M. Anton‡, Tor Savidge*, W. Allan Walker*, and Bobby J. Cherayil*

*Pediatric Gastroenterology Unit, Massachusetts General Hospital, 114 16th Street, Charlestown, MA 02129; and ‡Division of Gastroenterology, Beth Israel Deaconess Medical Center, Boston, MA 02215

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Necrotizing enterocolitis is a devastating inflammatory condition of the intestine that occurs almost exclusively in premature newborns. Although its exact pathogenesis is unclear, we have postulated that it may result from a predisposition of the immature intestine to mount an unusually robust and damaging response to microbial infection. In support of this idea, we report that the IL-8 response of an immature human enterocyte cell line to bacterial infection was significantly higher than that of a mature enterocyte cell line. The response in both cell lines was flagellin-dependent. Corresponding to the difference in IL-8 production, the immature enterocytes expressed appreciably lower levels of specific IκB genes when compared with the mature enterocytes. Similar developmentally regulated differences in cytokine response and IκB expression were also seen in primary rat enterocytes, indicating that these observations were not peculiarities of the cell lines. Furthermore, when the level of IκBα expression was increased in the immature cell line by transfection, the flagellin-dependent IL-8 response was attenuated. Thus, we have demonstrated a previously undescribed developmental regulation of IκB expression in the intestine involved in modulating the IL-8 response to bacterial infection, which may contribute to the pathogenesis of age-specific inflammatory bowel diseases such as necrotizing enterocolitis.

Enterocytes are active participants in host defense against microbial invasion. Certain aspects of this function are developmentally regulated, resulting in distinct differences between the immature and mature intestine with respect to interactions with microorganisms (1–4). Immaturity of epithelial barrier function, relative deficiencies in the expression of antimicrobial factors, and developmental variations in the pattern of epithelial surface glycosylation all contribute to the susceptibility of early postnatal intestine to bacterial infection (5–7). Concurrently, the secretion of proinflammatory cytokines is greater at earlier stages of development than in the adult. Specifically, IL-8 production in response to IL-1β and tumor necrosis factor α has been shown to be significantly increased in the human fetal intestinal epithelial cell (IEC) line H4 and fetal intestinal organ cultures compared with the adult IEC line Caco2 and biopsies from older children (8). The combination of weaker antimicrobial defense mechanisms and exaggerated cytokine responses may render the immature gut prone to extensive inflammatory damage after infection. These properties could thus contribute to the pathogenesis of age-specific diseases such as necrotizing enterocolitis (NEC), an ischemic and inflammatory bowel necrosis that primarily affects premature neonates after the initiation of enteral feeding.

We have postulated a model of NEC pathogenesis in which early feeding results in exposure to numbers and types of bacteria that the premature intestine is ill-equipped to handle (9). The exaggerated inflammatory responses that are characteristic of the gut at this stage of development could then lead to increased tissue injury. Using models of immature and mature IEC, we have previously shown that immature enterocytes have greater IL-8 secretion in response to the endogenous inflammatory mediators IL-1β and tumor necrosis factor α than do mature enterocytes (8, 10). As a next step, we hypothesized that the immature IEC would also have a more pronounced inflammatory response to exogenous mediators, specifically bacteria. We demonstrate here that a human immature enterocyte cell line and primary IEC from immature rodents have higher expression of inflammatory cytokines in response to bacterial infection than their more mature counterparts. A mechanistic explanation for these observations was suggested by the finding that the immature cells had appreciably lower expression of specific IκB genes, key regulators of NF-κB-dependent inflammatory pathways. The importance of differential IκB expression was confirmed by transfection studies showing that increased levels of IκBα dampened the IL-8 response to bacteria in the immature enterocyte cell line. These results document a previously undescribed developmental regulation of IκB expression in the intestine that modulates the IL-8 response to bacteria and suggest its involvement in the pathogenesis of age-specific inflammatory diseases of the bowel such as NEC.

Materials and Methods

Cell Culture. T84 cells originate from adult colonic adenocarcinoma cells. Cells were grown in a 1:1 (vol/vol) mixture of DMEM and F12 medium (Invitrogen) with 10% heat-inactivated FCS, 1% glutamine, 50 units/ml penicillin, and 50 μg/ml streptomycin. Cell passages 50–64 were used. H4 cells are a human fetal nontransformed primary small IEC line that has been characterized in this laboratory (11). They were cultured in DMEM with 10% heat-inactivated FCS, 1% glutamine, 1% sodium pyruvate, 1% amino acids, 1% Hepes, 50 units/ml penicillin, 50 μg/ml streptomycin, and 0.2 units/ml insulin. Cell passages 11–21 were used. All cells were grown at 37°C in a 5% CO_2 atmosphere.

Induction of IL-8. Nonpolarized H4 and T84 cells were grown to 80% confluence in 24-well tissue culture dishes in antibiotic-free medium. The cells were then infected with 10^8 organisms of a wild-type, virulent *Salmonella enterica* serovar Typhimurium strain (SL3201); its isogenic flagellin-deficient derivative, SL3201 FljB$^-$/FliC$^-$ (both *Salmonella* strains were obtained from Andrew Gewirtz, Emory University, Atlanta); a noninvasive, flagellin-containing, commensal strain of *Escherichia coli* (F18, kindly provided by Beth McCormick, Massachusetts General Hospital); or the flagellin-deficient *E. coli* laboratory strain DH5α for 1 h. Nonadherent bacteria were washed away, and the cells were incubated for 5 h in medium containing 100 μg/ml gentamicin (12). The cell supernatants were then collected for IL-8 measurement by ELISA as described below.

Abbreviations: IEC, intestinal epithelial cell; NEC, necrotizing enterocolitis.

†To whom correspondence should be addressed. E-mail: eclaud@partners.org.

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IL-8 Measurement. Ninety-six-well microtiter plates (Nunc-Maxisorp, Fisher Scientific) were coated overnight with anti-human IL-8 (R & D Systems) and then incubated for 70 min at 37°C with 100 μl of the cell supernatants. After sequential incubations with the detecting antibody, rabbit anti-human IL-8 (Endogen, Woburn, MA), the second antibody, horseradish peroxidase-conjugated goat anti-rabbit IgG (BioSource International, Camarillo, CA), and 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid), the absorbance was measured at 405 nm by using a microplate reader, and IL-8 concentrations were determined from a standard curve generated with purified recombinant human IL-8 (R & D Systems). IL-8 values were normalized to total cellular protein as determined by DC Protein assay (Bio-Rad) according to the manufacturer's recommendations.

Immunoblotting. The cells were washed in ice-cold PBS and then lysed in 1% Triton X-100 in 10 mM Tris (pH 8) and 150 mM NaCl, with 10 μg/ml aprotinin and leupeptin and 2 mM phenylmethylsulfonylfluoride. After estimating the protein concentrations of the cleared lysates, equal amounts of total protein were separated by SDS/PAGE on 10% polyacrylamide gels. Electrophoresed proteins were transferred from the gel to a nitrocellulose membrane by using a semidry transfer apparatus as described in ref. 13. The blot was blocked with 5% nonfat dry milk in Tris-buffered saline with Tween 20 and incubated with the primary antibody (anti-IκBα, anti-IκBβ, or anti-IκBε, all from Santa Cruz Biotechnology) and then with the appropriate horseradish peroxidase-conjugated secondary antibody. The blot was developed by using the West Pico SuperSignal chemiluminescence reagent (Pierce). The membranes were then stripped and reprobed with an antibody to GAPDH (Research Diagnostics Institute, Flanders, NJ) to confirm equal loading of lanes.

Isolation of Primary Rat Enterocytes. Using a modification of the Weiser technique, preweaned (10 days old) or postweaned (5 weeks old) Sprague–Dawley rats (Charles River Breeding Laboratories) were killed by carbon dioxide asphyxia (14). Full-length small intestine was removed through an abdominal incision, inverted, rinsed in cold 154 mM NaCl, and placed in a solution of 1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM potassium dihydrogen phosphate, and 5.6 mM disodium phosphate (pH 7.3) for 15 min at 37°C. The intestine was then transferred to a solution of 1.5 mM EDTA/0.5 mM DTT in PBS and placed on a shaker at 37°C for 20 min. The released cells were rinsed in cold PBS three times and then lysed as described above. Cell viability was confirmed by trypan blue exclusion.

Rat Ileal Loops. Sixteen-hour-fasted preweaned and postweaned rats were anesthetized with a mixture of 0.9 ml ketamine and 0.1 ml xylazine in 9 ml of sterile water at a dose of 0.1 ml per 10 g of body weight. Laparotomy was performed and one 3- to 5-cm-long closed distal ileal loop was formed and injected with either sterile PBS containing 10^8 colony-forming units of wild-type *Salmonella* strain SL3201 or buffer alone (control). The animals were killed 4 h later, the ileal loops were removed, and intestinal tissue samples were collected for RNA preparation (15).

Real-Time Quantitative PCR. Total RNA was extracted from frozen tissues with TRIzol reagent (Invitrogen) and reverse-transcribed (GeneAmp RNA-PCR Kit, Roche, Nutley, NJ). Real-time PCR was performed on the cDNA by using the rat IL-6 real-time PCR primer set (BioSource International) and SYBR Green PCR Master Mix (Applied Biosystems) for 40 cycles on an Opticon 2 DNA Engine (MJ Research, Watertown, MA) (95°C for 10 min, 60°C for 1 min, 72°C for 1 min, and 95°C for 1 min; steps 2–4 repeated for 40 cycles). Duplicate reactions were carried out for each sample. The GAPDH primer set (R & D Systems) and TaqMan Universal PCR Master Mix (Applied Biosystems) were used to normalize values. The cycle number at which the SYBR Green fluorescence intensity crossed a set threshold value (C_T) was determined for IL-6 and GAPDH for each sample. The values were normalized by subtracting the mean GAPDH C_T value from the mean IL-6 C_T value (15).

Transfection and Luciferase Reporter Assay. Transient transfection of H4 cells was accomplished by using Lipofectamine 2000 following the manufacturer's instructions (Life Technologies, Rockville, MD) (16). Briefly, cells were grown to 75% confluence in antibiotic-free medium and incubated with Lipofectamine 2000 reagent plus an IL-8 promoter-dependent firefly luciferase reporter construct (kindly provided by A. C. Keates, Beth Israel Deaconess Medical Center), a constitutively expressed *Renilla* luciferase reporter (Promega) as an internal control for transfection efficiency, and an IκBα expression construct kindly provided by Ranjan Sen (Brandeis University, Waltham, MA). After 40 h, cells were stimulated with the culture supernatant of either wild-type *Salmonella* (SL3201) or its flagellin-deficient mutant (SL3201 FljB$^-$/FliC$^-$) for 5 h. Cells were then lysed, and the Dual-Luciferase Reporter Assay (Promega) was carried out following the manufacturer's recommendations. The luminescence generated by the firefly luciferase was measured first and then quenched, and a second measurement was obtained to determine *Renilla* luminescence. Firefly luminescence (normalized to *Renilla* luminescence) is expressed as fold increase over the value obtained for the control, unstimulated cells.

Statistical Analysis. Results are presented as mean values ± SEM. Statistical significance was determined by using the *t* test. $P < 0.05$ was considered statistically significant.

Results

Immature Enterocytes (H4 Cells) Have Increased IL-8 Secretion in Response to Bacteria Compared with Mature Enterocytes (T84 Cells). H4 cells are a human fetal nontransformed primary small IEC line that has been characterized in this laboratory (11). Previous studies have used this cell line to investigate differences in immature and mature intestine in response to lipopolysaccharide, IL-1β, and cholera toxin. Results obtained with the H4 line have been found to be comparable to those obtained with primary human fetal organ cultures (4, 8). These cells are morphologically immature and do not form tight junctions. Data from the H4 cells were compared with those obtained using nonpolarized monolayers of the well characterized adult human adenocarcinoma IEC line T84, which at confluence is considered to have characteristics of developmentally mature enterocytes (17).

H4 and T84 cells were infected with either the wild-type *Salmonella* strain SL3201 or the noninvasive commensal *E. coli* strain F18. As shown in Fig. 1, both cell types responded to the *Salmonella* and *E. coli* infections with an increase in secreted IL-8. However, the H4 cells secreted a significantly higher amount of IL-8 than the T84 cells [H4 cells, 3,200 pg/mg in response to *Salmonella* and 2,400 pg/mg in response to *E. coli*; T84 cells, 1,000 pg/mg in response to *Salmonella* and 600 pg/mg in response to *E. coli* ($P < 0.05$)].

IL-8 Secretion in H4 and T84 Cells in Response to *Salmonella* and *E. coli* Is Flagellin-Dependent. To begin to investigate the mechanism behind the difference in IL-8 secretion between immature and mature enterocytes, we examined the role of bacterial flagellin. Flagellin is a subunit of flagella, previously shown to be a major bacterial factor involved in eliciting IL-8 production by epithelial cells (18). When flagellin-deficient strains of *Salmonella* and *E. coli* were used to infect H4 and T84 cells, measured IL-8 levels

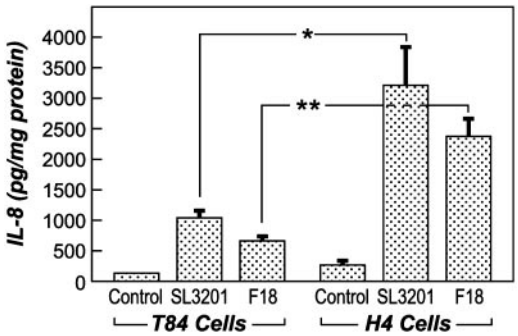

Fig. 1. Fetal H4 cells secrete significantly more IL-8 in response to the pathogenic *Salmonella* strain SL3201 and the commensal *E. coli* strain F18 than do the adult T84 cells. Cells were infected with the indicated bacteria, and IL-8 was measured by ELISA in the 5-h cell supernatants. IL-8 values were normalized to protein concentrations from cell lysates and are presented as mean ± SEM ($n \geq 4$). Asterisks denote significance at $P < 0.05$ for the comparison of the values indicated by the brackets.

were less than control in the supernatants of each cell line (Fig. 2). Similar results were obtained with a second flagellin-deficient *Salmonella* strain, SL3201 FlhD$^-$ (data not shown). These observations indicate that, in both the immature and mature cells, IL-8 secretion in response to bacterial infection is flagellin-dependent. The H4 and T84 cells expressed comparable levels of total and surface Toll-like receptor 5 (TLR5), the receptor for flagellin (19) (data not shown), indicating that the difference in IL-8 secretion by the two cell lines cannot be explained by differences in TLR5 expression.

Expression of IκB Genes in Immature and Mature Enterocytes. We proceeded to investigate postreceptor steps in signal transduction, focusing on the NF-κB pathway because of its role in inflammatory responses and NEC (20). The NF-κB transcription factor is retained in the cytosol by the inhibitor protein IκB (21). Signal-induced degradation of IκB allows NF-κB to move into the nucleus and activate the transcription of genes involved in several aspects of inflammation and innate immunity (22). Examination of protein lysates of H4 and T84 cells by immunoblotting revealed striking differences in IκB expression. Fig. 3 *Left* shows that both IκBα and IκBε were expressed at higher levels in T84 cells than in H4 cells, whereas IκBβ was expressed at similar levels in the two cell lines.

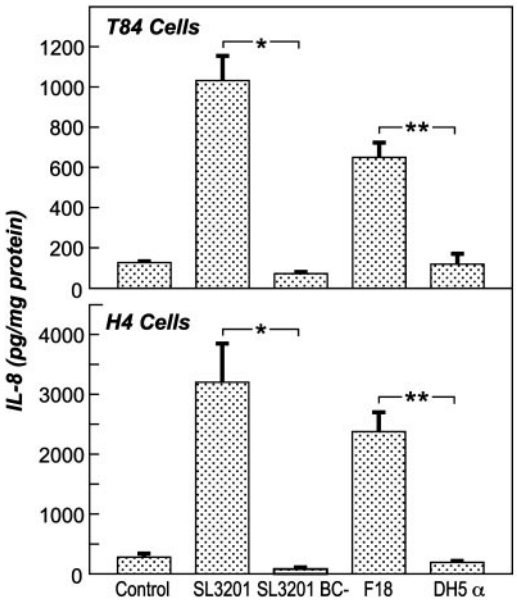

Fig. 2. Flagellin is required for IL-8 secretion in response to bacterial infection in both T84 and H4 cells. Cells were infected with the indicated bacteria, and IL-8 was measured by ELISA in the 5-h cell supernatants. IL-8 values were normalized to protein concentrations from cell lysates and are presented as mean ± SEM ($n \geq 4$). Asterisks denote significance at $P < 0.05$ for the comparison of the values indicated by the brackets. IL-8 secretion to the pathogenic *Salmonella* SL3201 strain is below control levels in the flagellin mutant SL3201 FljB$^-$/FliC$^-$ (SL3201 BC$^-$), and IL-8 secretion to the commensal *E. coli* strain F18 is below control levels in the *E. coli* flagellin mutant DH5α in T84 cells (*Upper*) and H4 cells (*Lower*).

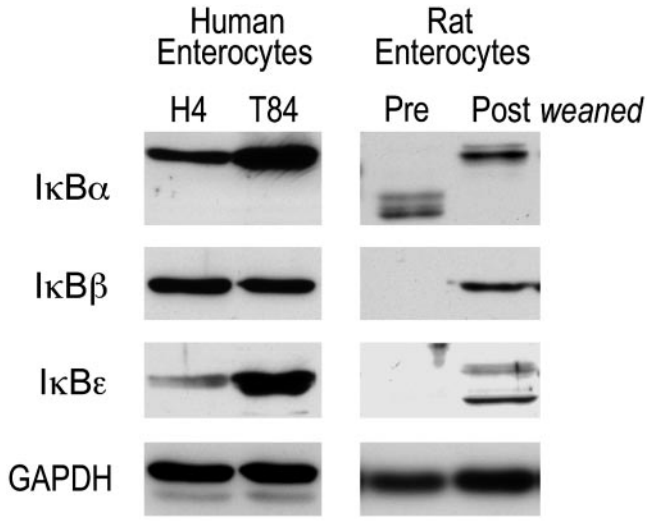

Fig. 3. Differential expression of IκB genes in immature and mature enterocytes. Cell lysates were obtained from human IEC cell lines (*Left*) or rat primary IECs (*Right*). The expression of IκBα, IκBβ, IκBε, and GAPDH was revealed by Western blotting with specific antibodies. The results shown are representative of four separate experiments in each category.

To confirm these developmental differences in IκB expression in primary IEC, we extended the immunoblotting studies to enterocytes harvested from either preweaned or postweaned rats. Intestine from preweaned rats <2 weeks of age is considered to be developmentally comparable to third-trimester human fetal intestine. After weaning at 3 weeks of life, rat intestine undergoes developmental "closure," no longer allowing passage of macromolecules (23). Postweaned rat intestine is thus considered developmentally mature. Comparisons between preweaned and postweaned rat intestine have been used as a model to approximate differences in immature and mature human intestine in other studies such as those investigating developmental responses to cholera toxin (24).

Protein extracts were made from enterocytes isolated from the small intestine of preweaned and postweaned rats and analyzed by Western blotting. As shown in Fig. 3 *Right*, clear differences in the expression of IκBα, IκBβ, and IκBε between the preweaned and postweaned animals were observed. Expression of all three IκB genes was readily detectable in all of the postweaned rats that we examined ($n = 4$) but was either absent or present at very low levels in the preweaned animals ($n = 4$). The level of expression of IκBα in the adult rat was similar to that in the postweaned animals (data not shown).

Preweaned Rats Have Increased IL-6 Expression in Response to Bacterial Infection When Compared with Mature Animals. The differences in IκB expression between the immature and mature rat enterocytes led us to ask whether these cells would also behave like the H4 and T84 cells with respect to their cytokine response to bacterial infection. Closed ileal loops were created in

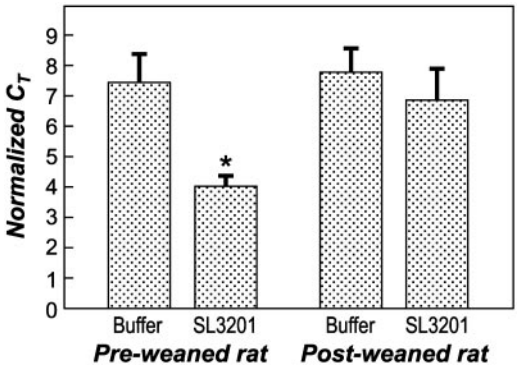

Fig. 4. Increased intestinal IL-6 expression in response to bacteria in preweaned rats compared with postweaned rats. Closed ileal loops were created and injected with buffer (negative control) or SL3201. Intestinal RNA samples were prepared 4 h later, and IL-6 was measured by real-time quantitative PCR. The experiment was performed in triplicate, and the results are presented as the mean ± SEM C_T for IL-6 normalized to the C_T value for GAPDH. An asterisk denotes significance at $P < 0.05$ as compared with buffer-treated animals.

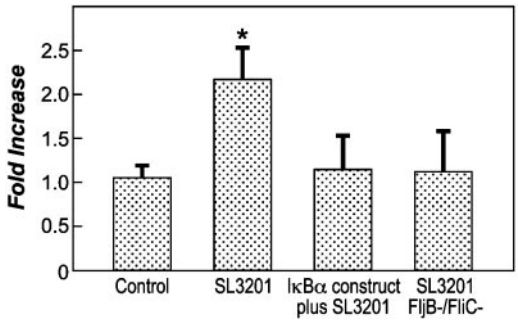

Fig. 5. Transfection of H4 cells with an IκBα expression construct attenuates IL-8 production in response to flagellin. H4 cells were transfected with an IκBα construct and an IL-8 promoter-driven firefly luciferase reporter construct along with a *Renilla* luciferase reporter as an internal control for transfection efficiency. Cells were then treated for 5 h with bacterial supernatants as indicated, and luminescence was measured. As described in *Materials and Methods*, results are presented as the mean ± SEM fold increase in firefly luminescence (normalized to *Renilla* luminescence) over control ($n = 6$). An asterisk denotes significance at $P < 0.05$ compared with control.

preweaned and postweaned rats and injected with the wild-type *Salmonella* strain SL3201. Intestinal tissue samples were collected 4 h later, and RNA was prepared. Real-time quantitative PCR was used to measure IL-6 and GAPDH mRNA levels. IL-6 is increased in the serum of patients with NEC, and levels of this inflammatory cytokine correlate with severity of illness (25, 26). Thus, because rodents lack an IL-8 gene, IL-6 was evaluated as a relevant, NF-κB-dependent cytokine. Fig. 4 shows that, after infection, there was a significant decrease in the IL-6 C_T value (normalized to GAPDH) in the preweaned animals but not in the postweaned animals. Thus, fewer PCR cycles were required to reach threshold in the preweaned animals, indicating a greater induction of IL-6 expression in this group. A trend similar to decreased C_T in preweaned animals was noted with tumor necrosis factor α primers (data not shown). These *in vivo* observations are consistent with those obtained with the cell lines, providing further support for the idea that the immature intestine has a more vigorous inflammatory response than the mature organ.

Exaggerated Flagellin-Induced IL-8 Production in the Immature H4 Cell Line Can Be Attenuated by Increased Levels of IκBα. Our data indicate a developmental regulation of IκB gene expression both in IEC lines and in primary enterocytes. To determine whether differences in IκB levels could contribute to alterations in the cytokine response to bacteria, we examined the effect of increasing expression of IκBα on the IL-8 response of H4 cells. H4 cells were transfected with an IL-8 promoter-driven firefly luciferase reporter construct, with or without cotransfection of an IκBα expression construct. Forty hours later, the cells were treated for 5 h with the flagellin-containing supernatant of wild-type *Salmonella* SL3201 or the supernatant of the flagellin-deficient *Salmonella* strain SL3201 FljB$^-$/FliC$^-$. Bacterial supernatants were used rather than whole bacteria to allow longer exposure of the cells to the flagellin without affecting cell viability. As can be seen in Fig. 5, exposure to the wild-type supernatant resulted in a statistically significant increase in normalized firefly luciferase activity, indicating increased transcription of the IL-8 promoter-dependent reporter, whereas the flagellin-deficient supernatant had no effect on expression of the reporter. Reproducibly, cotransfection of the IκBα expression plasmid inhibited the flagellin-induced increase in firefly luciferase expression. Thus, increased levels of IκBα attenuated the flagellin-induced transcriptional up-regulation of the IL-8 gene. This suggests that developmentally regulated differences in IκB expression could contribute to the differential responsiveness of immature and mature enterocytes to inflammatory stimuli.

Discussion

The findings reported here document a previously undescribed developmental regulation of IκB expression in IECs, as supported by observations in both human cell lines and primary rodent enterocytes (Fig. 3). The increase in IκB expression that occurs with maturation is associated with decreased expression of proinflammatory cytokines in response to bacterial infection (Figs. 1 and 4). The observation that increasing the expression of IκBα in the immature H4 enterocyte cell line leads to a decrease in flagellin-induced IL-8 expression (Fig. 5) indicates that the change in IκB expression is likely to be at least one factor contributing to the altered responsiveness. Taken together, our data offer a new mechanistic explanation for the heightened inflammatory responses of the immature intestine and suggest that low levels of intestinal IκB may partially explain the unique susceptibility of preterm infants to NEC.

It is a formal possibility that the differences in IκB expression between preweaned and postweaned rat enterocytes may reflect differences in the proportion of crypt cells to villus cells isolated at the two stages of development. Although we cannot definitively exclude this possibility, we think it is unlikely, because differences in IκB expression were also observed when comparing the immature H4 cell line with the mature T84 cell line. Both these cell lines are generally considered to correspond to the crypt cell phenotype (25, 26), indicating that developmental changes in IκB expression can occur within a single enterocyte subset.

NF-κB is a key factor in regulating the expression of genes involved in inflammatory and immune responses, including cytokines such as IL-6 and IL-8 (22). Importantly, studies in a rat model of NEC demonstrated increased NF-κB binding activity in the small intestine of the animals 0–3 h after the induction of the disease, suggesting that NF-κB activation is an early step in pathogenesis (20). The function of the IκB proteins in regulating NF-κB is well known, and in this respect IκBα, IκBβ, and IκBε, the three most widely expressed members of this family, play very similar roles. A recent detailed analysis of NF-κB activation and inflammatory gene expression in cells deficient in one or the other of the IκB proteins indicated that, in the absence of IκBα, an abnormally prolonged activation of NF-κB was observed, particularly after transient stimuli, associated with increased

expression of certain inflammatory cytokines (27). These observations are in keeping with *in vivo* data demonstrating a lethal, widespread inflammatory disorder in mice lacking IκBα (28, 29). Thus alterations in IκB expression can have a profound influence on the inflammatory response. A good deal of this dysregulation can be attributed to the failure, in the knockout animals and cells, of the NF-κB-dependent increase in IκBα expression that normally occurs after an activating stimulus and that functions as a negative feedback mechanism (27). However, there are also examples of inflammatory responses being influenced by a change in the steady-state level of IκB. Macrophage tolerance, a state of decreased responsiveness of inflammatory gene expression, has been shown to be associated with increased expression of the IκBε and IκBζ genes (30). Recently, increased IκBα expression has been linked to C5a-induced inhibition of lipopolysaccharide-dependent tumor necrosis factor α production in neutrophils (31). Other studies have demonstrated decreased NF-κB activation in response to increased levels of IκBα induced by transforming growth factor β1 in human salivary gland cells (32). Consistent with these earlier results, our observations lend further support to the idea that modulation of IκB levels may act as a rheostat that determines the responsiveness of a cell to inflammatory stimuli. In addition to the developmentally regulated expression of IκB, alterations in other aspects of NF-κB activity and regulation may occur during intestinal maturation and may influence the inflammatory response.

Many of the major complications of prematurity, including chronic lung disease, periventricular leukomalacia, and NEC are inflammatory in nature (33–35). Pilot data from the ongoing extremely low gestational age neonate (ELGAN) study suggested that, in the first few days of life, levels of cytokines such as IL-8 are twice as high in these preterm infants as in term newborns (36). One explanation offered is that ELGANs produce higher levels of inflammatory cytokines in response to infection than infants born at term. Looking specifically at the intestine, these data are consistent with previous findings from our laboratory demonstrating significantly higher IL-8 secretion by immature enterocytes in response to inflammatory stimuli compared with mature enterocytes (8, 10). It is an intriguing possibility that proinflammatory mediators produced by the immature intestine may contribute to the pathogenesis of prematurity-associated inflammatory conditions at other sites. In fact, a recent review of neurodevelopmental and functional outcomes of extremely low birth weight infants found that NEC was significantly associated with increased neurodevelopmental morbidity (37). Thus, the developmental regulation of IκB expression that we have demonstrated in the intestine may have consequences for inflammatory processes outside the gut. Although much work has looked at potential triggers of inflammation in preterm infants, including prenatal exposure to chorioamnionitis and sepsis, few studies have examined the mechanisms that regulate the magnitude and duration of the inflammatory response (34, 38). Induced maturation of the regulatory pathways controlling inflammation may be a new approach to treating these conditions.

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