

# Expression of 11 $\beta$ -hydroxysteroid dehydrogenase 1 and 2 in patients with chronic rhinosinusitis and their possible contribution to local glucocorticoid activation in sinus mucosa

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**Background:** It has been suggested that glucocorticoids might act in target tissues to increase their own intracellular availability in response to inflammatory stimuli. These mechanisms depend on the local metabolism of glucocorticoids catalyzed by 11 $\beta$ -hydroxysteroid dehydrogenase 1 (11 $\beta$ -HSD1) and 11 $\beta$ -hydroxysteroid dehydrogenase 2 (11 $\beta$ -HSD2).

**Objective:** This study is to investigate the effect of chronic rhinosinusitis (CRS) on expression of 11 $\beta$ -HSD1, 11 $\beta$ -HSD2, steroidogenic enzymes (cytochrome P450, family 11, subfamily B, polypeptide 1 [CYP11B1] and cytochrome P450, family 11, subfamily A, polypeptide 1 [CYP11A1]), and endogenous cortisol levels in human sinus mucosa. Expression levels were compared with those of healthy control subjects.

**Methods:** The expression levels of 11 $\beta$ -HSD1, 11 $\beta$ -HSD2, CYP11B1, CYP11A1, and cortisol were measured in healthy control subjects, patients with CRS with nasal polyps, and patients with CRS without nasal polyps by using real-time PCR, Western blotting, immunohistochemistry, and ELISA.

Expression levels of 11 $\beta$ -HSD1, 11 $\beta$ -HSD2, CYP11B1, CYP11A1, and cortisol were determined in cultured epithelial cells treated with CRS-relevant cytokines. The conversion ratio of cortisone to cortisol was evaluated by using the small interfering RNA technique, 11 $\beta$ -HSD1 inhibitor, and measurement of 11 $\beta$ -HSD1 activity.

**Results:** 11 $\beta$ -HSD1, CYP11B1, and cortisol levels increased in patients with CRS with nasal polyps and those with CRS without nasal polyps, but 11 $\beta$ -HSD2 expression decreased. In cultured epithelial cells treated with IL-4, IL-5, IL-13, IL-1 $\beta$ , TNF- $\alpha$ , and TGF- $\beta$ 1, 11 $\beta$ -HSD1 expression and activity increased in parallel with expression levels of CYP11B1 and

cortisol, but the production of 11 $\beta$ -HSD2 decreased. The small interfering RNA technique or the measurement of 11 $\beta$ -HSD1 activity showed that the sinus epithelium activates cortisone to cortisol in an 11 $\beta$ -HSD-dependent manner.

**Conclusion:** These results indicate that CRS-relevant cytokines can modulate the expression of 11 $\beta$ -HSD1, 11 $\beta$ -HSD2, and CYP11B1 in the sinus mucosa, resulting in increasing intracellular concentrations of bioactive glucocorticoids. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

**Key words:** 11 $\beta$ -Hydroxysteroid dehydrogenase 1, 11 $\beta$ -hydroxysteroid dehydrogenase 2, CYP11B1, CYP11A1, chronic rhinosinusitis with nasal polyps, chronic rhinosinusitis without nasal polyps, glucocorticoid, cortisol

Chronic rhinosinusitis (CRS) is defined as inflammation of the sinonasal mucosa lasting more than 12 weeks and is generally classified as either chronic rhinosinusitis with nasal polyps (CRSwNP) or chronic rhinosinusitis without polyps (CRSsNP).<sup>1-3</sup> The underlying causes are still unknown, and because of its heterogeneity, multiple medical therapies, including antibiotics, saline irrigations, and topical and systemic glucocorticoids, are widely advocated to achieve successful management.<sup>2,3</sup> Topical corticosteroids tend to constitute the first line of therapy in the medical management of CRS by reducing mucosal inflammation and improving symptoms associated with CRS. In addition, a short course of oral steroids is used to treat CRSwNP.<sup>2,4</sup>

The major anti-inflammatory mechanism of glucocorticoids is ascribed to numerous properties, including prevention of the release of various inflammatory cytokines, chemokines, and mediators from stimulated resident and inflammatory cells.<sup>5,6</sup> Like exogenously administered glucocorticoids, endogenous glucocorticoids have been importantly implicated in inhibiting the inflammatory responses of various organs, including the intestines, lungs, and joints.<sup>7-10</sup> Endogenous glucocorticoids are powerful modulators of inflammatory responses whose overall effects might result not only from circulating glucocorticoids through the hypothalamic-pituitary-adrenal axis but also through tissue-specific metabolism of glucocorticoids catalyzed by 2 isoforms of 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD).<sup>11</sup>

The 2 isoforms of 11 $\beta$ -HSD, 11 $\beta$ -hydroxysteroid dehydrogenase 1 (11 $\beta$ -HSD1) and 11 $\beta$ -hydroxysteroid dehydrogenase 2 (11 $\beta$ -HSD2), modulate endogenous glucocorticoid action within cells and tissues at the prereceptor level. 11 $\beta$ -HSD1 acts as an oxoreductase, generating active cortisol from cortisone, which potentiates the actions of glucocorticoids in tissues, whereas 11 $\beta$ -HSD2 inactivates cortisol to cortisone. Therefore 11 $\beta$ -HSD1

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**Abbreviations used**

|                   |                                                        |
|-------------------|--------------------------------------------------------|
| ACTH:             | Adrenocorticotrophic hormone                           |
| CRS:              | Chronic rhinosinusitis                                 |
| CRSsNP:           | Chronic rhinosinusitis without nasal polyps            |
| CRSwNP:           | Chronic rhinosinusitis with nasal polyps               |
| CYP11A1:          | Cytochrome P450, family 11, subfamily A, polypeptide 1 |
| CYP11B1:          | Cytochrome P450, family 11, subfamily B, polypeptide 1 |
| 11 $\beta$ -HSD1: | 11 $\beta$ -Hydroxysteroid dehydrogenase 1             |
| 11 $\beta$ -HSD2: | 11 $\beta$ -Hydroxysteroid dehydrogenase 2             |
| siRNA:            | Small interfering RNA                                  |
| SNOT-20:          | Sino-Nasal Outcome Test                                |

increases the local concentration of active glucocorticoids and 11 $\beta$ -HSD2 decreases the concentration, regulating local glucocorticoid concentrations.<sup>11</sup> Furthermore, it has been shown that 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 expression and activity are altered in various inflammatory diseases, leading to suggestion that deregulation of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 expression might contribute to resolving the chronic inflammation by inducing intracellular glucocorticoid action in various organs.<sup>7-10</sup> In patients with chronic inflammatory bowel diseases and arthritis, 11 $\beta$ -HSD1 expression was significantly increased in inflamed tissue, whereas 11 $\beta$ -HSD2 expression was decreased or absent.<sup>7,9</sup> *In vitro* studies have demonstrated that various inflammatory mediators, including cytokines, modulate the expression of these enzymes in various tissues.<sup>7,12</sup> In this respect these enzymes might play an important role in regulating the action of endogenous glucocorticoids in the sinonasal mucosa of patients with CRSwNP and those with CRSsNP.

Traditionally, it was thought that glucocorticoids were solely synthesized in the adrenal cortex. However, a growing body of evidence has demonstrated *de novo* synthesis of glucocorticoids in other organs, such as the thymus, brain, skin, and vascular endothelium.<sup>13</sup>

Other tissues, such as the lung and intestinal epithelium, have been described to express steroidogenic enzymes, including cytochrome P450, family 11, subfamily B, polypeptide 1 (CYP11B1), and were found to be potential extra-adrenal sources of glucocorticoids.<sup>14</sup> Epithelial surfaces in the gastrointestinal tract, lung, and skin are in constant contact with the outside environment and exposed to a plethora of pathogens. Thus local glucocorticoid synthesis occurs in these immunologically important tissues that are sites of exposure to antigen and lymphocyte activation and contributes to the maintenance of immune homeostasis.<sup>14,15</sup> On the other hand, the sinonasal mucosa lining the sinonasal cavity is also constantly exposed to ambient air, including bacteria and virus, linking the innate and adaptive immune systems.<sup>16</sup> Therefore we hypothesized that the sinus mucosa might express steroidogenic enzymes, inducing local reactivation of active corticosterone as another extra-adrenal source of cortisol.

Therefore the present study was undertaken (1) to investigate the synthesis of endogenous glucocorticoids and expression of 11 $\beta$ -HSD1, 11 $\beta$ -HSD2, CYP11B1, and cytochrome P450, family 11, subfamily A, polypeptide 1 (CYP11A1) in normal sinus mucosa of healthy control subjects and inflammatory sinus mucosa of patients with CRSwNP and CRSsNP; (2) to elucidate whether the expression levels of 11 $\beta$ -HSD1, 11 $\beta$ -HSD2, CYP11B1, CYP11A1, and cortisol might be altered by

stimulation of cytokines and mediators relevant to CRS; and (3) to determine the functional roles of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 in the conversion of cortisone to cortisol in the sinus mucosa.

**METHODS****Subjects**

As a control, normal ethmoid sinus mucosa was obtained from patients with blowout fractures during endoscopic reduction. Inflammatory ethmoid sinus mucosa was obtained from patients with CRSwNP and those with CRSsNP undergoing endoscopic sinus surgery. Endoscopic physical findings, computed tomographic scoring systems, and patients' symptoms were rated, as described previously.<sup>17-19</sup> The diagnosis of CRSwNP and CRSsNP was made according to the current European Position Paper on Rhinosinusitis and Nasal Polyps.<sup>1</sup> None of the control subjects or patients with CRS had a history of asthma or a positive skin prick test response to a standard panel of aeroallergens. In this study subjects were excluded if they took any oral or topical medication, including steroids, antihistamines, or antibiotics, at least 3 months before the surgery. Clinical data of patients are summarized in Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). Informed written consent was obtained from all patients before collecting samples for experimental use. The institutional review boards for human subjects at our institution approved the protocols and informed consent form.

Three pieces of ethmoid sinus mucosa were obtained from each subject. The first and second portions were stored at  $-80^{\circ}\text{C}$  for subsequent RNA and protein isolation. For immunohistochemistry, the third portion was embedded in paraffin wax. A part of the normal sinus mucosa was used for epithelial cell culture. Sections stained with hematoxylin and eosin were examined with a light microscope to obtain a general impression of the typical pathologic features encountered in normal and inflammatory sinus mucosa. The numbers of total inflammatory cells, eosinophils, mononuclear cells, and plasma cells in the subepithelial layer was counted at high-power magnification ( $\times 400$ ), and 5 high-power fields were randomly selected.

**Isolation and culture of epithelial cells from normal sinus mucosa**

Normal human sinus mucosa was treated with 0.5% dispase (GeneDEPOT, Baker, Tex) in a 1:1 mixture of Dulbecco modified Eagle medium and Ham nutrient F12 supplemented with penicillin G sodium (50 IU/mL) and streptomycin sulfate (50  $\mu\text{g/mL}$ ). Thereafter, epithelial cells were freed from the sinus mucosa and collected in a conical tube. The cells were cultured under an air-liquid interface in serum-free bronchial epithelial growth medium (Lonza, Walkersville, Md) containing no bovine pituitary extract and thyroid hormone. They were stimulated with cytokines (10 and 30 ng/mL IL-4, IL-5, IL-13, IL-1 $\beta$ , TGF- $\beta$ 1, TNF- $\alpha$ , or IFN- $\gamma$ ; PeproTech, Rocky Hill, NJ) for 12 and 24 hours in the presence or absence of cortisone (100 nmol/L). After stimulation, the cultured cells were harvested and used for real-time PCR or Western blotting to evaluate expression levels of 11 $\beta$ -HSD1, 11 $\beta$ -HSD2, CYP11B1, and CYP11A1. Furthermore, cultured epithelial cells were incubated with cortisone (10, 30, 50, 100, and 200 nmol/L; Sigma, St Louis, Mo), adrenocorticotrophic hormone (ACTH; 1 and 10 nmol/L, Sigma), and 11 $\beta$ -HSD1 inhibitor (1  $\mu\text{mol/L}$ ; Merck Millipore, Billerica, Mass), according to the experimental protocol specified in the Results section. In addition, the cultured cells were used to evaluate whether the glucocorticoid might affect the expression levels of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2.

**Sampling of blood and nasal secretions for analysis of cortisol**

Blood and nasal secretions were obtained between 8 and 9 AM, when the patients with CRS or healthy subjects visited the outpatient clinic at baseline. Nasal lavage was performed by slowly instilling 10 mL of sterile isotonic saline into each nostril by using a syringe. The solution was retained for approximately 10 seconds in the nasal cavities and then expelled by means of forward flexing the head and rinsing lavage liquid into a sterile plastic

beaker. After centrifugation, serum and nasal lavage fluid were stored at  $-80^{\circ}\text{C}$  until analysis.

### Small interfering RNA transfection experiments

Cultured epithelial cells were used as an *in vitro* model for functional assay of cortisol production. Small interfering RNA (siRNA) were synthesized as purified duplex (ST Pharm Oligo Center SH, Kyung Ki Do, Korea). The sequences of the sense and antisense strands of the human 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 siRNA were as follows: CCA AAG CAC UGA UAC UUA U (sense) and AUA AGU AUC AGU GCU UUG G (antisense) for 11 $\beta$ -HSD1 and CCA AGA AAC UGG ACU CCA U (sense) and AUG GAG UCC AGU UUC UUG G (antisense) for 11 $\beta$ -HSD2. Cells were plated in 6-well plates at a density of  $5 \times 10^5$  cells per well and transfected with siRNA duplexes by using Lipofectamine 2000 (Invitrogen, Carlsbad, Calif), according to the manufacturer's instructions. After transfection, cells were treated as specified in the Results section.

### Assay of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 activities

Confluent epithelial cells were treated with IL-4, IL-5, IL-13, IFN- $\gamma$ , IL-1 $\beta$ , TNF- $\alpha$ , and TGF- $\beta$ 1 and then lysed with assay buffer containing 1% Triton X-100 and 1 mmol/L EDTA to measure 11 $\beta$ -HSD1 activity in cytokine-treated epithelial cells. The enzyme assays were performed with 50  $\mu\text{g}$  of sample protein incubated for 1 hour at  $37^{\circ}\text{C}$  in 600  $\mu\text{L}$  of assay buffer containing 500  $\mu\text{mol/L}$  nicotinamide adenine dinucleotide phosphate for 11 $\beta$ -HSD1 and cortisone or NAD for 11 $\beta$ -HSD2. Thereafter, the samples were placed in a boiling water for 5 minutes. 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 activity was then assessed by measuring cortisol production with the Cortisol ELISA kit from R&D Systems (Minneapolis, Minn), and enzyme activity was expressed as picograms of cortisol produced per milligram of protein.

### Real-time RT-PCR analysis

Total RNA was extracted from frozen sinus tissues and cultured epithelial cells by using Trizol reagent (Life Technologies, Rockville, Md). Total RNA (1  $\mu\text{g}$ ) from each sample was reverse transcribed in 20  $\mu\text{L}$  of a reaction mixture containing 2.5 U of MMLV (RT; GIBCO BRL, Grand Island, NY). The primer sequences of each gene used in the study are listed in Table E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). The mean threshold cycle values were normalized to glyceraldehyde-3-phosphate dehydrogenase, and the relative mRNA levels of target genes were analyzed with the  $2^{-\Delta\Delta\text{Ct}}$  method.

### Immunohistochemical and Western blot analysis

Immunohistochemical staining was performed with a peroxidase-labeled streptavidin-biotin technique. Briefly, paraffin sections (4  $\mu\text{m}$ ) were incubated overnight at  $4^{\circ}\text{C}$  with a 1:100 dilution of anti-HSD1 antibody or a 1:100 dilution of anti-HSD2 antibody, a 1:500 dilution of anti-CYP11B1 antibody, or a 1:500 dilution of anti-CYP11A1 antibody (Santa Cruz Biotechnology, Santa Cruz, Calif). The color was developed with 3, 3'-diaminobenzidine.

For Western blot analysis, the proteins extracted from frozen sinus tissue and cultured epithelial cells were separated by using 12% SDS-PAGE and transferred to Immobilon membranes (Millipore, Bedford, Mass). The blots were incubated with a 1:1500 dilution of anti-HSD1 or a 1:1500 dilution of anti-HSD2 antibody or anti-CYP11B1 antibody or anti-CYP11A1 antibody in PBS-T overnight at room temperature. Antibody reactions were detected with the ECL detection kit (GE Healthcare, Amersham Biosciences, Little Chalfont, United Kingdom), followed by detection of chemiluminescence on x-ray film.

### ELISA

Cortisol levels in sinus mucosa, nasal lavage fluid, serum, and cultured cells were measured with an ELISA (R&D Systems), according to a commercial protocol.

### Statistical analyses

Statistical analyses were carried out with SPSS software for Windows (version 16.0.0; SPSS, Chicago, Ill). The age differences, Sino-Nasal Outcome Test (SNOT-20) scores, computed tomographic scores, endoscopic scores, and numbers of inflammatory cells in the 3 groups were calculated by using 1-way ANOVA, and the Bonferroni *post hoc* correction was applied. Pearson correlation coefficients were evaluated between the total SNOT-20 score and expression levels of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2. One-way ANOVA and the Bonferroni *post hoc* correction ( $\alpha = .0167$ ) and Tukey test were performed to establish statistically significant differences in values obtained from the sinus mucosa of healthy control subjects, patients with CRSwNP, and patients with CRSsNP, including the data evaluated by using real-time PCR, Western blot analysis, and ELISA. The Kolmogorov-Smirnov test showed that the distribution of each data set was normal. The level of significance was set at a *P* value of less than .05. Mann-Whitney *post hoc* tests were done to compare differences between different groups.

## RESULTS

### Expression levels and distribution pattern of 11 $\beta$ -HSD1, 11 $\beta$ -HSD2, CYP11B1, and CYP11A1 in sinus mucosa

Expression levels of 11 $\beta$ -HSD1 and CYP11B1 increased significantly in the inflammatory sinus mucosa of patients with CRSwNP and those with CRSsNP compared with levels seen in normal sinus mucosa. However, 11 $\beta$ -HSD2 expression decreased in inflammatory sinus mucosa, irrespective of the presence or absence of nasal polyps (Fig 1). However, the correlation between symptom severity and expression levels of 11 $\beta$ -HSD1 or 11 $\beta$ -HSD2 was not found in the present study (see Table E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). There were no significant differences in expression levels between patients with CRSwNP and those with CRSsNP. CYP11A1 is also present in normal sinus mucosa, but its expression levels were unchanged in inflammatory sinus mucosa (Fig 1). Expression of 11 $\beta$ -HSD1, 11 $\beta$ -HSD2, CYP11B1, and CYP11A1 was also detected in cultured epithelial cells obtained from human sinus mucosa (Fig 2). In normal and inflammatory sinus mucosa these enzymes were similarly located in the superficial epithelium, submucosal glands, and vascular endothelial cells (Fig 3).

### Regulation of 11 $\beta$ -HSD1, 11 $\beta$ -HSD2, CYP11B1, and CYP11A1 expression by cytokine stimulation in cultured epithelial cells

Expression levels of 11 $\beta$ -HSD1 and CYP11B1 increased 12 or 24 hours after stimulation with IL-4, IL-5, IL-1 $\beta$ , IL-13, TNF- $\alpha$ , and TGF- $\beta$ 1 compared with those seen in nontreated control subjects (Fig 2). By contrast, expression levels of 11 $\beta$ -HSD2 decreased after treatment with IL-4, IL-5, IL-1 $\beta$ , IL-13, TNF- $\alpha$ , and TGF- $\beta$ 1 (Fig 2). IFN- $\gamma$  had no effect on the expression levels of these enzymes, and CYP11A1 expression levels were not affected by stimulation of these cytokines (Fig 2).

### Evaluation of cortisol-synthesizing ability in human sinus epithelial cells and regulation of cortisol levels through 11 $\beta$ -HSD1 activity

To investigate the possible glucocorticoid synthesis in human sinus mucosa, we measured cortisol levels in sinus mucosa, nasal lavage fluid, and serum using ELISA. Cortisol levels in the sinus

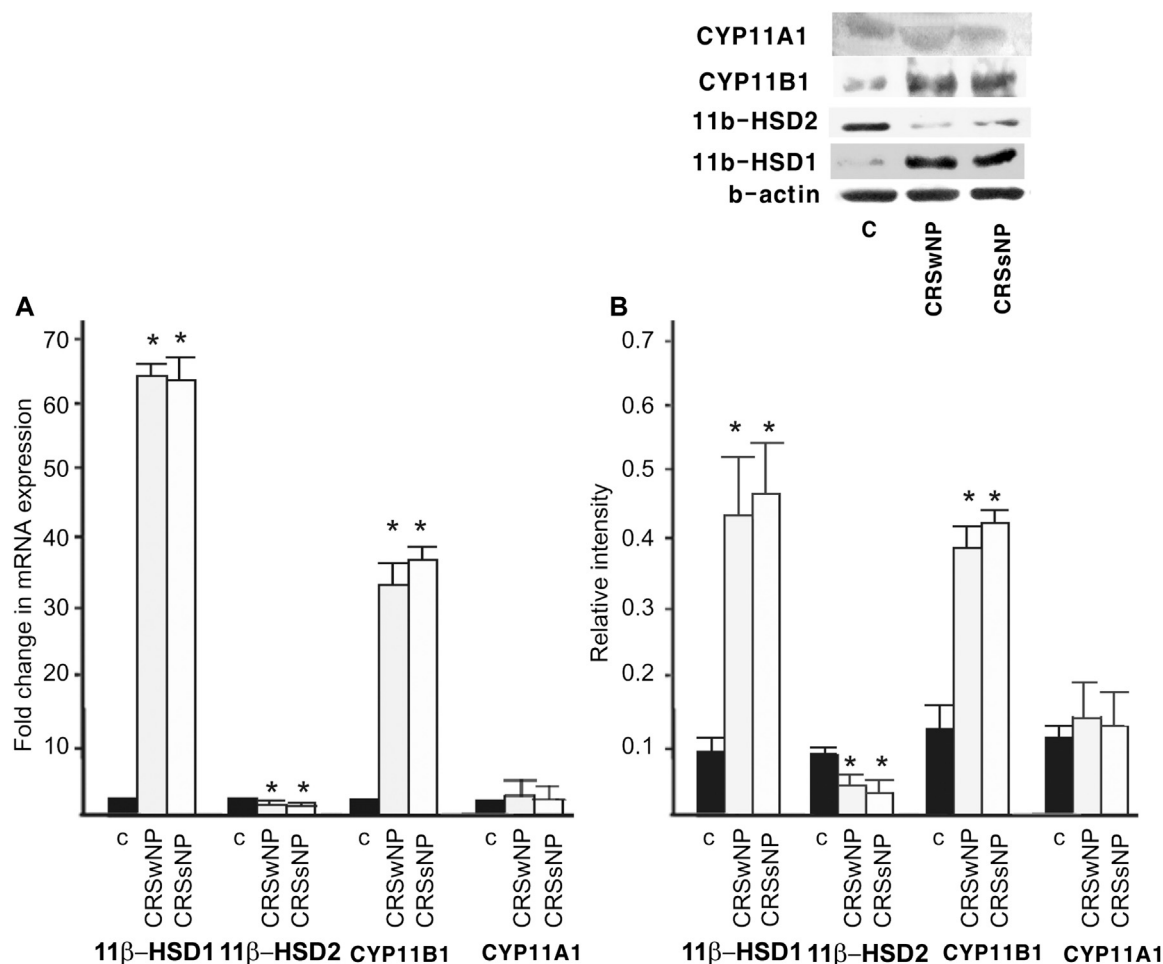


**FIG 1.** Real-time PCR (A) and Western blot analysis (B) of 11β-HSD1, 11β-HSD2, CYP11B1, and CYP11A1 expression in normal sinus mucosa of control subjects (C; n = 25) and inflammatory sinus mucosa of patients with CRSwNP (n = 25) and patients with CRSsNP (n = 25). Western blots show representative data. \*Significant difference in expression levels of 11β-HSD1, 11β-HSD2, and CYP11B1 between normal and inflammatory sinus mucosa ( $P < .05$ ). Results are expressed as means  $\pm$  SDs.

mucosa and nasal lavage fluid were increased significantly in patients with CRS, irrespective of polyp presence, compared with those seen in healthy subjects. However, the cortisol levels in serum were unchanged in patients with CRS compared with healthy control subjects (Fig 4, A-C).

We next evaluated whether the epithelial cells of the human sinus mucosa were capable of producing cortisol. Cells were cultured with ACTH, the 11β-HSD1 inhibitor, or both. Cortisol levels increased significantly in cells treated with ACTH compared with those seen in nonstimulated cells. However, when cells were treated with the 11β-HSD1 inhibitor in the presence or absence of ACTH, cortisol levels were decreased compared with those seen in cells treated with ACTH alone (Fig 4, D). These data show that sinus epithelial cells are not only able to activate cortisone to cortisol but also respond to stimulation with ACTH by initiating *de novo* cortisol production.

Cultured epithelial cells obtained from healthy control subjects were incubated with cortisone alone or in the presence of the 11β-HSD1 inhibitor to evaluate whether the epithelial cells lining the human sinus mucosa metabolize cortisol. In cells treated with cortisone alone, the level of cortisol increased in a dose-dependent manner (see Fig E1 in this article's Online

Repository at [www.jacionline.org](http://www.jacionline.org)), but when pretreated with 11β-HSD1 inhibitor, the cortisol levels decreased in comparison with those of cells incubated with cortisone alone. These findings indicate that human sinus mucosal epithelial cells can convert cortisone to cortisol through 11β-HSD1 enzyme activity (Fig 4, E).

To confirm the role of 11β-HSD1 and 11β-HSD2 in cortisol activation and deactivation, we used siRNA technology in the presence or absence of cortisone. Cells silenced with 11β-HSD1-specific siRNA produced lower levels of cortisol when compared with those seen in control subjects, whereas in 11β-HSD2-silenced cells cortisol levels increased in comparison with those in control subjects. These data indicate that both 11β-HSD enzymes could regulate the local concentration of cortisol in human sinus mucosal epithelial cells (Fig 4, F).

The activities of 11β-HSD1 and 11β-HSD2 in cultured epithelial cells in the presence or absence of various cytokines were measured by comparing the levels of cortisol accumulation in incubation medium after exposure to a physiologically relevant concentration of cortisone. The activities of the 11β-HSD1 enzyme increased after stimulation with IL-4, IL-5, IL-13, IL-1β, TNF-α, and TGF-β1 and were not induced by IFN-γ

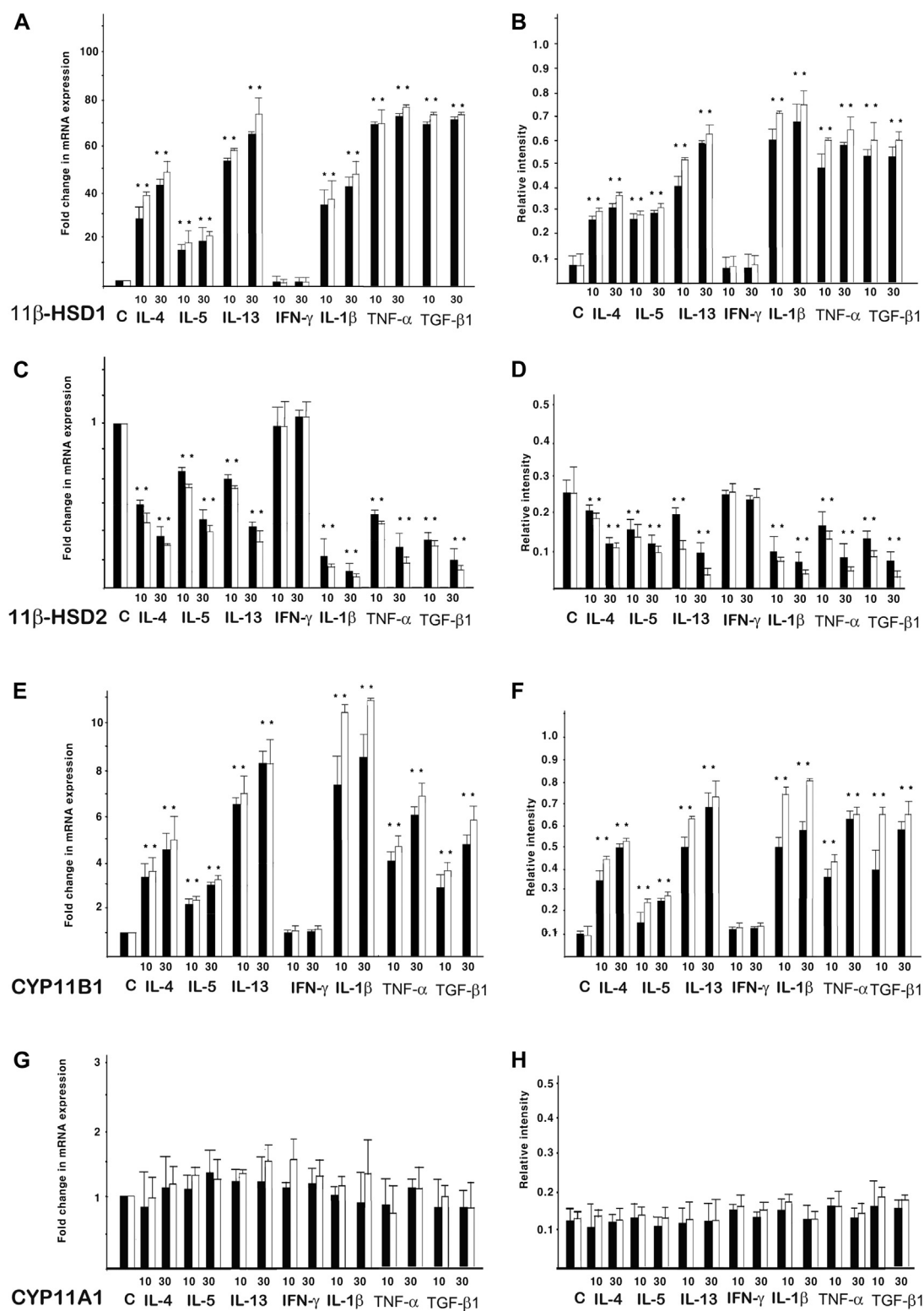


**FIG 2.** Real-time PCR (A, C, E, and G) and Western blot analysis (B, D, F, and H) of 11β-HSD1 (Fig 2, A and B), 11β-HSD2 (Fig 2, C and D), CYP11B1 (Fig 2, E and F), and CYP11A1 (Fig 2, G and H) expression levels in cultured epithelial cells after stimulation with IL-4, IL-5, IL-13, IFN-γ, IL-1β, TNF-α, and TGF-β1 (10 and 30 ng/mL), respectively, for 12 hours (solid bars, n = 6) or 24 hours (open bars, n = 6). Results are expressed as means ± SDs. \*Statistically significant difference in 11β-HSD1, 11β-HSD2, and CYP11B1 expression levels between cytokine-stimulated cells and nonstimulated control cells (Fig 2, C).

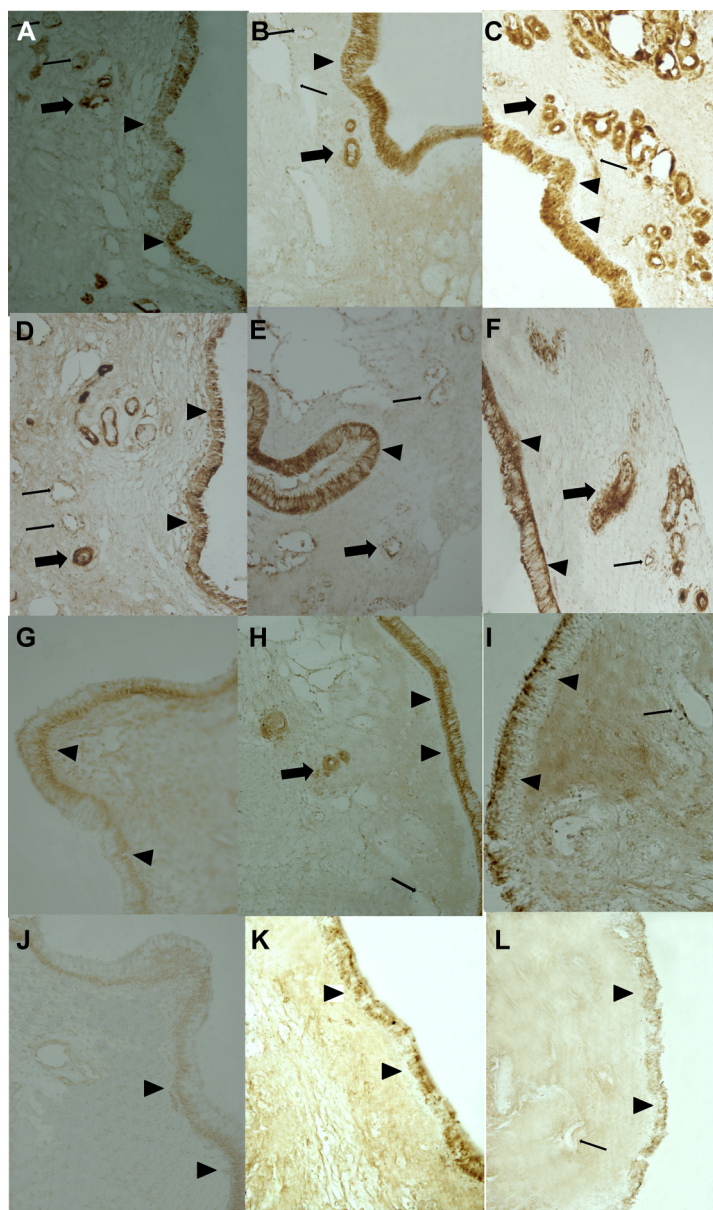


**FIG 3.** Immunohistochemical localization of 11 $\beta$ -HSD1 (A-C), 11 $\beta$ -HSD2 (D-F), CYP11B1 (G-I), and CYP11A1 (J-L) in normal sinus mucosa (Fig 3, A, D, G, and J) and inflammatory sinus mucosa of patients with CRSwNP (Fig 3, B, E, H, and K) and patients with CRSsNP (Fig 3, C, F, I, and L). 11 $\beta$ -HSD1, 11 $\beta$ -HSD2, CYP11B1, and CYP11A1 are detected in the surface epithelium (*arrowheads*), submucosal glands (*thick arrows*), and vascular endothelium (*thin arrows*). Original magnification  $\times 200$ .

(see Fig E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). However, the activities of the 11 $\beta$ -HSD2 enzyme decreased after stimulation with IL-4, IL-5, IL-13, IL-1 $\beta$ , TNF- $\alpha$ , and TGF- $\beta$ 1 (see Fig E2).

#### Effect of glucocorticoid treatment on 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 expression in cultured epithelial cells

In cultured epithelial cells stimulated with dexamethasone, the expression levels of 11 $\beta$ -HSD1 were increased compared with those in nontreated control subjects, whereas the expression levels of 11 $\beta$ -HSD2 were decreased (see Fig E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

#### DISCUSSION

In this study we found that normal sinus mucosa produces and metabolizes cortisol endogenously. First, we demonstrated the presence of detectable amounts of CYP11B1 and CYP11A1 mRNA transcripts and proteins, molecules involved in cortisol biosynthesis. Second, we confirmed that 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 enzymes are constitutively expressed in the normal sinus mucosa, thereby indicating that glucocorticoid regulation by the 11 $\beta$ -HSD isoforms does occur in the human sinus mucosa. Third, upregulation of 11 $\beta$ -HSD1 was found to be accompanied by downregulation of 11 $\beta$ -HSD2 in the inflammatory sinus mucosa of patients with CRS, irrespective of the presence of nasal polyps. Cortisol levels in sinus mucosa

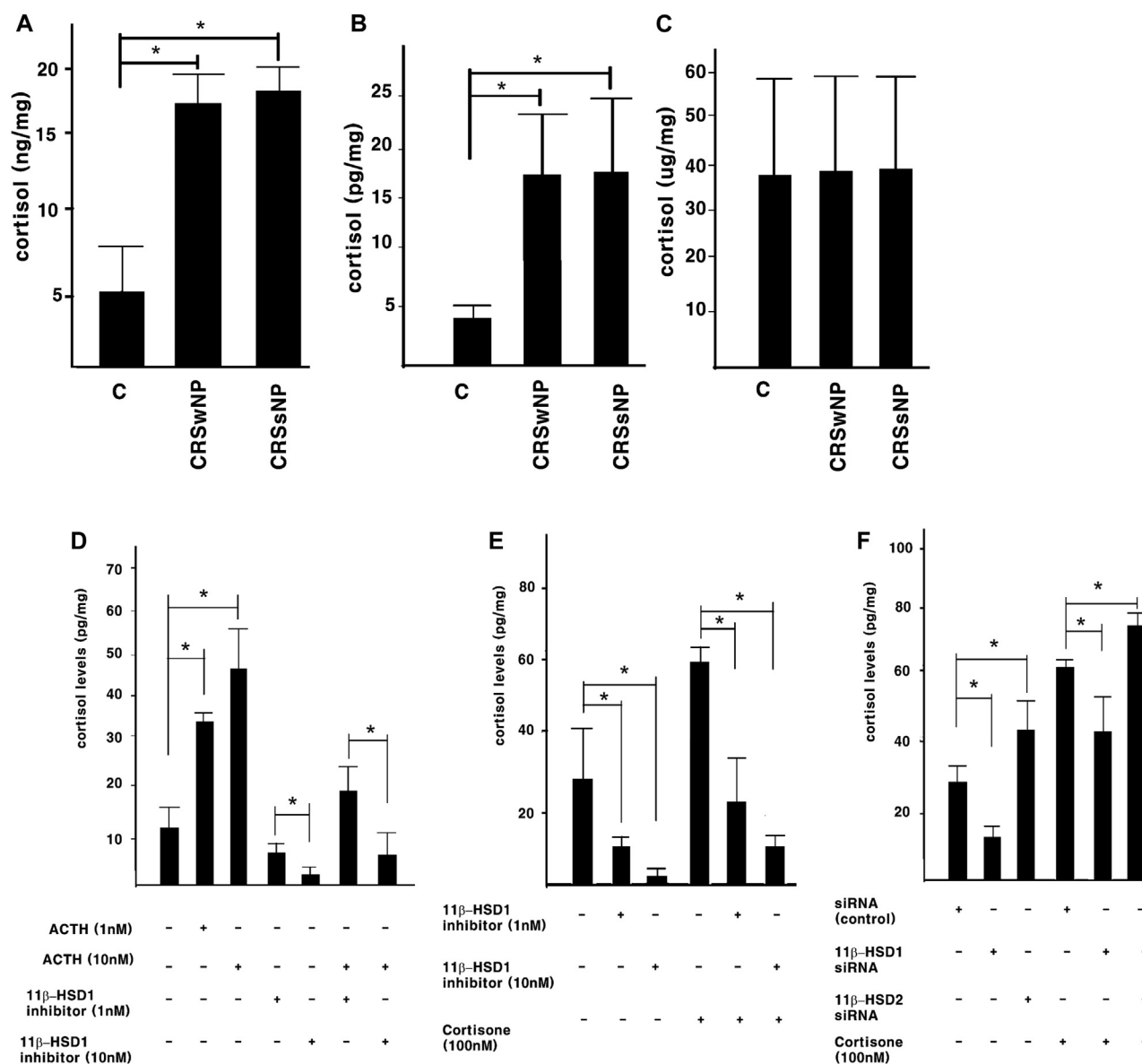


**FIG 4.** A-C, Analysis of cortisol levels in sinus mucosa (Fig 4, A), nasal lavage fluid (Fig 4, B), and serum (Fig 4, C) of healthy subjects ( $n = 25$ ), patients with CRSwNP ( $n = 25$ ), and patients with CRSsNP ( $n = 25$ ). D, Cortisol levels after incubation for 24 hours with ACTH (1 and 10 nmol/L). E, Cortisol levels after treatment with cortisone, 11 $\beta$ -HSD1 inhibitor, or both. F, Cortisol levels in siRNA transfection experiments. Results are shown as a mean of 6 independent experiments. \* $P < .05$ .

and nasal lavage fluid increased in patients with CRS compared with those in healthy subjects. Furthermore, expression levels of these enzymes were differentially enhanced by CRS-relevant cytokines. These results indicate that the inflammatory response in patients with CRS is associated with stimulation of 11 $\beta$ -HSD1 and inhibition of 11 $\beta$ -HSD2, resulting in increased generation of active glucocorticoids in the human sinus mucosa.

The 11 $\beta$ -HSD1 enzyme has an extensive distribution throughout human tissue, including the liver, skin, and bone.<sup>20</sup> The greatest 11 $\beta$ -HSD1 expression was reported in tissues that are known to be highly glucocorticoid sensitive, such as the liver, gonads, and brain. By contrast, 11 $\beta$ -HSD2 is expressed in the kidney, pancreas, and other mineralocorticoid-sensitive tissues.<sup>21-24</sup> To our knowledge, this study is the first to

demonstrate expression of both glucocorticoid-metabolizing enzymes (11 $\beta$ -HSD1 and 11 $\beta$ -HSD2) in the human sinus mucosa. The subsequent observation that local cortisol levels might be altered by the silencing of either 11 $\beta$ -HSD1 or 11 $\beta$ -HSD2 by siRNA or the selective 11 $\beta$ -HSD1 inhibitor led us to conclude that the epithelial cells of the sinus mucosa possess an endogenous system that regulates glucocorticoid levels and thereby contributes to the maintenance of physiologic balance between active and inactive cortisone. Similar findings have also been reported in cultured keratinocytes and airway smooth muscle cells, in which silencing of either 11 $\beta$ -HSD1 or 11 $\beta$ -HSD2 by siRNAs or inhibitors resulted in significant changes in the level of active cortisol.<sup>10,25</sup> The initial step in synthesis and secretion of glucocorticoids from cholesterol is the conversion of cholesterol to

pregnenolone, which is induced by the CYP11A1 enzyme. The final step of cortisol biosynthesis, converting 11-deoxycortisol to cortisol, is performed by the 11 $\beta$ -hydroxylase CYP11B1.<sup>26</sup> Therefore evidence of CYP11A1 and CYP11B1 expression supports the formation of glucocorticoids in tissue levels.<sup>26</sup> Furthermore, we found that cultured sinus mucosal epithelial cells could synthesize cortisol *de novo* after stimulation with ACTH, thereby acting as a glucocorticoid-producing organ. The expression of these steroidogenic critical enzymes was identified in the heart and intestine, suggesting the possibility of adrenal-independent glucocorticoid synthesis *in situ*.<sup>27,28</sup> Recently, cortisol production by fibroblasts, melanocytes, and isolated hair follicles was demonstrated *in vitro*.<sup>29-31</sup> A previous study showed that cultured keratinocytes could synthesize cortisol *de novo* after stimulation with ACTH.<sup>25</sup> These studies suggest the possibility that human sinus mucosa is another extra-adrenal site of cortisol synthesis. Further study is required to evaluate the full component of enzymes necessary for *de novo* production of glucocorticoids from cholesterol in human sinus mucosa.

There is a growing body of work demonstrating the importance of 11 $\beta$ -HSD1 in regulating local glucocorticoid levels during inflammation *in vivo*. In the colonic mucosa in patients with colitis or inflammatory bowel disease, 11 $\beta$ -HSD1 expression increased and 11 $\beta$ -HSD2 expression decreased compared with that seen in control subjects.<sup>8,9</sup> Similarly, a marked increase in 11 $\beta$ -HSD1 expression was observed in inflamed synovium isolated from patients with rheumatoid arthritis or osteoarthritis.<sup>32,33</sup> Furthermore, substantial upregulation of steroidogenic enzymes clearly induced the local synthesis of intestinal glucocorticoid in an experimental colitis model.<sup>9,28</sup> Overall, these findings suggest that inflammation is associated with stimulation of 11 $\beta$ -HSD1, leading to increased production of active glucocorticoids, namely cortisol. The hypothesis arising from these experimental data is that changes in glucocorticoid metabolism can contribute to negative feedback suppressing further inflammatory responses. This suggestion is supported by the observation that 11 $\beta$ -HSD1 is stimulated by IL-1 $\beta$  and TNF- $\alpha$  in renal mesangial cells, subsequently modulating intrarenal inflammatory responses.<sup>34</sup> Another study identified the T<sub>H</sub>2 cytokine-driven homeostatic feedback mechanism in airway smooth muscle, where there is an enhancement of endogenous glucocorticoid production by increasing 11 $\beta$ -HSD1 activity, which attenuates the adverse effects of the cytokines on airway function.<sup>10</sup> In a skin wound model cortisol synthesis in the epidermis was found to serve as a local negative feedback to proinflammatory cytokines.<sup>12</sup> On the other hand, in patients with rheumatoid arthritis, the level of 11 $\beta$ -HSD1 expression correlates with the degree of inflammation both histologically and based on clinical/biochemical measures of disease activity.<sup>33</sup> In the present study the reciprocal regulation of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 expression levels in patients with CRSwNP or patients with CRSsNP was observed compared with those seen in healthy control subjects. Taken together, these results suggest that reciprocal regulation of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 expression might reflect the appropriate autoregulatory response to inflammation. Therefore a maladaptive mechanism in patients with chronic inflammation resulting in imbalanced 11 $\beta$ -HSD expression might contribute to the failure of resolution during chronic inflammation. This suggestion is supported by results showing that the onset of inflammation

occurs earlier in an 11 $\beta$ -HSD1 knockout mouse model compared with a control mouse model and that resolution is delayed, highlighting a role for 11 $\beta$ -HSD1 both in restraining inflammation and promoting its resolution.<sup>35</sup>

On the basis of the evidences that 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 are reciprocally modulated by cytokines, one possible mechanism is that the increased levels of various cytokines in patients with CRS might result in upregulation of 11 $\beta$ -HSD1 and downregulation of 11 $\beta$ -HSD2. In vascular smooth muscle cells upregulation of 11 $\beta$ -HSD1 was induced after exposure to IL-1 $\beta$  and TNF- $\alpha$ , suggesting that the induction of 11 $\beta$ -HSD1 plays a role in regulating inflammatory responses in arterial vessels.<sup>36</sup> In a human bronchial epithelial cell line endotoxin inhibited 11 $\beta$ -HSD2 enzyme activity in a dose-dependent manner.<sup>35</sup> Likewise, TNF- $\alpha$  inhibited both the activity and mRNA expression of 11 $\beta$ -HSD2.<sup>37</sup> Induced expression of 11 $\beta$ -HSD1 by IL-1 $\beta$  and TNF- $\alpha$  was also found in human airway smooth muscle cells.<sup>32</sup> Another study showed that expression of 11 $\beta$ -HSD1 in airway smooth muscle cells was upregulated in the presence of IL-13, whereas 11 $\beta$ -HSD2 expression was inhibited.<sup>7</sup> Therefore it is likely that the reciprocal changes in 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 expression in the inflammatory sinus mucosa of patients with CRSwNP and those with CRSsNP might be induced by inflammatory mediators, including cytokines relevant to CRS. Our data were in accordance with results showing that inflammatory cytokines induces 11 $\beta$ -HSD1 expression and activity in synovial fibroblasts and osteoblasts.<sup>38</sup> On the basis of these results, we conclude that the reciprocal regulation of 11 $\beta$ -HSD isoforms by cytokines might contribute to the regulation of intracellular corticosteroid metabolism in inflammatory sinus mucosa, serving to enhance prereceptor glucocorticoid bioavailability and contributing to feedback control of chronic inflammation. Our findings support the notions that inflammatory cytokines can induce the formation of biologically active glucocorticoids through the interactions between enzymes relating to glucocorticoid metabolism and cytokines that can persist in the target tissue through autocrine or paracrine mechanisms.<sup>32,36,39-41</sup>

The action of glucocorticoids at sites of inflammation is attributed to the prevention of the release of various inflammatory cytokines in inflammatory sinus mucosa. Interestingly, in the present study, when cultured epithelial cells were stimulated with glucocorticoids or cytokines, the expression levels of 11 $\beta$ -HSD1 were increased, whereas 11 $\beta$ -HSD2 expression levels were decreased. Our data were in accordance with results showing that glucocorticoids induce 11 $\beta$ -HSD1 expression and activity in synovial fibroblasts and osteoblasts.<sup>38</sup> Furthermore, combined treatment with inflammatory cytokines and glucocorticoids synergistically induced 11 $\beta$ -HSD1 expression and activity in these cells.<sup>38</sup> How glucocorticoids potentiate the effects of inflammatory cytokines on 11 $\beta$ -HSD1 expression remains unclear. Understanding the interaction of glucocorticoids and cytokines on the expression of 11 $\beta$ -HSD isoforms in sinus mucosa could provide important clues in revealing the therapeutic effects of glucocorticoids in patients with CRS.

In conclusion, compared with normal sinus mucosa, the inflammatory sinus mucosa of patients with CRSwNP and those with CRSsNP showed increased 11 $\beta$ -HSD1 expression and a reciprocal decrease in 11 $\beta$ -HSD2 expression, suggesting that these changes might induce the production of endogenous cortisol in the human sinus mucosa.

**Clinical implications: Reciprocal expression of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 in the inflammatory sinus mucosa of patients with CRSwNP and those with CRSsNP might play an important role in the pathogenesis of CRS, contributing to the increased local supply of glucocorticoids.**

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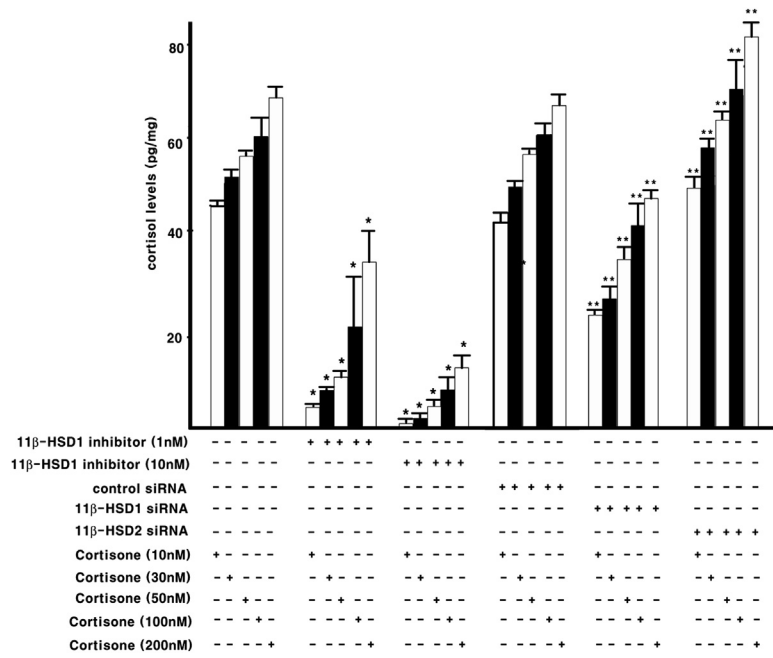


**FIG E1.** Concentration-dependent effects of cortisone on production of cortisol after treatment with 11β-HSD1 inhibitor, 11β-HSD1 siRNA, or 11β-HSD2 siRNA. \*Significant difference between groups stimulated with 11β-HSD1 inhibitor versus unstimulated control subjects. \*\*Significant difference between 11β-HSD1 or 11β-HSD2 siRNA versus control siRNA.

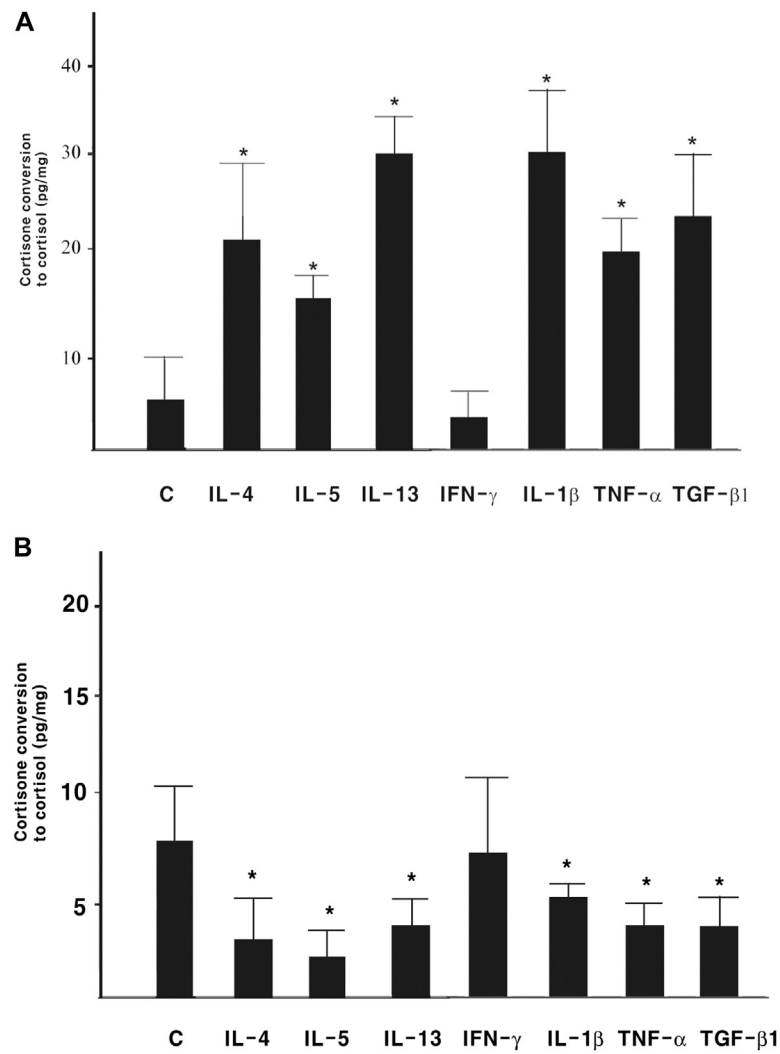


**FIG E2.** 11 $\beta$ -HSD1 (**A**) and 11 $\beta$ -HSD2 (**B**) activity measured after stimulation with IL-4, IL-5, IL-13, IFN- $\gamma$ , IL-1 $\beta$ , TNF- $\alpha$ , and TGF- $\beta$ 1 at a concentration of 10 ng/mL, respectively, in the presence of cortisone (100 nmol/L). \*Statistically significant difference:  $P < .05$ .

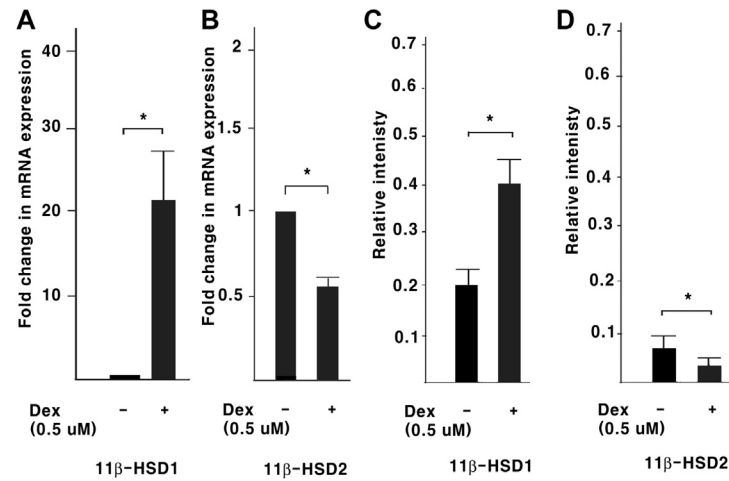


**FIG E3.** Real-time PCR (A and B) and Western blot analysis (C and D) of 11β-HSD1 (Fig E3, A and C) and 11β-HSD2 (Fig E3, B and D) expression levels in cultured epithelial cells after stimulation with dexamethasone (Dex) for 24 hours. \*Statistically significant difference:  $P < .05$ .

**TABLE E1.** Patients' characteristics

|                          | Control subjects | Patients with CRSwNP | Patients with CRSsNP |
|--------------------------|------------------|----------------------|----------------------|
| No. of patients          | 25               | 25                   | 25                   |
| Age (y), mean (range)    | 39.7 (18-33)     | 36.5 (20-40)         | 37.7 (22-43)         |
| No. (female/male)        | 6/19             | 8/17                 | 9/16                 |
| Asthma history           | 0                | 0                    | 0                    |
| Skin test result         | Negative         | Negative             | Negative             |
| No. of sinus surgeries   | 0                | 0                    | 0                    |
| SNOT-20 score            | 2.6 ± 1.3        | 26.9 ± 1.5           | 20.5 ± 2.7           |
| CT grade                 | 0                | 18 ± 2.1             | 6.6 ± 2.7            |
| Endoscopy score          | 0                | 8.6 ± 0.8            | 2.7 ± 0.6            |
| Total inflammatory cells | 13.6 ± 4.8       | 87.5 ± 9.8           | 71.8 ± 8.5           |
| Eosinophils              | 1.0 ± 0.4        | 5.9 ± 1.3            | 1.3 ± 1.2            |
| Mononuclear cells        | 13.5 ± 5.9       | 55.3 ± 10.1          | 33.7 ± 12.2          |
| Plasma cells             | 1.5 ± 1.2        | 6.2 ± 1.2            | 2.8 ± 1.3            |

CT, Computed tomography.  
\*  $P < .05$ .

**TABLE E2.** Sequences of PCR primers

| Primer           | Sequence                                                                             |
|------------------|--------------------------------------------------------------------------------------|
| 11 $\beta$ -HSD1 | Sense: 5'-ACACACACACACACACACACAC-3'<br>Antisense: 5'-ACCATGAGGTAGAAGCCACGTGTT-3'     |
| 11 $\beta$ -HSD2 | Sense: 5'-ACATTAGCCGCGTGCTAGAGTTCA-3'<br>Antisense: 5'-CGCGCCAAAGAAATTCACCTCCAT-3'   |
| CYP11B1          | Sense: 5'-AAGGTGTCAGCAGGTTCTGTGTCT-3'<br>Antisense: 5'-TCTCTGTGAGCTGTCTTGCCCTTT-3'   |
| CYP11A1          | Sense: 5'-ACATCAAGGCCAACGTCACAGAGA-3'<br>Antisense: 5'-TTGCGTGCCATCTCATACAAGTGC-3'   |
| IL-4             | Sense: 5'-TTGCTGCCTCCAAGAACAACACTG-3'<br>Antisense: 5'-TTCCTGTCGAGCCGTTTCAGGAAT-3'   |
| IL-5             | Sense: 5'-TAGCTCTTGGAGCTGCCTACGTGTAT-3'<br>Antisense: 5'-AAGCAGTGCCAAGGTCTCTTTCAC-3' |
| IL-1 $\beta$     | Sense: 5'-TGGACAAGCTGAGGAAGA-3'<br>Antisense: 5'-CCCATGTGTCGAAGAAGATAG-3'            |
| IL-13            | Sense: 5'-TGGTCAACATCACCCAGAACCAGA-3'<br>Antisense: 5'-AGCCTGACACGTTGATCAGGGATT-3'   |
| IFN- $\gamma$    | Sense: 5'-TGCAGGTCATTGATGTAGCGGA-3'<br>Antisense: 5'-TGTCTTCCTTGATGGTCTCCACACTC-3'   |
| TNF- $\alpha$    | Sense: 5'-AAGCCCTGGTATGAGCCCATCTAT-3'<br>Antisense: 5'-ATGATCCCAAAGTAGACCTGCCCA-3'   |
| TGF- $\beta$ 1   | Sense: 5'-CGACTACTACGCCAAGGA-3'<br>Antisense: 5'-GAGAGCAACACGGGTTCA-3'               |
| GAPDH            | Sense: 5'-ATCATCCCTGCCTCTACTGG-3'<br>Antisense: 5'-GTCAGGTCCACCACTGACAC-3'           |

*GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase.

**TABLE E3.** Pearson correlation coefficient between SNOT-20 scores and expression levels of 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2

|                      | Symptoms | 11 $\beta$ -HSD1<br>( <i>P</i> value) | 11 $\beta$ -HSD2<br>( <i>P</i> value) |
|----------------------|----------|---------------------------------------|---------------------------------------|
| Control subjects     | SNOT-20  | 0.286 (.166)                          | −0.008 (.970)                         |
| Patients with CRSwNP | SNOT-20  | 0.035 (.867)                          | −0.238 (.252)                         |
| Patients with CRSsNP | SNOT-20  | −0.095 (.651)                         | 0.172 (.411)                          |