Glucocorticoid-regulated genes in eosinophilic esophagitis: A role for *FKBP51*

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Background: Eosinophilic esophagitis (EE) involves marked accumulation of eosinophils in the esophageal mucosa that responds to swallowed fluticasone propionate (FP) in a subset of patients.

Objectives: We aimed to uncover the mechanism of action of swallowed FP in patients with EE by providing evidence for a topical effect in the esophagus by identifying a molecular signature for FP exposure *in vivo*.

Methods: Global microarray expression profiles, immunofluorescence microscopy, and cell signaling in esophageal tissue and cell lines were analyzed.

Results: Thirty-two transcripts exhibited altered expression in patients who responded to swallowed FP treatment. Esophageal FK506-binding protein 5 *(FKBP51)* mRNA levels were increased ($P < .05$) in FP responders compared with those seen in control subjects and patients with untreated active EE. After FP treatment of esophageal epithelial cells, *FKBP51* mRNA and protein levels were increased in a dose- and time-dependent manner by FP treatment *in vitro*. FP-induced *FKBP51* was steroid receptor dependent because RU486 completely inhibited gene and protein induction. The half-life of *FKBP51* mRNA was 16 to 18 hours independent of FP treatment. *FKBP51* overexpression reduced FP action as assessed by FP inhibition of IL-13–induced eotaxin-3 promoter activity.

Conclusions: Our results suggest that swallowed glucocorticoid treatment directly affects esophageal gene expression in patients with EE. In particular, increased *FKBP51* transcript levels identify glucocorticoid exposure *in vivo* and distinguish FP responders from untreated patients with active EE and patients without EE. In addition, *FKBP51* reduces glucocorticoid-mediated inhibition of IL-13 signaling in epithelial cells *in vitro*, suggesting that *FKBP51* might influence FP responsiveness. We propose that esophageal *FKBP51* levels have diagnostic and prognostic significance in patients with EE. (J Allergy Clin Immunol 2010;125:879-88.)

Key words: *Glucocorticoids, eosinophilic esophagitis, FKBP51, IL-13, esophageal epithelial cells*

Eosinophilic esophagitis (EE) is characterized by an increased accumulation of eosinophils in the esophageal mucosa that is refractory to acid-suppressive therapy.[1] Evidence suggests that the disease strongly associates with atopy and an antigen-driven, T_H2-type immune response.[2] EE predominantly affects male subjects in a ratio of approximately 3:1 (male/female).[3,4] This chronic disease affects pediatric populations because age of onset can be as early as during the first year of life.[3] Treatment strategies for EE include dietary restriction of food allergens or systemic glucocorticoid therapy. In addition, a subset of patients respond to swallowed glucocorticoid therapy delivered by means of asthma inhalation medications. The mechanism of action of swallowed glucocorticoids has not been examined, but the observation that patients with EE receiving inhaled corticosteroid therapy benefit from swallowing the same medication provides evidence for a local effect, although other reasons for the improvement exist (eg, increased systemic levels or delivery to the oral or gastric mucosa). Although swallowed or systemic glucocorticoids represent an effective therapy, their action is not sustained on discontinuation of the medicine.[5] Additionally, a recent controlled clinical trial has reported that only 50% of patients with EE treated with swallowed fluticasone propionate (FP) respond to treatment compared with 9% in the placebo group.[6] Determining the mechanism of action of swallowed corticosteroid therapy is likely to help improve efficacy, develop better drug formulation strategies, and identify mechanisms of steroid resistance, a common problem seen in patients with EE, as well as other allergic inflammatory disorders.[7]

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Abbreviations used
CHX: Cycloheximide
EE: Eosinophilic esophagitis
FKBP51: FK506-binding protein 5
FP: Fluticasone propionate
F3: Coagulation factor III gene
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase gene
GRE: Glucocorticoid response element
hpf: High-power field
UTR: Untranslated region

Glucocorticoid treatment induces an anti-inflammatory effect by targeting various cell types involved in both the innate and adaptive immune responses. In the respiratory tract the action of glucocorticoids is mediated through effects on epithelial cells, at least in part.[8,9] Several lines of evidence suggest that esophageal epithelial cells (keratinocytes) comprise a key cellular component involved in the pathogenesis of EE. Notably, on exposure to IL-13, a cytokine overproduced in patients with EE, esophageal epithelial cells express a transcriptome that markedly overlaps the EE transcriptome,[10] a set of genes differentially expressed between biopsy specimens from control subjects and patients with EE.[11] The gene exhibiting the highest increase in patients with EE and in IL-13–treated esophageal epithelial cells is eotaxin-3, an eosinophil chemoattractant and activator. It has therefore been proposed that IL-13 signaling in esophageal epithelial cells contributes to the pathogenesis of EE, particularly through induction of eotaxin-3. Global transcript profiling shows that 98% of the EE transcriptome is reversed to normal levels in patients who respond to FP.[10] Given the importance of esophageal epithelial cells in patients with EE, these cells can serve as relevant targets of glucocorticoid treatment, particularly swallowed FP.

Glucocorticoids exert their action through a variety of mechanisms, including transcriptional inhibition of specific promoter response elements, destabilization of cytokine mRNA, and direct induction of cellular apoptosis. The action of glucocorticoids varies among cell types, and few studies exist[12-15] that have examined the action of glucocorticoids on the esophagus *in vivo* or on esophageal cell lines. Accordingly, in this study we aimed to identify targets of swallowed FP treatment in the esophagus of patients with EE to further understand the molecular mechanisms underlying patient response to this drug. Using global expression profile analysis of esophageal tissue, we identified a panel of 32 transcripts altered by FP treatment *in vivo* in responders compared with untreated control patients and patients with active EE. Of these transcripts, only 1 gene exhibited levels that were differentially regulated in both responders and nonresponders; the remainder exhibited altered regulation only in responders and not in nonresponders. This suggested that nonresponders might exhibit a fundamental dysfunction in the glucocorticoid signaling pathway in their esophagus. Notably, levels of mRNA for *FKBP51*, a known steroid-induced gene in respiratory epithelial cells and lymphocytes,[16-19] were increased in FP responders. *FKBP51* was directly induced by glucocorticoids in esophageal epithelial cells, and we developed evidence that it acts as a negative regulator of FP action. We propose that swallowed FP therapy acts topically and mediates its effects by directly regulating gene expression in esophageal epithelial cells. Furthermore, we suggest that levels of esophageal transcripts, in particular *FKBP51*, serve as *in vivo* signatures for steroid exposure.

METHODS

Study design

Patients included in the study include those recruited from either Cincinnati Children's Hospital Medical Center (50 patients) or Children's Hospital, San Diego (1 patient). Patients ranged in age from 1.5 to 22.4 years (mean age, 8.9 ± 4.8 years). The population included 38 male and 13 female subjects. Samples were divided into groups according to the following characteristics: control patients (n = 14)—no history of EE, 0 eosinophils per high-powered field (hpf) in the esophagus at the time of biopsy, and no concomitant swallowed glucocorticoid treatment; patients with EE (n = 14)—24 or more eosinophils/hpf at the time of biopsy and no concomitant swallowed glucocorticoid treatment); patients with EE who responded to FP treatment (n = 13)—history of EE, 0 to 1 eosinophils/hpf at the time of biopsy, and concomitant swallowed fluticasone treatment; and patients with EE who did not respond fully to FP treatment (n = 8)—history of EE, 6 or more eosinophils/hpf at the time of biopsy, and concomitant swallowed FP treatment (see Table E1 in this article's Online Repository at www.jacionline.org). It was noted that no significant difference existed in the average eosinophil count of untreated biopsy samples between FP responders and nonresponders (see Figure E1 and Table E2 in this article's Online Repository at www.jacionline.org).

Patients taking swallowed FP were generally instructed to use an inhaler without a spacer, spray the medication into the pharynx, and not eat, drink, or rinse for 30 minutes after administration. Doses of swallowed FP are indicated for individual patients; when available, the time of the last dose of FP before endoscopy is listed (see Table E3 in this article's Online Repository at www.jacionline.org). Biopsy specimens were collected from the distal esophagus on routine endoscopy and submerged in RNAlater (for RNA isolation), modified F-media (for culture), or 4% paraformaldehyde (for immunofluorescence studies). This study was approved by the Institutional Review Board of the Cincinnati Children's Hospital Medical Center.

Microarray analysis

RNA extraction and microarray analysis were performed as previously described.[11] To identify glucocorticoid-regulated genes, those transcripts differentially regulated between control subjects (n = 14) and patients with EE (n = 14) were identified by using the *t* test ($P < .01$). Genes differentially regulated between control subjects and FP responders (n = 13) were then identified by using a 2-fold change filter. Subsequently, the genes differentially regulated between control subjects and patients with EE were subtracted from those differentially regulated between control subjects and FP responders. These transcripts were then subjected to ANOVA ($P < .01$). Although not involved in the initial analysis, transcript values were also included for FP nonresponders (n = 8).

Quantitative real-time PCR

Real-time PCR was performed with the IQ5 system (Bio-Rad Laboratories, Hercules, Calif). Reactions were carried out with SYBR green mix (Bio-Rad Laboratories). The value obtained for each primer set was normalized to the glyceraldehyde-3-phosphate dehydrogenase *(GAPDH)* value for the corresponding sample. Primer sequences are listed in Table E4 (available in this article's Online Repository at www.jacionline.org).

Primary epithelial cell culture

Primary esophageal epithelial cells were generally cultured as previously described.[10] Detailed methods of culture and treatment can be found in the Methods section in this article's Online Repository at www.jacionline.org. This study was approved by the Institutional Review Board of the Cincinnati Children's Hospital Medical Center.

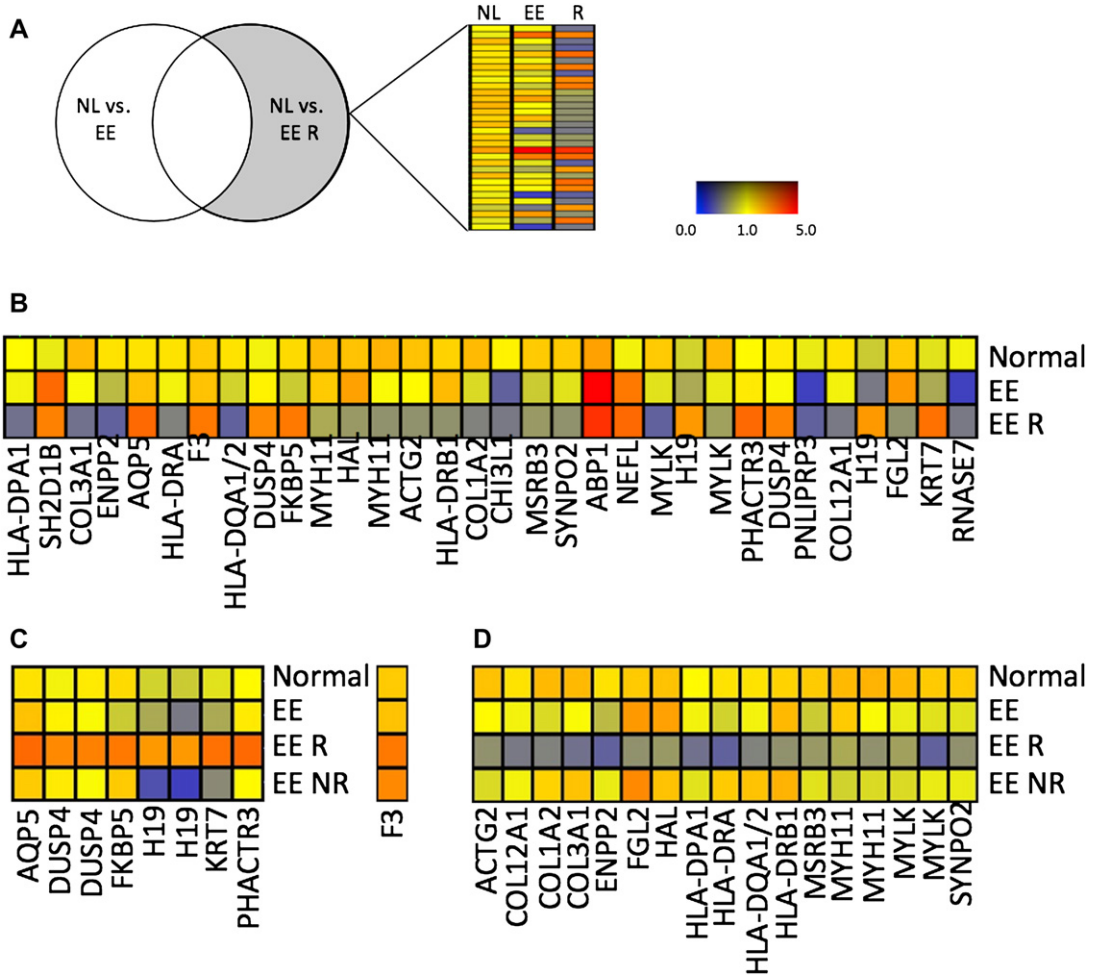

FIG 1. Identification of glucocorticoid-regulated genes in patients with EE who respond to FP treatment. **A**, The average relative gene expression for each patient group. **B**, Average expression levels of transcripts identified in Fig 1, *A*. The scale is represented in Fig 1, *A (right)*. **C**, Average expression of genes exhibiting increased transcript levels in FP responders. **D**, Average expression of genes showing decreased transcript levels in FP responders. *EE*, Untreated patients with EE; *NL*, control patients; *NR*, FP nonresponders; *R*, FP responders.

TE-7 cell culture

The human esophageal cell line TE-7 was kindly provided by Dr Hainault (IARC, Lyon, France). These cells were maintained in RPMI medium (Invitrogen, Carlsbad, Calif) supplemented with 5% FBS (Atlanta Biologicals, Lawrenceville, Ga) and 1% penicillin/streptomycin (Invitrogen). Details regarding cell treatment and transfection can be found in the Methods section in this article's Online Repository.

RNA isolation and reverse transcription

RNA was isolated from biopsy specimens as previously described[11] by using the RNeasy kit (Qiagen, Hilden, Germany) per the manufacturer's instructions. RNA was isolated from cells with Trizol (Invitrogen) according to the manufacturer's instructions, except in actinomycin experiments, in which RNA was isolated with the RNeasy kit (Qiagen). RNA samples (100-1000 ng) were subjected to reverse transcription with Superscript II Reverse Transcriptase (Invitrogen) per the manufacturer's protocol.

Protein extracts and Western blot analysis

TE-7 or primary esophageal epithelial cells were washed with PBS and incubated with MPER lysis buffer (Thermo Fisher Scientific, Rockford, Ill) supplemented with aprotinin (10 μg/mL), leupeptin (10 μg/mL), pepstatin (10 μg/mL), and sodium orthovanadate (1 mmol/L). Total protein (5-10 mg) was loaded onto 4-12% NuPage Tris-bis gels (Invitrogen), electrophoresed for 1.5 hours at 150 V, and transferred to nitrocellulose membranes, followed by Western blot analysis. Primary antibodies were diluted in TBS/0.1% Tween 20 containing 5% milk: goat anti-FKBP51 (1:1,000; R&D Systems, Minneapolis, Minn) and murine anti–β-actin (1:5,000; Sigma-Aldrich, St Louis, Mo). Secondary antibodies were incubated with the membranes: anti-goat horseradish peroxidase (1:10,000; Santa Cruz Biotechnology, Inc, Santa Cruz, Calif) and anti-mouse horseradish peroxidase (1:10,000; Cell Signaling Technology, Inc, Danvers, Mass). Blots were developed with ECL Plus reagent (GE Healthcare, Piscataway, NJ). Densitometric measurements were performed with Multi Gauge V3.0 (Fujifilm, Tokyo, Japan).

Immunofluorescence microscopy

Biopsy samples were collected as described above. After incubation in 4% paraformaldehyde, biopsy specimens were submerged in 30% sucrose and then embedded in OCT compound. Frozen sections cut from OCT compound–embedded esophageal biopsy samples were mounted on slides. Sections were fixed in ice-cold acetone for 10 minutes, incubated in blocking buffer (PBS, 1% saponin, and 3% FBS), and then incubated with either primary antibody or an equal concentration of control antibody in blocking buffer: anti-FKBP51, 10 μg/mL (R&D Systems, Inc); control, normal goat IgG (R&D Systems,

Inc). Sections were incubated with Alexa 594–conjugated secondary antibody (1:250, Invitrogen). Sections were washed 3 times with PBS after each antibody incubation. Fluromount G containing 4′-6-diamidino-2-phenylindole, dihydrochloride was used for mounting. Sections were visualized with the BX51 microscope and MagnaFire imaging software (Olympus America, Inc, Center Valley, Pa).

Constructs

pHRL-TK, pGL3-Basic, and pCDNA3.1 were obtained from Promega (Madison, Wis). Construction of pEotaxin-3 has been described.[20] The pEotaxin-3 3′ untranslated region (UTR) consists of coordinates 394 to 562 of NM_006072.4 (GenBank) in the *Xba*I/*Sal*I sites of the pGL3-Promoter. pFKBP51 was constructed by inserting the *FKBP51* open reading frame (coordinates 154 to 1535 of NM_004117.2, GenBank) into the pCDNA3.1 polylinker.

Statistical methods

Data are expressed as means ± SEMs. Statistical significance was determined by using the Student *t* test (normal distribution, equal variance), 1-way ANOVA followed by the Tukey post test (>2 groups), or the Kruskal-Wallis test followed by a Dunn multiple comparison test (nonparametric test, >2 groups) with Prism 5.0 Software.

RESULTS

Identification of glucocorticoid-regulated genes in the esophagus

We performed genome-wide expression analysis of esophageal biopsy samples derived from 4 distinct patient populations, including control patients (n = 14), untreated patients with EE (n = 14), patients with EE who responded to FP treatment (n = 13), and patients with EE who did not respond fully to FP treatment (n = 8; see Table E1). We aimed to identify the subset of genes regulated exclusively by FP treatment and not by EE disease. More specifically, we sought to identify genes differentially regulated between untreated control subjects and FP responders, excluding genes that were dysregulated in untreated patients with EE. Accordingly, genes exhibiting altered expression between control subjects and untreated patients with EE were subtracted from the subset of genes that were differentially expressed between control subjects and FP responders (Fig 1, *A*). Thirty-two transcripts exhibited differential expression in FP responders compared with control subjects but were not included in the genes differentially expressed in untreated patients with EE at baseline (Fig 1, *B*, and see Table E5).

Eight transcripts represented 6 genes that exhibited increased expression in responders but not in nonresponders (Fig 1, *C*). Of the 17 transcripts (representing 15 genes) with decreased levels in responders, all showed levels in nonresponders that were similar to those seen in control subjects (Fig 1, *D*). Notably, 4 MHC class II genes were identified, as well as 3 genes of the collagen family. Six transcripts identified in the original analysis (Fig 1, *B*) were not included in either Fig 1, *C* or *D*, because they exhibited a change in expression in control subjects versus that seen in untreated patients with EE despite the analysis scheme devised to eliminate such transcripts. Of the subset of transcripts with increased levels in responders, 1 gene (*F3*, encoding for coagulation factor III or tissue factor) was identified that exhibited increased expression in both responders and nonresponders (Fig 1, *C*). Taken together, these results indicate that nonresponders have markedly blunted steroid-induced signaling responses in the esophagus because only 1 of 32 of the genes exhibited differential regulation in both responders and nonresponders.

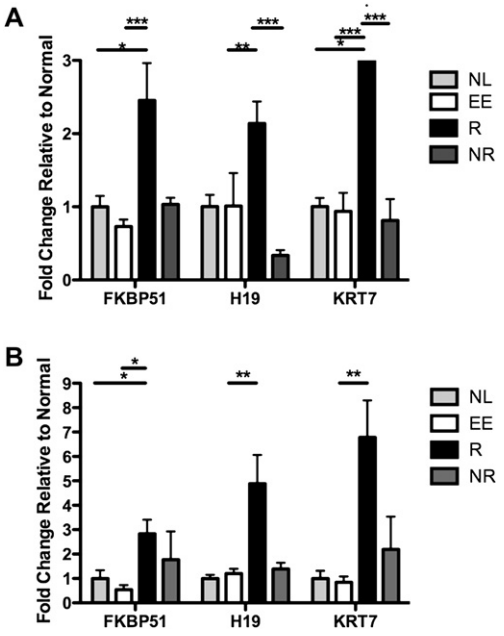

FIG 2. Verification of transcript levels of glucocorticoid-regulated genes by means of real-time PCR analysis. **A**, Average gene expression determined by means of microarray analysis is expressed as the fold change compared with that seen in control subjects. **B**, Transcript levels for the indicated gene were quantified by means of real-time PCR and normalized to *GAPDH* levels; samples used were collected from the same patients used for microarray analysis. The graph displays the fold change compared with that seen in control subjects. *EE*, Untreated patients with EE; *NL*, control patients; *NR*, FP nonresponders; *R*, FP responders. **P* < .05. ***P* < .01. ****P* < .001.

We next validated the expression pattern of 3 of the identified candidate genes using real-time PCR analysis. Genes were selected based on their known biology. FK506-binding protein 5 *(FKBP51)*, which has a known role in glucocorticoid receptor biology; keratin 7 *(KRT7)*, which promotes epithelial cell integrity and protects against physical damage; and *H19*, which is processed to a microRNA, showed increased transcript levels in FP responders compared with untreated patients by means of both microarray (Fig 2, *A*) and real-time PCR (Fig 2, *B*) analysis. On average, *FKBP51* mRNA levels were 2-fold higher in FP responders compared with those seen in control subjects, whereas nonresponders exhibited levels of this transcript that were not significantly higher than those seen in control subjects (Fig 2, *A*).

FKBP51 expression patterns in esophageal tissue

We delineated the cell type or types responsible for *FKBP51* expression in patients' biopsy samples. Esophageal biopsy specimens were immunostained with antibody specific for *FKBP51*, and nuclei were stained with 4′-6-diamidino-2-phenylindole, dihydrochloride. In biopsy specimens obtained from untreated patients with EE, the basal layer of the esophageal tissue exhibited the strongest signal (Fig 3, *A*). The signal appeared most intense in the nuclei, although some cytoplasmic staining was apparent. Weaker cytoplasmic staining was also observed in the cells near the luminal side of the biopsy specimen (Fig 3, *A*). When compared with the hematoxylin and eosin–stained biopsy specimen (data not shown), the expression pattern of

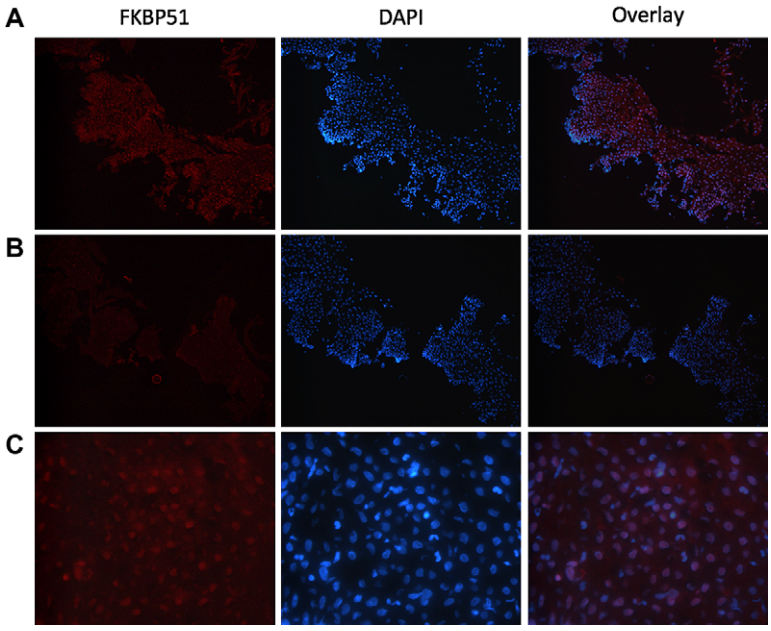

FIG 3. Localization of FKBP51 expression in patients' biopsy specimens. Esophageal biopsy sections were immunostained to visualize FKBP51 (*red*), and 4′-6-diamidino-2-phenylindole, dihydrochloride *(DAPI)* was used to visualize nuclei (*blue*). Magnification is ×200 for Fig 3, *A* and *B*, and ×800 for Fig 3, *C*. **A**, Esophageal biopsy specimen from an untreated patient with EE. **B**, Negative control (control antibody) for Fig 3, *A*. **C**, High-power magnification of biopsy specimen in Fig 3, *A*.

FKBP51 in patients' biopsy specimens was consistent with its being present in epithelial cells. Staining with control antibody confirmed that the observed signal was specific for the *FKBP51* antibody (Fig 3, *B*).

FKBP51 induction in esophageal epithelial cells

To determine whether FP directly induced *FKBP51* in esophageal epithelial cells, we treated primary esophageal epithelial cells with FP and observed an increase (2.15 ± 0.4–fold) in FKBP51 protein expression after FP treatment (Fig 4, *A*). In addition, FP also induced *FKBP51* mRNA and protein in the esophageal epithelial cell line TE-7 (Fig 4, *B-D*). Transcript levels of *FKBP51* were increased by 4 hours after treatment and were further increased after 8 hours, after which a plateau was observed (Fig 4, *C*). Similarly, FKBP51 protein levels in TE-7 cells increased in a time-dependent manner, with maximum levels of protein observed by 24 hours after treatment (Fig 4, *D*). The induction of *FKBP51* by FP was not specific for this steroid because *FKBP51* mRNA (Fig 5, *A*) and protein (Fig 5, *B* and *C*) levels increased after treatment with dexamethasone. FKBP51 protein levels were similarly increased in primary esophageal epithelial cells after dexamethasone treatment (Fig 5, *D*).

Glucocorticoid-induced FKBP51 is steroid receptor dependent

Cells were pretreated with the glucocorticoid receptor antagonist RU486 followed by FP or dexamethasone treatment. Western blot analysis showed that RU486 treatment abolished the FP- and dexamethasone-mediated increase in FKBP51 protein levels. Dexamethasone required an equimolar amount of RU486 for the inhibition, whereas FP-mediated induction of FKBP51 protein levels was inhibited by a 100-fold molar excess of RU486 (Fig 4, *E*). As such, FKBP51 induction is mediated through a glucocorticoid receptor ligand binding–dependent process.

TE-7 cells were treated with FP for 24 hours, followed by addition of actinomycin D to inhibit *de novo* transcription, to determine whether glucocorticoid treatment increased the stability of *FKBP51* mRNA. At the indicated time points after initiation of actinomycin D treatment, *FKBP51* mRNA levels were monitored. The half-life of *FKBP51* mRNA in the absence and presence of FP treatment was similar (17.84 ± 2.03 hours and 16.44 ± 1.11 hours, respectively; $P = .35$; Fig 4, *F*). We next tested whether *de novo* protein synthesis was required for the increased transcript levels of *FKBP51*. TE-7 cells were pretreated with cycloheximide (CHX) at a dose (10 μg/mL) that inhibited protein synthesis in these cells more than 95%, as measured based on [^{35}S]-methionine incorporation into protein (data not shown) followed by addition of vehicle or FP (10^{-6} mol/L) for 24 hours. Although an increase of *FKBP51* transcripts was observed at baseline after CHX treatment, *FKBP51* mRNA levels were even further increased in FP-treated cells in which protein synthesis was inhibited (Fig 4, *G*), suggesting that *de novo* protein synthesis was not absolutely required for subsequent FP-mediated induction of *FKBP51* gene expression.

Effect of *FKBP51* on glucocorticoid and IL-13 signaling in esophageal epithelial cells

IL-13 signaling in esophageal epithelial cells has been shown to have a critical role in EE pathogenesis by inducing the EE transcriptome, at least in part.[10] When TE-7 cells were treated with IL-13, an increase in eotaxin-3 transcript levels was observed (Fig 6, *A*). When the cells were treated concomitantly

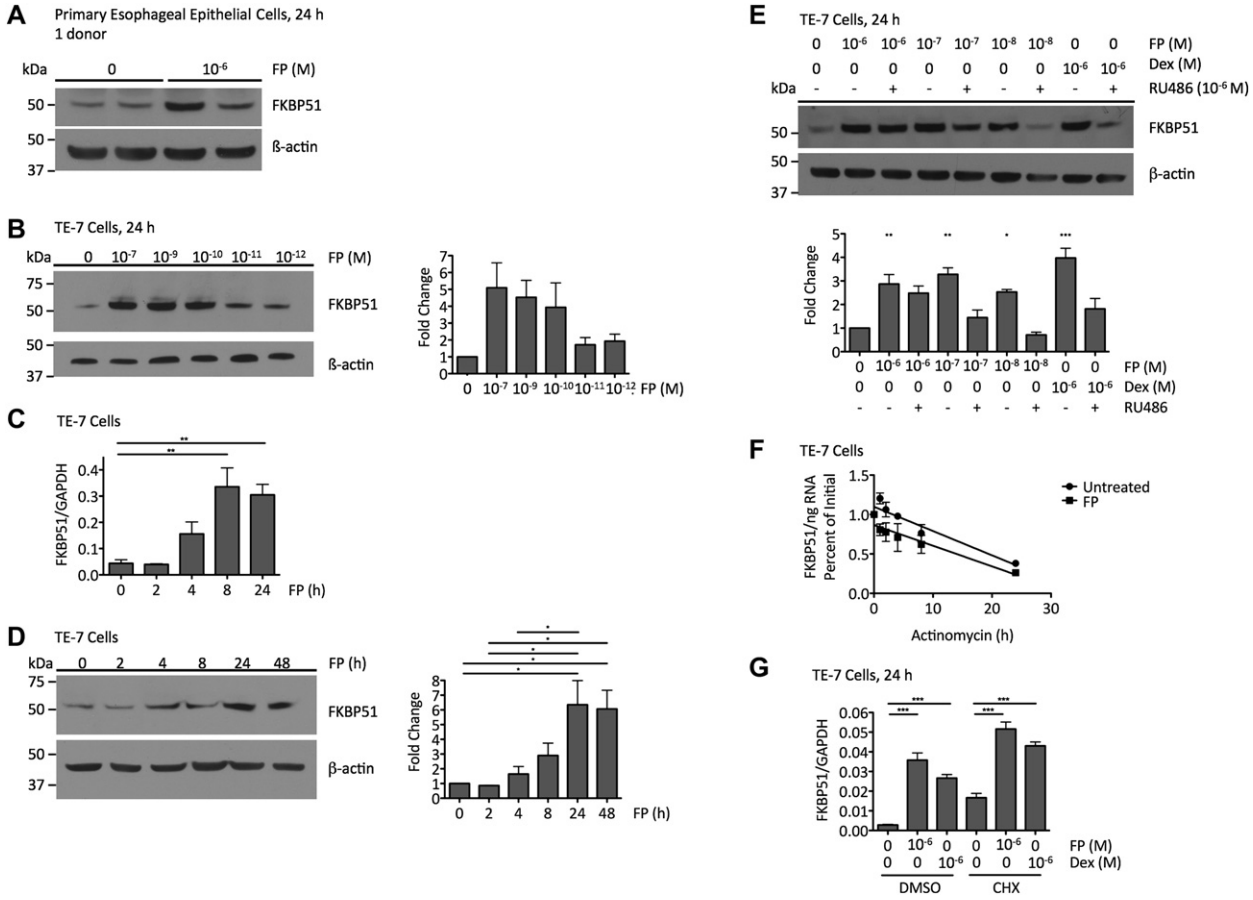

FIG 4. *FKBP51* induction after glucocorticoid treatment of esophageal epithelial cells. For Fig 4, *A*, *B*, *D*, and *E*, protein extracts were subjected to SDS-PAGE and Western blot analysis for FKBP51 and actin; protein levels were quantified by means of densitometric analysis. The average fold change in the ratio of FKBP51 to actin for each sample compared with the untreated sample for 3 experiments is shown. For Fig 4, *A* through *E*, results are representative of 3 experiments. **A**, Primary esophageal epithelial cells were treated with 10^{-6} mol/L FP for 24 hours. **B**, TE-7 cells were treated with FP for 24 hours. **C**, TE-7 cells were treated with 10^{-7} mol/L FP. RNA was isolated, cDNA synthesis was performed, and real-time PCR analysis to detect *FKBP51* and *GAPDH* transcripts was done. **D**, In a separate experiment TE-7 cells were treated for the indicated time with 10^{-7} mol/L FP. $*P < .05$. $**P < .01$. **E**, TE-7 cells were pretreated with 10^{-6} mol/L RU486 for 30 minutes. FP or dexamethasone *(Dex)* was then added for 24 hours. **F**, TE-7 cells were treated with 10^{-6} mol/L FP for 24 hours. Actinomycin D was then added (10 μg/mL). Cells were incubated for the indicated number of hours, at which time they were subjected to RNA isolation followed by cDNA synthesis. *FKBP51* mRNA levels determined by means of real-time PCR were normalized to nanograms of reverse-transcribed RNA. The graph shows the ratio of FKBP51 per nanogram RNA as a percentage of the initial time point; each time point represents the mean ± SEM of 3 independent experiments. Half-life was calculated as the mean ± SEM of the half-life value for each of the 3 independent experiments. **G**, TE-7 cells were pretreated with 10 μg/mL CHX for 30 minutes. Subsequently, FP or dexamethasone was added for 24 hours. The graph shows *FKBP51* mRNA expression normalized to *GAPDH* expression. The results are representative of 3 experiments. *DMSO*, Dimethyl sulfoxide.

with FP, the IL-13–mediated increase in eotaxin-3 mRNA and protein decreased significantly at all doses tested (Fig 6, *A* and *B*).

To understand the contribution of the promoter and 3′ UTR sequences of eotaxin-3 to the control of its gene expression in esophageal epithelial cells after IL-13 and FP treatment, we performed luciferase assays with TE-7 cells transiently transfected with constructs containing the eotaxin-3 5′ UTR upstream or the 3′ UTR downstream of a luciferase expression cassette. The cells were transfected with a luciferase reporter construct containing either 800 bp of the eotaxin-3 promoter 5′ of the firefly luciferase gene in the pGL3-Basic vector or a plasmid containing 164 bp of the eotaxin-3 3′ UTR downstream of the firefly luciferase gene in the pGL3-Promoter vector (Fig 7, *A*).[10,11] Experiments with the promoter construct demonstrated a decrease in IL-13–induced eotaxin-3 promoter activity on concomitant FP treatment (Fig 7, *B*). Experiments with the 3′ UTR construct did not reveal significant modulation of mRNA stability on IL-13 or FP treatment (Fig 7, *C*). Taken together, these findings indicate that at least part of the observed decrease in IL-13–induced eotaxin-3 mRNA levels after FP treatment is mediated through modulation of the promoter activity through cis-acting sequences located within the immediate 5′ UTR of eotaxin-3.

To test whether increased *FKBP51* levels affected the FP-mediated decrease in IL-13–induced eotaxin-3 promoter activity, TE-7 cells were transfected with either empty vector (pCDNA3.1) or an expression construct that contained *FKBP51*

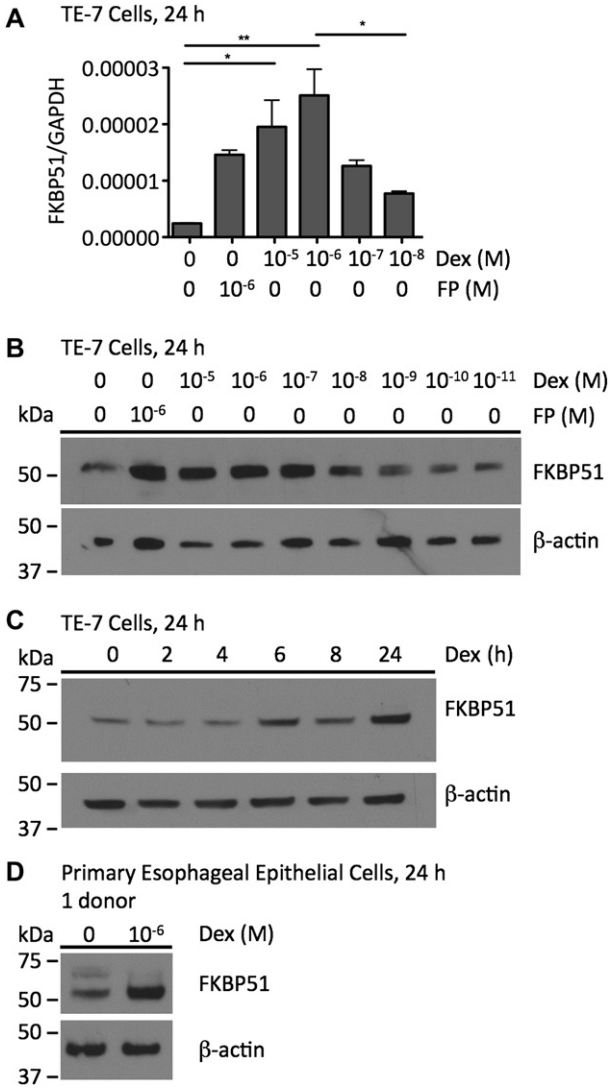

FIG 5. *FKBP51* transcript and protein levels are increased by dexamethasone *(Dex)* treatment of esophageal epithelial cells. **A**, RNA isolated from TE-7 cells treated with dexamethasone or FP for 24 hours was subjected to cDNA synthesis. *FKBP51* transcript levels determined by means of real-time PCR analysis were normalized to *GAPDH* levels. **B-D**, Western blot analysis for FKBP51 and β-actin. Fig 5, *B*, TE-7 cells were treated with dexamethasone for 24 hours. Fig 5, *C*, TE-7 cells were treated with 10^{-6} mol/L dexamethasone. Fig 5, *D*, Primary esophageal epithelial cells were treated with 10^{-6} mol/L dexamethasone for 24 hours. In Fig 5, *A-D*, data are representative of 3 experiments per figure.

under the control of the CMV promoter (pFKBP51; Fig 7, *A*) to simulate high baseline levels of *FKBP51*. Cells transfected with the empty vector showed a 2-fold increase in eotaxin-3 promoter activity on IL-13 treatment, and this was reduced by 50% when the cells were concomitantly treated with FP (Fig 7, *D*). However, when cells were transfected with the *FKBP51* expression vector, the FP-mediated decrease in IL-13–induced eotaxin-3 promoter activity was reduced (Fig 7, *D*).

DISCUSSION

In this study we identified a set of genes that exhibited differential expression in the esophagus of treated, FP-responsive patients with EE compared with untreated subjects. Our analysis uncovered several genes previously shown to be glucocorticoid responsive, including those encoding FKBP51, MHC class II, and collagen molecules. Glucocorticoids upregulate *FKBP51*, whereas they decrease expression of MHC class II and collagen genes in several cell types.[16-19,21,22] The identification of such genes validates the analysis performed. Of the transcripts identified, all but 1 exhibited expression in nonresponders similar to that observed in untreated patients. This indicates that nonresponders might have a fundamental dysfunction in glucocorticoid signaling, at least in the esophagus. Tissue-specific glucocorticoid resistance has been reported in other inflammatory diseases, including rheumatoid arthritis, osteoarthritis, Crohn disease, ulcerative colitis, and asthma.[23] Multiple mechanisms have been shown to account for glucocorticoid resistance, including aberrant interactions between transcription factors, coactivators, and corepressors; posttranslational modification of inflammatory mediators; and expression of glucocorticoid receptor isoforms.[23] Interestingly, only 1 gene (*F3*) showed differential expression in both FP responder and FP nonresponders compared with untreated subjects. This implies that *F3* gene expression could be influenced by a nongenomic mechanism of glucocorticoid signaling. This gene might be especially useful to monitor patient compliance because it is expressed in all patients who take the drug, regardless of responsiveness.

The observed change in patients' biopsy specimen gene expression in addition to previous literature reporting a low systemic bioavailability of oral FP[24] supports the interpretation that swallowed FP treatment exerts a topical effect in the esophagus. The oral bioavailability of FP has been reported to be less than 1% at a dose greater than 20-fold higher than the dose for patients with EE in this current study[24]; this, coupled with the fact that FP undergoes extensive first-pass metabolism in the liver,[25] makes it less likely that swallowed FP acts through a systemic route to alter gene expression in the esophagus. To our knowledge, this represents the first report suggesting direct evidence of a topical effect of glucocorticoids in the esophagus.

Here we report that *FKBP51* was upregulated at the mRNA and protein levels in esophageal epithelial cells, including primary esophageal epithelial cells that were cultured from esophageal biopsy specimens. Based on the ability of RU486 to inhibit FKBP51 protein induction, the increased FKBP51 levels are likely glucocorticoid receptor dependent. We observed that increased transcript stability did not account for the upregulation of *FKBP51* mRNA. Instead, increased *FKBP51* mRNA levels are likely caused by increased transcription of the gene. Numerous putative glucocorticoid response elements (GREs) exist within the *FKBP51* promoter and introns, and such sequences might mediate the increased transcription in cell types, including A549 cells.[26] We observed that *FKBP51* levels were increased at baseline on CHX treatment, suggesting that inhibition of protein synthesis upregulates *FKBP51*. This could occur if a repressor of *FKBP51* transcription required ongoing protein synthesis; alternatively, *FKBP51* levels might be upregulated as part of a stress response. Despite this increased baseline expression, *FKBP51* transcription still increased on glucocorticoid treatment in the presence of CHX, suggesting that at least a portion of the increased *FKBP51* transcripts do not require *de novo* protein synthesis. These data collectively suggest that glucocorticoid signaling directly affects *FKBP51* transcription in esophageal epithelial cells.

We observed that FP treatment inhibited IL-13–mediated eotaxin-3 promoter activity. This appears to be mediated through

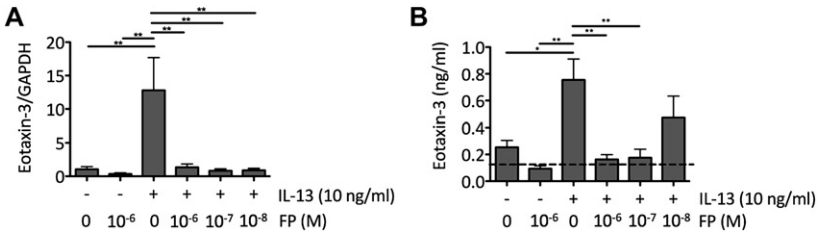

FIG 6. IL-13–induced transcript and protein levels of eotaxin-3 are reversed by FP treatment of esophageal epithelial cells. **A**, RNA isolated from TE-7 cells treated with IL-13, FP, or both for 24 hours was subjected to cDNA synthesis. Transcript levels of eotaxin-3 were determined by means of real-time PCR analysis and normalized to *GAPDH* levels. **B**, TE-7 cells were treated with IL-13, FP, or both for 48 hours. ELISA was performed to detect eotaxin-3 in the supernatants. The *dashed line* indicates the detection limit of the assay. For Fig 6, *A* and *B*, data are representative of 3 experiments per figure. $*P < .05$. $**P < .01$.

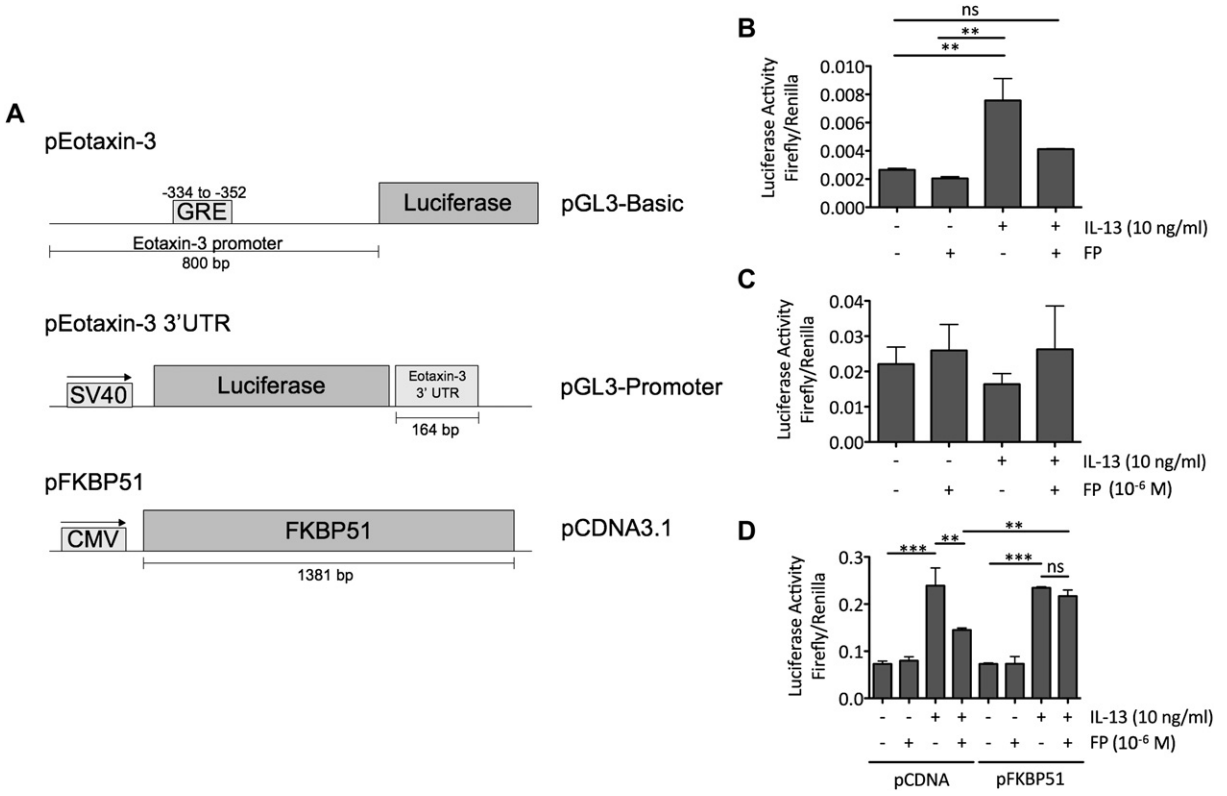

FIG 7. Increased baseline *FKBP51* levels affect glucocorticoid-mediated repression of IL-13–induced eotaxin-3 promoter activity. **A**, Constructs used in this study. *CMV*, Cytomegalovirus promoter. Cells were transfected with pHRL-TK and either pEotaxin-3 **(B)** or pEotaxin-3-3′UTR **(C)** or pHRL-TK, pEotaxin-3, and either pCDNA3.1 or pFKBP51 **(D)**. Fig 7, *B-D*, After transfection, cells were treated with IL-13, FP, or both for 24 hours. Firefly and Renilla luciferase activities for each sample were then quantified. Eotaxin-3 promoter activity is expressed as the ratio of firefly to Renilla luciferase activity. For Fig 7, *B-D*, panels are representative of 3 experiments. *ns*, Nonsignificant. $**P < .01$. $***P < .001$.

the 800 bp of promoter that contains 1 canonical GRE sequence. Previous reports have suggested that repression of IL-4–induced eotaxin-3 expression by FP occurs independently of this GRE sequence in lung epithelial cells.[27] It remains to be tested whether this is the case in esophageal epithelial cells.

FKBP51 has been shown to act as a negative regulator of glucocorticoid signaling.[28,29] In fact, baseline *FKBP51* levels in airway epithelial cells negatively correlate with response to glucocorticoid treatment in asthmatic patients.[30] Additionally, new world primates exhibit high levels of *FKBP51*, and this correlates with general glucocorticoid resistance in these animals.[17,31,32] Herein, we show that increasing baseline *FKBP51* levels in the esophageal cell line TE-7 correlate with a decreased ability of glucocorticoid to repress IL-13–mediated eotaxin-3 promoter activity (Fig 8).

Biopsy specimens of patients who respond to swallowed FP treatment exhibit high levels of *FKBP51*, a negative regulator of glucocorticoid signaling.[28,29,31,32] However, we note that the observed high *FKBP51* transcript levels occurred after treatment. In contrast, we showed that high baseline *FKBP51* levels before

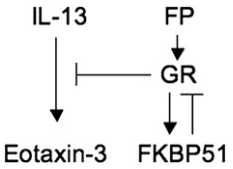

FIG 8. Model to describe the regulation and potential function of *FKBP51* in patients with EE. FP represses IL-13–induced eotaxin-3 expression while inducing *FKBP51* gene expression through a mechanism likely dependent on the glucocorticoid receptor *(GR). FKBP51* additionally inhibits glucocorticoid receptor (GR)–mediated signaling and thus dampens glucocorticoid-mediated repression of IL-13–induced eotaxin-3 promoter activity.

treatment negatively affect glucocorticoid signaling *in vitro*. Therefore we propose that although increased *FKBP51* levels after FP treatment serve as a readout of functional glucocorticoid signaling, the actual biological function of *FKBP51* in part involves negative regulation of glucocorticoid signaling to dampen the anti-inflammatory response so that homeostatic conditions can be restored after the glucocorticoid-mediated resolution of inflammation.

In summary, we have shown that (1) FP responders express a unique set of esophageal genes (including *FKBP51*), providing evidence that swallowed glucocorticoids mediate their role through a topical effect in the esophagus *in vivo*, and (2) FP nonresponders have a blunted expression of steroid-induced esophageal transcripts, suggesting impaired glucocorticoid signaling in these patients. Further proof for the ability of glucocorticoids to induce esophageal transcripts is derived by *in vitro* studies with esophageal epithelial cells, which have demonstrated that (1) glucocorticoids directly induce *FKBP51* through a RU486-inhibited mechanism, indicating the direct involvement of the glucocorticoid receptor; (2) *FKBP51* mRNA half-life is approximately 16 to 18 hours and importantly not regulated by glucocorticoids; and (3) *FKPB51* overexpression inhibits glucocorticoid-mediated transcriptional repression of eotaxin-3. Taken together, our data provide molecular evidence that swallowed glucocorticoids mediate their action through a topical effect in the esophagus and that posttreatment *FKBP51* esophageal levels serve as a measure of functional glucocorticoid signaling.

We thank Drs Michael Farrell, Michael Bates, Kathleen Campbell, and Mitchell Cohen for their referral of patients. We are grateful to Bridget Buckmeier, Emily Stucke, and Tommie Grotjan for research assistance, as well as to the Food Allergy Project, the Campaign Urging Research for Eosinophilic Disorders (CURED), and the Buckeye Foundation for their generous support.

Clinical implications: Swallowed glucocorticoids induce disease remission by regulating local gene expression in the esophagus. Understanding the mechanism by which topical glucocorticoids induce remission from EE will provide insight into disease pathogenesis, steroid resistance, and additional molecular pathways to serve as targets for therapeutic intervention.

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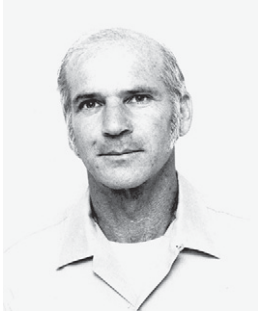

ALLERGY ARCHIVES

B LYMPHOCYTES, LYMPHOID ORGANS, AND IMMUNE RESPONSES

A major lead to elucidating the association of lymphoid tissue cells and the immune response was provided by poultry scientist Glick (1927-2009). In 1956, with associates at Ohio State University, Glick[1] demonstrated that surgical removal of the bursa of Fabricius, an avian lymphoid organ in the lower intestine, in 2-week old chicks led to their subsequent inability to produce specific antibodies to immunizing *Salmonella typhimurium.* Additionally discovered was that bursectomy in a later period of life after hatching, when the bursa began to atrophy, did not affect antibody production in the chicks. The finding suggested that, after differentiation and maturation in the bursa, antibody-producing lymphocytes relocate systemically.

Glick's seminal observation led to studies of thymus and bone marrow control of antibody-forming lymphocytes. Investigating outcomes of thymectomy in mice at the Chester Beatty Institute in London in 1961, Miller[2] demonstrated a lateral functional role of thymus-derived lymphocytes in immune genesis and development. Neonatally thymectomized mice were totally immunoincompetent, and if reaching maturity, they exhibited serious immunologic defects. Adult thymectomized mice were unaffected.

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Images
Bruce Glick (gift of Dr Glick).

Jacques F. A. P. Miller (gift of Dr Miller).

METHODS

Primary epithelial cell culture

Briefly, after informed consent was obtained, 1 distal esophageal biopsy specimen was collected during routine endoscopy. After digestion with trypsin/EDTA, biopsy samples were cultured in modified F-medium (3:1 F-12/Dulbecco modified Eagle medium) supplemented with FBS (5%), adenine (24.2 μg/mL), cholera toxin (10^{-4} μmol/L), insulin (5 μg/mL), hydrocortisone (0.4 μg/mL), and epidermal growth factor (10 ng/mL) in the presence of penicillin, streptomycin, and amphotericin (Invitrogen). Cultures were supplemented with 1 to 5×10^5 feeders (NIH 3T3 J2 cells irradiated with 6,000 rad). Media were changed twice weekly. On reaching confluence, cells were trypsinized and replated for experiments.

Cell treatment

IL-13 (Peprotech, Rocky Hill, NJ) was added to culture media at 10 or 100 ng/mL for 24 or 48 hours. FP, dexamethasone, and RU486 (Sigma-Aldrich) were dissolved in ethanol and added to culture media (10^{-5} mol/L to 10^{-12} mol/L, 10^{-5} mol/L to 10^{-11} mol/L, and 10^{-6} mol/L, respectively), as indicated. Primary esophageal epithelial cells treated with FP or dexamethasone were cultured for 24 hours in modified F-media lacking hydrocortisone. Actinomycin D (Sigma-Aldrich) dissolved in ethanol (1 μg/μL) was added at 10 μg/mL. CHX (Sigma-Aldrich) dissolved in dimethyl sulfoxide (10 μg/μL) was added at 10 μg/mL. An equivalent amount of ethanol (10 μL/mL for actinomycin D experiments and 0.1-1 μL/mL for FP/dexamethasone/RU486 experiments) or dimethyl sulfoxide (1 μl/mL) was added to control cells to serve as vehicle control. The appropriate dose of CHX was determined by treating TE-7 cells with various doses of CHX (0, 0.1, 1, and 10 μg/mL) in methionine-free RPMI supplemented with 15 μCi/mL [^{35}S]-methionine for 24 hours, followed by trichloroacetic acid (TCA) precipitation and solubilization of protein in 0.5 mol/L NaOH; equal aliquots were then counted by means of liquid scintillation.

Transient transfections

TE-7 cells were transfected with Lipofectamine reagent (Invitrogen), as previously described.[E1] Briefly, approximately 10^4 cells per well were plated in 24-well plates. Five hundred nanograms of reporter plasmid was mixed with 3 mL of Lipofectamine reagent (Invitrogen) in 100 μL of serum-free RPMI medium. In transfection experiments involving expression constructs, 250 ng of expression plasmid or control plasmid was included. In all transfection experiments 50 ng of a plasmid containing Renilla luciferase under the control of a constitutive promoter (pHRL-TK) was included as a control for transfection efficiency. After 48 hours, cells were treated with IL-13, FP, or both, as indicated, for 24 hours.

Luciferase assay

TE-7 cells transfected with reporter constructs as described were lysed in 1× passive lysis buffer (Promega). Firefly and Renilla luciferase activities were quantified by using the Synergy 2 plate reader (BioTek, Winooski, Vt) with the Dual Luciferase Reporter assay system (Promega).

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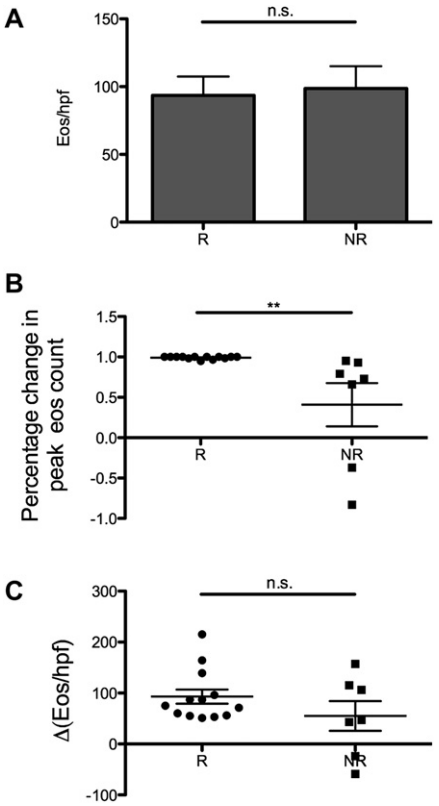

FIG E1. Characterization of peak eosinophil counts of biopsy specimens from FP responders and nonresponders at times after FP treatment and with no FP treatment. **A**, Mean peak eosinophil *(Eos)* counts per 400× hpf for FP responder *(R)* and nonresponder *(NR)* patients' esophageal biopsy specimens obtained at a time when the patients were not receiving swallowed FP treatment. This graph represents the mean ± SEM of the numbers obtained from Table E2. *n.s.*, Nonsignificant. **B**, Percentage change in peak eosinophil count was calculated by dividing the peak eosinophil count derived from the post-FP biopsy specimen by the peak eosinophil count derived from the biopsy specimen obtained at a time when the patient had active EE and was not being treated with swallowed FP. A negative percentage indicates that the peak eosinophil count was increased after FP treatment compared with that seen in the untreated, active EE biopsy specimen. These numbers were derived from Table E2. $^{**}P < .01$. **C**, Changes in eosinophil counts were calculated by subtracting the peak eosinophil count in the post-FP treatment biopsy from the peak eosinophil *(Eos)* count of the untreated, active EE biopsy specimen. These numbers were derived from Table E2. *n.s.*, Nonsignificant.

TABLE E1. Patients' characteristics

Patient no.	Sex	Age (y)	Ethnicity*	Esophageal disease	Treatment†	Maximum eosinophils/hpf	Asthma‡	Allergic conjunctivitis‡	Allergic rhinitis‡	Hay fever‡	Urticaria/angioedema‡	Eczema‡	Anaphylaxis, nonfood‡	Anaphylaxis, food‡	Drug allergy‡	Food allergy‡	Environmental allergy‡
Microarray																	
1	F	9.0	O	NL	PPI	0											
2	M	7.7	PI	NL	Miralax, H1RA	0					Yes	Yes					
3	M	4.2	C	NL	INHGC, LTRA, B2RA	0						Yes					
4	M	1.5	C	NL	None	0											
5	M	3.1	C	NL	None	0											
6	F	10.0	C/NH	NL	PPI	0											
7	F	3.7	C/NH	NL	None	0											
8	M	8.0	C/NH	NL	None	0	No	Yes	Yes	No	No	No	No	No	No	No	Yes
9	F	1.5	C/NH	NL	PPI, mesalamine	0											
10	M	10.5	C	NL	INHGC	0											
11	F	6.7	C	NL	H2RA	0						Yes					
12	F	12.9	C/NH	NL	None	0											
13	M	10.7	C	NL	PPI, LTRA	0											
14	F	6.7	C	NL	H2RA	0						Yes					
15	F	3.5	C/NH	EE	antibiotic	25		Yes	Yes	Yes	No	Yes	No	No	No		
16	M	14.7	C/NH	EE	None	51	Yes	No	No	No	No	No	No	No	No		
17	M	11.5	C/NH	EE	INHGC, B2ARA	71	Yes					Yes					
18	M	5.2	C/NH	EE	None	58						Yes					
19	M	13.4	C/NH	EE	INHGC, LTRA, H1RA	42	Yes	No	No	No	No	Yes	No	No	No		
20	F	4.8	C/NH	EE	PPI	218		No	Yes	No	No	No	No	No	No		
21	M	11.7	C/NH	EE	INHGC, PPI	248	Yes	Yes	Yes	Yes	No	No	No	No	No		
22	F	16.7	C/NH	EE	INHGC, PPI	50	Yes										
23	F	14.1	C/NH	EE	INHGC, PPI, H1RA, B2ARA	72	Yes	No	Yes	No	No	Yes	No	No	No		
24	M	3.1	C/NH	EE	None	56	No	No	No	No	No	Yes	No	No	No		
25	M	15.9	C	EE	B2ARA	31	Yes	Yes	Yes								Yes
26	M	13.2	C	EE	None	32											
27	M	1.8	C/NH	EE	None	83	No	No	Yes	No	Yes	No	No	Yes	No	Yes	
28	M	13.8	C/NH	R	SWGC	1	Yes	Yes	Yes	No	Yes	No	No	Yes	No	Yes	Yes
29	M	8.2	C	R	SWGC	0	Yes	Yes	Yes	No	No	Yes	No	No	No	No	No
30	M	6.6	C/NH	R	SWGC	0											
31	M	10.0	C/H	R	SWGC, PPI	0	Yes					No			Yes		
32	M	11.7	C/NH	R	SWGC	0	Yes					Yes					
33	M	5.7	C/NH	R	SWGC	1						Yes				Yes	
34	M	3.4	C	R	SWGC	0	Yes	No	Yes	No	No	Yes	No	No	No	Yes	Yes
35	M	6.1	C/NH	R	SWGC	2		No	No	No	No	No	No	No	No		
36	M	3.5	C/NH	R	SWGC	0	No	No	No	No	No	Yes	No	No	No		
37	M	22.4	C/NH	R	SWGC, PPI	0	Yes	No	No	No	No	No	No	No	No	Yes	
38	M	4.3	I/NH	R	SWGC, PPI	0	No	Yes	Yes	No	No	No	No	No	No		
39	M	8.0	C	R	SWGC	0	Yes	Yes	Yes	No	No	Yes	No	No	No	No	No
40	M	7.7	C/NH	R	SWGC, H1RA, mesalamine, antibiotic	4	Yes	No	Yes	No	No	Yes	No	No	No	Yes	
41	M	8.8	U	NR	SWGC	89											

(Continued)

TABLE E1. *(Continued)*

Patient no.	Sex	Age (y)	Ethnicity*	Esophageal disease	Treatment†	Maximum eosinophils/ hpf	Asthma‡	Allergic conjunctivitis‡	Allergic rhinitis‡	Hay fever‡	Urticaria/ angioedema‡	Eczema‡	Anaphylaxis, nonfood‡	Anaphylaxis, food‡	Drug allergy‡	Food allergy‡	Environmental allergy‡
42	M	4.8	C	NR	SWGC, INHGC, LTRA, H2RA, B2ARA	9	Yes		Yes							Yes	
43	M	6.3	C/NH	NR	SWGC	24	No					Yes					
44	M	12.8	C/NH	NR	SWGC, PPI	8	Yes	No	Yes	No	No	No	No	No	No		
45	M	8.7	C/NH	NR	SWGC	53	No	No	No	No	No	No	No	No	No		
46	F	11.2	C	NR	SWGC	130	No	No	No	No	No	No	No	Yes	No	Yes	No
47	M	14.6	C	NR	SWGC, PPI	16	No										
48	M	14.3	C/NH	NR	SWGC, OGC	30	No	No	Yes	No	No	No	No	No	No		
Epithelial cells																	
49	M	6.9	C/NH	NR	SWGC, B2ARA, methylphenidate	50	Yes					Yes				Yes	
50	M	19.0	C/NH	R	SWGC, PPI	0	Yes	Yes	Yes			Yes				Yes	
Immunofluorescence																	
51	F	11.3	C/NH	EE	None	150	No	No	Yes	No	No	Yes	No	No	No	Yes	Yes

This table lists the characteristics of patients at the time that the biopsy specimen was collected.

B2ARA, β_2-Adrenergic receptor antagonist; *F*, female; *H1RA*, H1 receptor antagonist; *H2RA*, H2 receptor antagonist; *INHG*C, inhaled glucocorticoid; *LTRA*, leukotriene receptor antagonist; *M*, male; *NL*, control subject; *NR*, FP nonresponder; *OGC*, oral glucocorticoid (prednisone); *PPI*, proton pump inhibitor; *R*, FP responder; *SWGC*, swallowed glucocorticoid (FP).

*Ethnicity: *C*, white; *H*, Hispanic; *I*, Indian; *NH*, non-Hispanic; *O*, other; *PI*, native Hawaiian/Pacific Islander; *U*, unknown.

†Treatment at the time the biopsy specimen was obtained.

‡History of past or present condition as reported in patient history. A blank space indicates that the information is unknown.

TABLE E2. Changes in eosinophil counts in biopsy specimens of patients receiving FP treatment

Patient no.*	Esophageal disease	Non-FP peak eos count†	FP peak eos count‡	δ(Peak eos count)§	Time elapsed between biopsies (d)‖	Percentage change¶
28	R	54	1	53	899	0.98
29	R	87	0	87	233	1.00
30	R	164	0	164	90	1.00
31	R	51	0	51	76	1.00
32	R	71	0	71	94	1.00
33	R	61	1	60	155	0.98
34	R	139	0	139	101	1.00
35	R	57	2	55	644	0.96
36	R	56	0	56	140	1.00
37	R	215	0	215	142	1.00
38	R	96	0	96	−896	1.00
39	R	87	0	87	143	1.00
40	R	79	4	75	136	0.95
41	NR	65	89	−24	132	−0.37
42	NR	166	9	157	−372	0.95
43	NR	71	24	47	105	0.66
44	NR	114	8	106	269	0.93
45	NR	N.A.	53	N.A.	N.A.	N.A.
46	NR	71	130	−59	140	−0.83
47	NR	59	16	43	551	0.73
48	NR	145	30	115	791	0.79

For responders or nonresponders, biopsy specimens were obtained for the same patient at a time he or she had active EE and was not treated with swallowed FP. When possible, biopsy counts were obtained for the biopsy specimen obtained most immediately before the biopsy specimen that was included in the study that met these criteria. When this was not possible, the count was obtained from a biopsy specimen obtained at a time after the study biopsy specimen showing that the patient had active EE and was not being treated with swallowed FP. Distal esophageal peak eosinophil counts per 400× hpf were determined for these biopsy specimens.

Eos, Eosinophil; *NR*, FP nonresponder; *R*, FP responder.

*Patient number as denoted in Table E1.

†Peak eosinophil count per 400× hpf of a biopsy specimen taken from the given patient at a time when the patient had active EE but was not receiving swallowed FP treatment. When possible, the biopsy specimen obtained at the most proximal time point before the biopsy specimen obtained in the study was used. However, this was not possible in all cases. When not possible, the count was obtained from a biopsy specimen that indicated active EE which was taken at a time after not receiving swallowed FP treatment.

‡Peak eosinophil count per 400× hpf of the biopsy specimen used in the study.

§Values from the "Non-FP peak eos count" column minus those from the "FP peak eos count" column.

‖Number of days elapsed between the collection of the biopsy specimen in the "Non-FP peak eos count" column and that from the "FP peak eos count" column. A positive number indicates that the biopsy specimen in the "Non-FP peak eos count" column was collected before the biopsy specimen in the "FP peak eos count" column. A negative number indicates the opposite.

¶Values in the "δ(peak eos count)" column divided by those in the "Non-FP peak eos count" column.

TABLE E3. Swallowed FP dose of responders and nonresponders

Patient no.*	Swallowed FP Dose (μg)†	Times per day†	Total dose per day (μg)‡	Time of last reported dose of swallowed FP before endoscopy§
28	440	2	880	Morning of endoscopy
29	NA	NA	NA	NA
30	440	2	880	NA
31	220	2	440	NA
32	440	2	880	NA
33	440	2	880	NA
34	440	2	880	Morning of endoscopy
35	440	1	440	Night before endoscopy
36	440	2	880	Night before endoscopy
37	440	2	880	Day before endoscopy
38	NA	2	NA	Day before endoscopy
39	NA	2	NA	NA
40	440	2	880	NA
41	440	2	880	NA
42	NA	NA	NA	Night before endoscopy
43	440	2	880	NA
44	440	2	880	NA
45	440	2	880	NA
46	440	2	880	Night before endoscopy
47	440	2	880	Three days before endoscopy
48	NA	3	NA	NA

The dose of swallowed FP is indicated that had been prescribed to the patients during the time period immediately preceding the endoscopy during which the study biopsy specimen was obtained. When recorded, the last dose of swallowed FP relative to the time of endoscopy is indicated.

NA, Not available.

*The patient numbers correspond to those used in Table E1.

†Dose of FP prescribed in the time period immediately before the endoscopy during which the study sample was acquired. "Swallowed FP dose" indicates the amount taken in micrograms, and "Times per day" indicates the number of times per day the indicated dose was taken.

‡The total amount of swallowed FP per day (ie, "Swallowed FP dose" × "Times per day").

§Time the patient reported last taking swallowed FP before the endoscopy during which the study sample was acquired.

TABLE E4. Primers used in this study

Gene	Forward primer	Reverse primer
Eotaxin-3	5′-aactccgaaacaattgtgactcagct-3′	5′-gtaactctgggaggaaacaccctctc-3′
FKBP51	5′-ggatatacgccaacatgttcaa-3′	5′-ccattgctttattggcctct-3′
H19	5′-ttacttcctccacggagtcg-3′	5′-gctgggtagcaccatttctt-3′
KRT7	5′-ctgaggctgaagcctggta-3′	5′-gggtattccggaggtcgt-3′
GAPDH	5′-tggaaatcccatcaccatct-3′	5′-gtcttctgggtggcagtgat-3′

TABLE E5. Genes identified as being glucocorticoid regulated in the esophagus of patients with EE treated with FP

Affymetrix ID	Gene name	Gene description	Fold change			GenBank†
			EE*	R*	NR*	
213537_at	*HLA-DPA1*	MHC, class II, DP α 1	0.936	0.459	0.877	AI128225
1553177_at	*SH2D1B*	SH2 domain containing 1B	2.946	2.375	3.865	BC022407
201852_x_at	*COL3A1*	Collagen, type III, α 1	0.731	0.332	0.926	AI813758
209392_at	*ENPP2*	Ectonucleotide pyrophosphatase/ phosphodiesterase 2 (autotaxin)	0.702	0.359	0.858	L35594
213611_at	*AQP5*	Aquaporin 5	1.220	2.656	1.149	BF726531
210982_s_at	*HLA-DRA*	MHC, class II, DR α	0.910	0.481	1.226	M60333
204363_at	*F3*	Coagulation factor III (thromboplastin, tissue factor)	1.052	2.001	1.730	NM_001993
204014_at	*HLA-DQA1/ HLA-DQA2/ LOC650946*	MHC, class II, DQ α 1 /// MHC, class II, DQ α 2 /// similar to HLA class II histocompatibility antigen, DQ(1) α chain precursor (DC-4 α chain)	0.789	0.380	1.154	BG397856
212671_s_at	*DUSP4*	Dual-specificity phosphatase 4	1.042	2.118	1.057	NM_001394
206643_at	*FKBP5*	FK506 binding protein 5	0.721	2.130	1.108	AI753747
201496_x_at	*MYH11*	Myosin, heavy polypeptide 11, smooth muscle	0.887	0.482	0.606	NM_022844
224840_at	*HAL*	Histidine ammonia-lyase	1.297	0.479	1.001	NM_002108
201497_x_at	*MYH11*	Myosin, heavy polypeptide 11, smooth muscle	0.697	0.402	0.590	S67238
202274_at	*ACTG2*	Actin, γ 2, smooth muscle, enteric	0.781	0.451	0.669	NM_001615
209395_at	*HLA-DRB1*	MHC class II, DR β 1	1.165	0.484	1.240	AJ297586
225782_at	*COL1A2*	Collagen, type I, α 2	0.650	0.375	0.856	NM_000089
215193_x_at	*CHI3L1*	Chitinase 3-like 1 (cartilage glycoprotein-39)	0.372	0.483	0.467	M80927
202404_s_at	*MSRB3*	Methionine sulfoxide reductase B3	0.650	0.492	0.730	AW027333
221805_at	*SYNPO2*	Synaptopodin 2	0.733	0.466	0.783	AI634580
225895_at	*NEFL*	Neurofilament, light polypeptide 68 kd	18.660	2.486	20.129	AL537457
203559_s_at	*ABP1*	Amiloride binding protein 1 (amine oxidase [copper-containing])	2.498	2.841	0.998	NM_001091
224646_x_at	*MYLK*	Myosin, light polypeptide kinase	0.714	0.312	0.743	AA526844
224823_at	*H19*	H19, imprinted maternally expressed untranslated mRNA	0.823	2.155	0.373	BF569051
202555_s_at	*MYLK*	Myosin, light polypeptide kinase	0.665	0.462	0.623	NM_005965
227949_at	*PHACTR3*	Phosphatase and actin regulator 3	1.024	2.794	0.998	AL357503
204015_s_at	*DUSP4*	Dual-specificity phosphatase 4	0.932	2.122	0.959	BC002671
1558846_at	*PNLIPRP3*	Pancreatic lipase-related protein 3	0.291	0.414	0.251	AL833418
204834_at	*COL12A1*	Collagen, type XII, α 1	0.879	0.431	0.895	AA788946
209016_s_at	*H19*	H19, imprinted maternally expressed untranslated mRNA	0.620	2.255	0.325	AL575306
224997_x_at	*FGL2*	Fibrinogen-like 2	1.455	0.483	1.703	NM_006682
225664_at	*KRT7*	Keratin 7	0.729	2.893	0.588	BC002700
234700_s_at	*RNASE7*	Ribonuclease, RNase A family, 7	0.268	0.494	0.154	AJ131212

Genes identified by means of the analysis described in Fig 1, *A*, are listed, as well as their average expression calculated as a fold change in expression compared with that seen in control subjects.

EE, Untreated patients with EE; *NR*, FP nonresponders; *R*, FP responders.

*Average fold change in gene expression compared with that seen in control subjects. Patient designation is as described in the text.

†Annotation according to GenBank.