Hydrocortisone-induced anti-inflammatory effects in immature human enterocytes depend on the timing of exposure

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Rautava S, Walker WA, Lu L. Hydrocortisone-induced anti-inflammatory effects in immature human enterocytes depend on the timing of exposure. Am J Physiol Gastrointest Liver Physiol 310: G920–G929, 2016. First published April 7, 2016; doi:10.1152/ajpgi.00457.2015.—The immature human gut has a propensity to exaggerated inflammatory responses that are thought to play a role in the pathogenesis of necrotizing enterocolitis (NEC). Prenatal exposure to corticosteroids has been reported to reduce the risk of NEC, while postnatal dexamethasone treatment is associated with adverse neurodevelopmental outcomes in preterm infants. The aim of this study was to investigate the direct role of hydrocortisone in gene expression patterns and inflammatory responses in immature human enterocytes. Time-dependent hydrocortisone effects in nontransformed primary human fetal intestinal epithelial cell line H4 were investigated by cDNA microarray. Fetal intestinal organ culture and cell culture experiments were conducted. Inflammatory responses were induced by stimulation with IL-1β and TNF-α with and without hydrocortisone. IL-8 and IL-6 expression and secretion were measured as functional readout. Here we report time-dependent hydrocortisone-induced changes in gene expression patterns detected by cDNA microarray. Hydrocortisone significantly attenuated IL-1β-induced inflammatory responses in the immature human gut when administered at the time of the proinflammatory insult: IL-1β-induced IL-8 and IL-6 secretion in the fetal ileum as well as H4 cells were significantly reduced. Hydrocortisone also inhibited IL-8 secretion in response to TNF-α. In contrast, TNF-α-induced IL-8 secretion was not reduced in cells treated with hydrocortisone for 48 h before stimulation. Our observations provide a physiological basis for understanding the differential clinical effects of corticosteroids in the immature human gut depending on the timing of treatment.

MATERIALS AND METHODS

Materials. Hydrocortisone hemisuccinate, collagenase type IV, protease inhibitor cocktail, and phosphatase inhibitor cocktail I and II were obtained from Sigma-Aldrich (St. Louis, MO). Recombinant human insulin (Novolin R) was obtained from Novo Nordisk (Bagsvaerd, Denmark). Extracellular matrix ECL was obtained from Upstate Biotechnology (Lake Placid, NY). The cytokines IL-1β and TNF-α as well as all ELISA reagents were obtained from R&D Systems (Minneapolis, MN). The BCA Protein Assay kit was obtained from Thermo Scientific (Rockford, IL). The LDH Cytotoxicity Detection Kit was obtained from Roche (Mannheim, Germany). RNeasy Mini kit and SuperScript III Platinum SYBRgreen One-Step qRT-PCR kits were obtained from Qiagen (Valencia, CA).

Media (DMEM-F-12, CMRL, and Opti-MEM I) and other reagents for tissue culture were obtained from Invitrogen-GIBCO (Carlsbad, CA). Comparison of allergen-specific IgE and IgG4 antibodies was performed with flow cytometry using a FACSCalibur system (Becton Dickinson, San Jose, CA). The cytokines IL-1β and TNF-α were detected by a sandwich ELISA method using the Cytometric Bead Array platform (BD Biosciences, San Jose, CA). Hydrocortisone-hemisuccinate (Sigma-Aldrich, St. Louis, MO) was dissolved in OPTI-MEM I (Life Technologies, Carlsbad, CA) and used at stock concentrations of 100 μM.

THE MUCOSA OF THE SMALL INTESTINE has many important functions in addition to nutrient absorption and fluid secretion. These functions, which include host defense and immune responses, may be developmentally regulated. The host inflammatory response is a primary defense mechanism following injuries or infection, which is necessary to restore intestinal homeostasis. The immature human gut is more susceptible to inflammatory responses in the immature human gut depending on the timing of treatment. The host inflammatory response (3). Many of these genes, including TNF-α, GM-CSF, IL-1β, IL-6, and IL-8, are commonly also overexpressed during inflammation in the immature human gut (9).

Using a time course of GC exposure, we and others have reported that GCs exhibit pleiotropic effects in multiple cell types (1, 21, 42). In this study, we investigated the time-dependent impact of hydrocortisone (HC) on gene expression patterns and inflammatory responses in immature human IECs.
CA). M3D medium was purchased from Incell (San Antonio, TX). All other chemicals were purchased from Sigma-Aldrich unless otherwise specified.

**Cell culture and growth conditions.** This study employed human IEC lines: H4, primary human fetal intestinal epithelial cells (hFIECs), and the mature IEC line NCM460. H4 cells are a human fetal nontransformed small IEC line that has previously been characterized in this laboratory (34). H4 cells were routinely maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and human recombinant insulin (0.5 U/ml). H4 cells were incubated with H4 medium containing 1 μM HC for 12 h, 24 h, 48 h, or 5 days (27).

**Human fetal intestinal organ culture and IEC isolation and culture.** Fetal intestinal organ culture was prepared from fetal tissue obtained from therapeutic abortions as described previously (1). Briefly, fetal small intestine was stripped of its mesentery, split longitudinally, and cut into explants (5 × 5 mm), which were cultured in a Falcon organ culture dish at 37°C with 95% O2. Isolation and culture of primary small intestine epithelia were modified from procedures described by Quaroni and Beaulieu (30). Segments of fetal small intestine were minced and incubated with a 1.25% trypsin-0.5 mM EDTA solution at room temperature for 10 min. Crypts were collected and digested with collagenase type IV (200 U/ml) for 90 min. The dissociated epithelial cells were then plated in a tissue culture petri dish with Opti-MEM supplemented with 20 ng/ml human epidermal growth factor, 10 μg/ml insulin (human recombinant), and 4% FBS and incubated with 5% CO2 at 32°C.

Fetal tissue was obtained with approval from the Partners Human Study Committee and with signed permission (IRB no. 1999p003833).

**RNA isolation and amplification.** Human IECs were lysed in RLT buffer (containing guanidine isothiocyanate) from Qiagen. Total RNA was isolated with the RNeasy kit according to the manufacturer’s instructions. Human Genome U133 Plus 2.0 GeneChip microarrays were purchased from Affymetrix (Santa Clara, CA). Preparation of labeled cRNA, hybridization, and scanning of microarray analysis were performed with standard protocols and reagents as described in the Affymetrix Technical Manual (Revision 3) as described previously in Reference 21.

**Data filtering and analysis.** These procedures were described previously and were submitted to the GEO repository (accession code: GSE22106) (21). In brief, data were analyzed with linear modeling of microarray data (AffyLMGUI), and the fluorescence intensity of each spot was normalized by robust multiarray averaging with R/Bioconductor. We defined differentially expressed genes as those whose expression value changed at least twofold (≥ 2-fold change) in response to HC. MetaCore (GeneGo) was used to perform the gene enrichment analysis among the groups and to identify canonical pathways and networks that were most significant to the data set. Fischer’s exact test was used to calculate a P value determining the probability that the association between the genes in the data set and the canonical pathway was due to chance.

**qRT-PCR.** IL-6 and IL-8 mRNA expressions were measured with semiquantitative RT-PCR (real-time RT-PCR) from RNA isolated as described above. All RT-PCR reactions were done with the SuperScript III One-Step RT-PCR Kit according to the manufacturer’s protocol. Total RNA (10 ng) was added to each reaction in duplicates with the annealing temperature set at 60°C and 40 cycles.

**Effect of HC on IL-6 and IL-8 secretion.** Cells were grown to confluence in 24-well plates. Medium was then changed, and cells were pretreated with HC for 48 h at 1 μM for prolonged pretreatment of the cells. All other cells were incubated with either 10 ng/ml TNF-α or 1 ng/ml IL-1β in the presence or absence of HC for 18 h. Comparable cell viability under these pretreatment and treatment conditions was confirmed in initial assays using a LDH cytotoxicity assay. Supernatants were collected for IL-6 and IL-8 determination and stored at −20°C. Cell lysates were obtained for measurement of protein concentration.

**Cytokine measurement.** IL-8 and IL-6 concentrations were measured by an ELISA as described by McCormick et al. (23). Briefly, 96-well plates (Nunc Maxisorp) were coated overnight with either 8 or 6 μg/ml goat anti-human IL-8 or IL-6 polyclonal antibody. The plate was then washed (PBS + 5% neonatal goat serum, 1% Tween 20 diluted 1:10) and incubated with samples at 37°C. After a second wash, the plate was incubated with 8 μg/ml rabbit anti-human IL-8 or IL-6 antibody. The plate was washed again and incubated with 80 ng/ml horseradish peroxidase goat anti-rabbit IgG. Finally, the plate

![Fig. 1. Principal component analysis (PCA) of early and late HC-induced differentially expressed genes in H4 cells: results of PCA on the HC time series data.](http://ajpgi.physiology.org/)

The figure shows the principal component analysis (PCA) of early and late HC-induced differentially expressed genes in H4 cells. The PCA was performed on the HC time series data, and the results indicate a significant overlap between early and late HC-induced genes in H4 cells.
was washed and incubated with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). Absorbance was measured at OD 405. Samples were run in quadruplicate, and IL-8 and IL-6 values were normalized to measured protein concentration to allow comparison among cell types.

For all reported data, at least two independent experiments were conducted in which each condition was tested in triplicate. One representative experiment for each parameter is included in this report. The key experiments for H4 cells were repeated six times (with triplicates every time), with similar results.

**Protein concentration determination.** For protein concentration determination, cells were lysed for 30 min on ice in the 96-well plate with 40 µl/well lysis buffer consisting of 1% Nonidet P-40, 150 mM NaCl, 1x PBS, 20 mM EDTA, 20 mM EGTA, 4 mM Na3VO4, and 100 mM DTT.

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**Fig. 2. MetaCore gene enrichment analysis of HC effects in H4 cell microarray.** HC-induced differentially expressed genes (fold change ≥ 2) were further analyzed by GeneGo MetaCore gene enrichment analysis. A: top 10 canonical pathways that were affected by HC-upregulated genes (fold change ≥ 2) in H4 cells. HC treatment, particularly long term (>48 h), has a global effect on gene expression and a large effect on development genes. B: top 10 canonical pathways that were affected by HC-downregulated genes (fold change ≤ -2) in H4 cells. C: top-ranked networks where the HC-downregulated genes are overrepresented. Short-term HC exposure (12 h) has a stronger anti-inflammatory and immunosuppression action compared with long-term HC exposure (48 h).
40 mM NaF. After centrifugation, 5 μl/well lysate was used for the Bio-Rad DC Protein Assay per the manufacturer’s protocol.

**Statistical analysis.** Statistical analysis was reviewed with a statistician. Results are presented as mean ± SE values of IL-8 or IL-6 (pg/mg protein). Statistical significance was evaluated by two-tailed t-test. \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**Time effect of hydrocortisone on gene expression patterns in H4 cells.** HC has been reported to induce differential expression of gene transcripts either rapidly (2–24 h) or slowly (>48 h) in mammalian enterocytes in vivo and in vitro (1, 36, 42). Using NIA Array Analysis Tool (http://lgsun.grc.nia.nih.gov/) we performed principal component analysis (PCA) using the singular value decomposition method of the log-transformed data matrix (4, 9a). As shown in Fig. 1, samples were clustered by the length of HC exposure: the shorter HC-treated samples (12 and 24 h) and the longer HC-treated samples (48 h and 5 days). This result suggested that there is a time-dependent pattern of gene expression in response to HC exposure. There are 206, 265, 1,003, and 1,152 differentially expressed genes (fold change ≥ ±2) in H4 cells treated with HC for 12, 24, 48 and 120 h, respectively. Moreover, the shorter HC exposure (12 and 24 h) exhibits a major inhibitory effect on gene expression, with 68% and 76% downregulated genes, respectively, whereas longer HC treatment (48 h) exhibits a major transcriptional activation effect, with 60% upregulated genes in H4 cells. We previously reported that HC may induce either a

![Fig. 3](http://ajpgi.physiology.org/)

**Fig. 3.** Pathway-specific analysis of HC-induced differentially expressed genes in H4 cells: comparison of the differentially expressed genes in short-term HC exposure (12 h) and long term HC exposure (48 h) presented in canonical IL-1β signaling pathway (A and B) and in canonical TNF signaling pathway (C and D) with Ingenuity Pathway Analysis software. Red, upregulated genes; green, downregulated genes; black arrow, differentially expressed negative regulator of inflammatory response genes (TOLLIP, A20).
transient or a persistent change in gene expression in H4 cells that depends on the duration of HC exposure; moreover, many persistently upregulated genes are involved in the developmental processes of the organisms including IEC differentiation/maturation (21). In this study, we mainly examined the downregulated genes and their functional pattern. For this purpose the differentially expressed genes were analyzed with knowledge-based integrated pathway enrichment software by MetaCore. The top 10 scored canonical pathways and gene networks are displayed in Fig. 2. HC-upregulated genes are involved in IEC differentiation/maturation, including the organization of extracellular matrix, TGF-β and WNT-mediated cytoskeleton remodeling, cell adhesion, and cell-matrix interactions (Fig. 2A). In contrast, HC-downregulated genes, particularly the short-term HC-responsive genes, were overrepresented in pathways associated with innate immune/inflammatory responses (Fig. 2B). More strikingly, the downregulated genes induced by short-term HC treatment were overrepresented in networks involving IL-1, IL-6, TLR, and TNF-α signaling (Fig. 2C). This result suggested that short-term HC exposure exhibits a strong anti-inflammatory effect in H4 cells.

**HC effects on genes associated with inflammatory responses in H4 cells.** The immunosuppressive actions of GCs have been reported to be predominantly mediated by interference with signaling by the key inflammatory transcriptional regulators: nuclear factor (NF)-κB and c-Fos/c-Jun (AP-1) (15, 16). To examine the role of short-term/long-term HC treatment in IL-1β and TNF signaling activity, Ingenuity Pathway Analysis software was used. As shown in Fig. 3, HC induced downregulation of many gene transcripts that are overrepresented in the canonical IL-1β signaling pathway. Moreover, compared with the longer exposure, short-term HC treatment elicits not only strong inhibitory effects on IL-1β signaling-associated genes but also upregulation of the gene TOLLIP, a known inhibitory regulator of innate immune/inflammatory response, resulting in a suppression of MAPK/p38, JNK, and NF-κB activity (Fig. 3, A vs. B). Although both short- and long-term HC treatment inhibit expression of many key TNF-α signaling-associated genes such as TRADD, RIP, TRAF, NIK, IKK, and NF-κB, short-term HC treatment induced an upregulation of A20 expression (Fig. 3, C vs. D). A20 is known to inhibit inflammation and autoimmunity (11, 39) as well as preserve tissue homeostasis by protecting cells from apoptosis (40).

Using microarray analysis of H4 cells, we have shown that HC may induce a transcriptional change in genes involved in IL-1 signaling. To validate the microarray data and investigate the functional relevance, we examined the gene expression changes of two well-known NF-κB-targeted proinflammatory...
cytokines (IL-8 and IL-6) in response to HC and IL-1β treatment. First, we investigated whether HC can inhibit IL-1β-induced upregulation of IL-8 and IL-6 mRNA expression. IL-1β induced a rapid increase in the expression of both genes after 6 h and peaked or plateaued between 6 and 18 h, respectively. Coincubation with HC significantly decreased IL-1β-upregulated IL-8 and IL-6 mRNA expression; the inhibitory effect of HC increased in a time-dependent manner and peaked by 18 h of treatment (Fig. 4, A and B). Next, we examined the long-term HC inhibitory effect on IL-1β-induced response in H4 cells. Our data indicate that the inhibitory effect on IL-1β-induced IL-8 and IL-6 gene expression was not augmented by longer HC exposure (48 h) in H4 cells. Furthermore, a prolonged pretreatment of H4 cells with HC further diminished the inhibitory effect of HC on IL-1β-induced cytokine gene expression as shown in Fig. 4, C and D.

Effect of HC on IL-8 production in response to inflammatory cytokines. We first examined whether HC inhibits IL-1β-induced inflammatory responses in organ cultures of fetal small intestinal tissue. In these experiments, IL-1β induced an appreciable IL-8 response, and this response was completely suppressed by concurrent exposure to HC (Fig. 5A).

Next, we investigated whether there is a biphasic effect of HC on inflammatory responses in IECs. For long-term HC treatment, human immature IECs (H4 and hFIECs) were incubated with HC for 48 h before the addition of proinflammatory cytokines: IL-1β or TNF-α. In other experiments, IECs were treated with HC and inflammatory cytokines concurrently for 18 h. IL-8 concentration was measured by ELISA and normalized to cell protein concentration to allow comparison among cell lines. In H4 cells, IL-1β increased IL-8 secretion from undetectable to 3,784 ± 41 ng/mg protein (P < 0.0001) and this response was significantly reduced to 1,546 ± 89 ng/mg protein (P < 0.0001) when HC was present. Pretreatment of HC for 48 h inhibited IL-1β-induced IL-8 secretion to the level of 1,974 ± 96 ng/mg protein (P < 0.0001). Similar results were observed in primary culture of hFIECs after exposure to HC and IL-1β (Fig. 5C). Consistent with IL-8 mRNA expression data, prolonged HC treatment did not augment inhibitory effect on IL-1β responses in immature enterocytes.

![Graphs showing IL-8 secretion in response to inflammatory cytokines and HC exposure.](http://ajpgi.physiology.org/)(Fig. 5. HC differentially modulates IL-8 secretion in immature human epithelial cells depending on timing of exposure. A–C: IL-1β induced a significant increase in IL-8 secretion in fetal ileal organ culture (SI; A), H4 cells (B), and primary culture of human fetal intestinal epithelial cells (hFIECs; C). D and E: in a similar fashion, a robust IL-8 response was detected in response to TNF-α in H4 cells (D) and hFIECs (E). IL-8 responses were significantly reduced by HC administered at the time of proinflammatory stimulation in all these models. Prolonged HC exposure (>48 h) elicited significant though more modest inhibition of IL-8 secretion in response to IL-1β in H4 cells (B). In contrast, 48-h treatment with HC exhibited no inhibition of IL-8 secretion in response to TNF-α in H4 cells (D); nor did exposure to HC before stimulation have inhibitory effect on IL-1β- or TNF-α-induced IL-8 secretion in hFIECs (C and E). Significance between IL-1β-induced cytokine levels and IL-1β-stimulated cytokine levels after HC exposure: **P ≤ 0.01, ***P ≤ 0.001.)
IECs (Fig. 5, B and C), HC also inhibited IL-8 secretion in response to TNF-α in H4 cells (Fig. 5D) and hFIECs (Fig. 5E) when administered simultaneously with the proinflammatory insult. In contrast, TNF-α-induced IL-8 secretion was not reduced in cells pretreated with HC for 48 h (Fig. 5, D and E).

Effect of HC on IL-6 production in response to inflammatory cytokines in immature human IECs. Since IL-6 is a pleiotropic cytokine with pathological roles in various disease conditions, such as inflammatory, autoimmune, and malignant diseases (22, 37), we also examined the HC effect in IL-6 production in H4 cells after inflammatory insults. IL-1β increased IL-6 secretion significantly. To determine the biphasic effect of HC, H4 cells were either incubated with HC and IL-1β concurrently or pretreated with HC for 48 h before the addition of IL-1β. HC, with either simultaneous addition or pretreatment in H4 cells, exhibited a strong inhibitory effect on IL-6 secretion after inflammatory cytokine challenge (Fig. 6A). In primary culture of hFIECs, both long- and short-term HC treatment had the same inhibitory effect on IL-6 production in response to IL-1β or TNF-α (Fig. 6B). In contrast to the HC effect on IL-8 described above, there is no biphasic effect of HC on IL-6 production in immature enterocytes (Fig. 6, C and D).

Next, to determine whether there is an effective length of HC exposure and corresponding inhibitory effect on inflammatory cytokine expression, we conditioned H4 cells with HC for 2, 6, 24, or 48 h and then stimulated with IL-1β. IL-1β-induced IL-8 (Fig. 7A) and IL-6 (Fig. 7B) secretion were significantly reduced after 2–48 h of exposure to HC. We also observed that prolonged pretreatment of HC (48 h) exhibited no greater anti-inflammatory effects in H4 cells. However, both IL-8 (Fig. 7A) and IL-6 (Fig. 7B) secretion were more profoundly inhibited when HC was administered at the time of IL-1β stimulation, and this effect could also be seen if cells, after exposure to HC for 48 h, were treated with freshly administered HC at the time of IL-1β stimulation. These data suggested that timing and duration of exposure might be important factors in the efficacy of HC-mediated anti-inflammatory responses in human IECs.

DISCUSSION

GCs are the most widely used and effective treatment for control of inflammatory and autoimmune diseases. GCs not only inhibit many of the initial events in an inflammatory response such as vasodilation, increased vascular permeability, and leukocyte infiltration into inflamed sites (reviewed in Ref. 28) but also modulate leukocyte/lymphocyte trafficking (24), apoptosis/survival (29), and differentiation programming (2, 33, 38), thus shaping the subsequent responses including 1)
Among these mechanistic networks, many genes associated with IL-1β and TNF-α signaling pathway were moderately downregulated and resulted in decreased NF-κB and cFos/cJun (AP-1) activity (Fig. 3). It is widely accepted that the potent anti-inflammatory actions of GCs are due to inhibition of transcription factors, such as AP-1 and NF-κB, that are involved in activation of proinflammatory genes (9). Our data suggested that in immature human IECs HC also altered the gene expression program of these cells, leading to a downregulation of NF-κB and cFos/cJun activity. Moreover, our data suggest that short-term HC treatment (12 h) induces gene expression of several important inhibitory modulators of innate immune/inflammatory response in the human IECs, such as TOLLIP for IL-1β and TLRα-signaling pathways and A20 (TNFAIP3) for TNF-mediated NF-κB activation. In contrast, a longer HC exposure (48 h) downregulates the expression of those genes that inhibit IL-1β and/or TNF-α signaling pathways. Moreover, a longer HC exposure also upregulates major signaling molecules TRAF and JNK expression in H4 cells (Fig. 3D). In combination, this might be the basis for the diminished inhibitory effect of long-term HC on TNF-α-induced cytokine production. This is consistent with our results that short-term HC treatment exhibits more potent anti-inflammatory effects in human IECs. Longer exposure might induce desensitization of GC receptors, and the finding that a fresh dose of HC rescued full inhibitory effect in H4 cells pretreated with HC for 48 h supports this argument (Fig. 7). However, the exact mechanism is unclear, and further studies are needed to examine the effective dose and timing of GC in long-term treatment of inflammatory conditions.

Fig. 7. Effect of HC on IL-1β-induced inflammatory cytokine secretion in H4 cells depends on timing and duration of exposure. H4 cells were pretreated with or without HC for 2, 6, 24, or 48 h and then stimulated with IL-1β for 18 h. IL-1β-induced IL-8 (A) and IL-6 (B) secretion were significantly reduced after 2–48 h of exposure to HC. However, both IL-8 (A) and IL-6 (B) secretion were more profoundly inhibited with concurrent HC exposure at the time of IL-1β stimulation. Significance between IL-1β-induced cytokine levels and IL-1β-stimulated cytokine levels after HC exposure: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.01.

redirection of lymphocyte traffic, 2) inhibition of cytokine gene expression, and 3) inhibition of the expression of adhesion molecules. Most of the anti-inflammatory actions are characterized in lymphocytes and myeloid and hematopoietic cells, but little has been reported on the direct anti-inflammatory effects of GCs on IECs. In the present study, we focus on inflammatory cytokines produced by immature human IECs in response to inflammatory insults and on regulation of their expression by HC. In our previous study, we used microarray analysis to identify transcripts that are differentially up- and downregulated in response to the duration of HC exposure in H4 cells. The observed pattern of H4 cell responsiveness to HC indicates an early and a late phase of transcription regulation. Many transcripts that are most affected by longer HC treatment (with a similar number of up- or downregulated genes) were found in cell adhesion, TGF-β and WNT-regulated cell proliferation, cytoskeleton reorganization, and ECM remodeling categories, implicating effects of HC on differentiation and maturation (21). In contrast to prolonged HC exposure (≥48 h), a shorter HC exposure exhibits a larger effect on transcription repression than on activation (21). Moreover, the downregulated genes were overrepresented in networks involved in inflammatory/innate immune responses (Fig. 2C). When we extracted the pattern of differentially expressed gene sets, we found that TNF and IL-1 are the top scored upstream regulators for the genes affected by shorter HC (<24 h) treatment. Among these mechanistic networks, many genes associated with IL-1β and TNF-α signaling pathway were moderately
The transcription factor NF-κB activates genes coding for many inflammatory cytokines including IL-1β, TNF-α, IL-6, and IL-8. Previous studies from our group have shown that immature human IECs produce a large amount of IL-6 and IL-8 upon inflammatory stimulation or infection (5, 7, 32). Therefore it seemed appropriate to investigate the anti-inflammatory effect of HC on IECs by monitoring NF-κB-targeted genes such as IL-6 and IL-8 in response to inflammatory insults. The series studies presented here provide evidence that HC attenuates inflammatory responses in immature human IECs. Although cell culture findings have limitations and the clinical relevance needs to be confirmed in in vivo models, these findings suggest that, in addition to its function as a potent trophic factor promoting intestinal maturation/differentiation, HC exhibited a direct anti-inflammatory effect on human IECs in general and immature IECs in particular. However, the anti-inflammatory potential of HC on IECs is target specific and time dependent. IECs can produce and secrete cytokines such as IL-8 and IL-6 in response to stimuli, and that acute induction of IL-8 in the intestinal epithelium results in neutrophil recruitment and activation (14, 17, 18, 26, 31). Thus suppression of IL-8 production in the enterocytes by early, short-term, and effective GC treatment may play a pivotal role in acute and chronic inflammatory disease progression.

We previously reported that HC inhibits the cholera toxin-induced secretory response in the immature gut (19). However, this antisecretory action of HC is due to its trophic effects on the immature IECs, such as maturation of the plasma membrane leading to the downregulation of clathrin-mediated cholera toxin uptake, and decreased transcytosis and signal transduction efficiency (19). Moreover, cholera toxin-induced Cl⁻ secretion can only be blocked via the prolonged pretreatment with HC in immature IECs in human and rat models of secretory diarrhea (6, 20). Shorter exposure to HC (<48 h) augmented the cholera toxin-induced Cl⁻ secretion in immature IECs (unpublished data). In contrast, a shorter and concurrent HC treatment elicited a strong and consistent inhibitory effect on human IECs in response to inflammatory insults. This action is likely due to the inhibitory effect on transcriptional factors such as NF-κB and cFos/cJun, thus decreasing the mRNA expression levels of inflammatory cytokines. It is worth noting that HC elicits a substantially larger anti-inflammatory effect on fetal intestinal organ culture than on IEC culture. The whole fetal intestinal tissue also consists of lymphocytes, myofibroblasts, and myeloid and hematopoietic cells. These cells are major sources of innate immune/inflammatory cytokines (10, 35) and also major sites of GC anti-inflammatory actions. Our data suggest a concerted anti-inflammatory effect on both immune cells and IECs in the intestinal organ culture.

Here we report that a time-dependent HC-induced change in gene expression patterns detected by cDNA microarray was reflected in functional differences in anti-inflammatory potential of HC resulting from timing of the exposure.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s). All authors had access to the study data and reviewed and approved the final manuscript.

AUTHOR CONTRIBUTIONS

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BIPHASIC EFFECTS OF HYDROCORTISONE IN FETAL ENTEROCYTES


