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Effect of prednisone on nasal symptoms and peripheral blood T-cell function in chronic rhinosinusitis

So Watanabe, MD^{1,2}, Jayant M. Pinto, MD¹, Mohamed Elfatih H. Bashir, PhD¹, Marcella De Tineo, BSN¹, Harumi Suzuki, MD², Fuad M. Barood, MD¹, Robert M. Naclerio, MD¹, and Shilpy Sharma, PhD¹

¹Department of Surgery, Section of Otolaryngology–Head and Neck Surgery, The University of Chicago, Chicago, IL, USA

²Department of Otorhinolaryngology, Showa University School of Medicine, Tokyo, Japan

Abstract

Background—Systemic corticosteroids are the most effective anti-inflammatory drugs used for controlling chronic rhinosinusitis (CRS) symptoms. The potential mechanisms for their beneficial effects include increasing the number and function of T regulatory cells (Tregs), as reported in the local tissue post–intranasal steroid treatment. We investigated the effect of systemic corticosteroids on peripheral blood (PB) Tregs in subjects with CRS.

Methods—Twenty CRS subjects and 19 controls were recruited. PB mononuclear cells (PBMCs) were isolated from CRS subjects before and after systemic corticosteroid administration in the course of clinical treatment. Control subjects received no treatment and were studied at one visit. Nasal symptoms were recorded. CD4⁺CD25⁺Foxp3⁺ cells (Tregs) were analyzed by flow cytometry. Messenger RNA (mRNA) levels for interferon γ (IFN- γ), interleukin 4 (IL-4), IL-10, IL-13, IL-17A, transforming growth factor β 1 (TGF- β 1), forkhead box P3 (FoxP3), and GATA-binding factor 3 (GATA-3) were measured in PBMCs using real-time polymerase chain reaction (PCR).

Results—CRS subjects reported improved nasal symptoms ($p = 0.005$) and significantly reduced PB Tregs after treatment with corticosteroids ($p = 0.042$). The transcript levels of IL-4 and GATA-3 were significantly higher in the CRS subjects at their first visit when compared to controls ($p = 0.019$ and $p = 0.05$, respectively). Corticosteroid treatment lowered the transcript levels of immunoregulatory transcription factors [FoxP3 ($p = 0.048$) and GATA-3 ($p = 0.012$)] and IFN- γ ($p = 0.036$) in PB.

Conclusion—In contrast to prior work in local nasal tissue, our study reports reduced PB Tregs and decreased T helper 1 (T_H1) and T_H2 function after treatment with systemic corticosteroids.

Correspondence to: Shilpy Sharma, PhD, Department of Biotechnology, University of Pune, Ganeshkhind, Pune - 411007, Maharashtra, India; shilpy.sharma@gmail.com.

Current address of Shilpy Sharma: Department of Biotechnology, University of Pune, Pune, Maharashtra, India

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Public clinical trial registration: <http://clinicaltrials.gov/show/NCT01002313>. Effect of Prednisone on Peripheral Blood T-cell Function (Tregs and Th17) in Patients With Chronic Rhinosinusitis (CRS).

These data indicate that corticosteroid effects on Tregs in CRS are complex involving local signals in the tissue that are distinct from those in circulating cells.

Keywords

chronic rhinosinusitis; FoxP3; GATA-3; IL-4; IFN- γ ; nasal polyps; prednisone; peripheral blood mononuclear cells; systemic steroids; T regulatory cell

Chronic rhinosinusitis (CRS) imposes a significant health burden that affects 5% to 15% of the U.S. population.^{1,2} The hallmark of CRS is extensive inflammation of the sinonasal mucosa and has been divided into 2 broad categories: CRS with nasal polyposis (CRSwNP) and CRS without nasal polyposis (CRSsNP).^{1,3} Studies report a predominant T helper 1 (T_H1) polarization in CRSsNP with increased levels of interferon γ (IFN- γ) and transforming growth factor β 1 (TGF- β 1).³ In contrast, CRSwNP has typically been characterized by an eosinophilic T_H2 milieu associated with elevated levels of interleukin 5 (IL-5) and immunoglobulin E (IgE) and decreased levels of TGF- β 1.^{1,4} However, some data also show that there is an overlap in these phenotypes in both the groups.

T regulatory cells (Tregs) are known to be involved in peripheral tolerance and maintenance of normal immune homeostasis in the airways.⁵ Because of their ability to migrate to inflammatory sites and suppress immune responses via direct cellular contact and secretion of inhibitory cytokines including IL-10 and TGF- β 1, a number of studies indicate that abnormalities in numbers and/or functions of Tregs are related to autoimmune diseases⁶ and other immune disorders, including allergies,^{7,8} CRS,^{3, 9,10} and asthma.^{5,7} Although a number of markers have been identified, Tregs are most typically characterized by the presence of CD4 and CD25 surface markers and nuclear expression of the transcription factor forkhead box P3 (FoxP3).

Most studies linking CRS to Tregs demonstrate a decreased expression of FoxP3 and TGF- β 1 in sinonasal tissue samples from subjects with CRSwNP compared with CRSsNP or control subjects assessed by a variety of methods.^{3, 4,11–13} Our recent study in a severely affected, mixed ethnicity population of subjects with CRSsNP or CRSwNP, from Chicago, IL, demonstrated a lower percentage of peripheral blood (PB) Tregs from CRS subjects along with lower TGF- β 1 and IL-10 release from the peripheral blood mononuclear cells (PBMCs) when compared to controls.¹⁴ Another recent study, however, also used PB and found a significantly increased percentage of Tregs in PB of CRSsNP subjects (but not CRSwNP) when compared to controls.¹⁰

A number of pharmacological interventions aiming at the alleviation of symptoms of CRS exist. However, none have proven sufficiently effective. The only medications with demonstrated efficacy include leukotriene modulators (montelukast and zileuton), which have shown some utility in certain settings,¹⁵ and oral and topical steroids in patients with nasal polyps.^{16–18} Short courses of systemic corticosteroids are often employed in CRS, but their continuous use is associated with risk of significant comorbidity.^{15, 19,20} Both inhaled and systemic corticosteroid treatment have been shown to restore low CD4⁺CD25⁺ cell numbers, FoxP3 messenger RNA (mRNA) expression, and IL-10 production in asthma, a disease which often is associated with CRS.^{21,22} Expression of Foxp3 mRNA has been

shown to be downregulated in the affected sinonasal tissues of CRSwNP subjects, while treatment with a topical steroid enhanced the expression of Foxp3 mRNA and IL-10 and also increased Treg accumulation in nasal polyps.⁹ This study suggested that treatment with intranasal steroids attenuated the chronic inflammatory response by enhancing the expression and function of Foxp3 in the affected tissues, an important finding because intranasal steroids are a mainstay of therapy for CRS. To date, there have been a few studies on the effects of systemic steroids in this area.

In the present study, we hypothesized that systemic corticosteroids would restore the levels of Tregs in PB of CRS subjects and this would correlate with symptom improvement observed after administration of these drugs. We aimed to (1) determine the effect of systemic corticosteroids on CD4⁺CD25⁺Foxp3⁺ Treg cells in PB in CRS subjects, and (2) assess the transcript levels of cytokines and transcription factors expressed by PBMCs, before and after administration of systemic corticosteroids in these subjects.

Subjects and methods

Subjects and specimens

CRS subjects who had not taken oral and/or topical corticosteroids and any other sinonasal medications for at least 1 month prior to the study were recruited from the Otolaryngology–Head and Neck Surgery clinic at The University of Chicago Medical Center, Chicago, IL. All subjects met the diagnostic criteria of CRS,² including the presence of symptoms for >12 weeks and confirmatory nasal endoscopy and imaging. Subjects who met the treating otolaryngologist's clinical criteria for systemic steroid treatment were recruited and enrolled. Subjects received courses of oral prednisone with dose and length of treatment solely determined by the treating surgeon according to clinical judgment. Subjects answered a validated questionnaire²³ about their nasal symptoms (runny nose, stuffy nose, and sneezing); the symptoms were scored from 0 (no symptom) to 3 (severe) at each visit. The changes in nasal symptoms were used for assessing the effect of prednisone treatment in these subjects.

Paranasal sinus computed tomography (CT) scans were obtained for 18 of the 20 subjects at visit 1, prior to steroid therapy, and were scored for severity of disease by the Lund-Mackay staging system.²⁴ We excluded subjects with confounding diagnoses (cystic fibrosis, an established immunodeficiency, and allergic fungal rhinosinusitis) and pregnancy from our study.

Nineteen control subjects were recruited from the University of Chicago employees. These subjects had no evident history of CRS or asthma and had normal sinonasal examinations by anterior rhinoscopy. Information about their medical histories and nasal symptoms were also collected.

PB was drawn from all subjects by venipuncture. For CRS subjects, the blood was drawn before and after the treatment with prednisone (24 hours after the last dose), whereas for the controls it was drawn at a single time-point. Atopic status was confirmed by *in vitro* testing to a standard screening panel of representative allergens in Chicago. The study protocol was

approved by the Institutional Review Board of The University of Chicago and registered with clinicaltrials.gov (NCT01002313). Written informed consent was obtained from all subjects prior to entry into the study.

PBMC isolation

PBMCs were isolated from whole blood using Ficoll density gradient centrifugation. Briefly, whole blood was diluted 1:1 in Hanks' buffered salt solution (Invitrogen, Carlsbad, CA) and layered over a Ficoll Paque gradient (Ficoll-Paque PLUS; GE Healthcare, Waukesha, WI). The PBMCs were then washed 2 times with phosphate-buffered saline (PBS), suspended in RecoveryTM Cell Culture Freezing Medium (Invitrogen) and frozen at -80°C until further use. After storage, the PBMCs were defrosted, and the cell viability was found to be $>90\%$, as determined by Trypan blue staining.

Treg counts with flow cytometry

PBMCs (1×10^6 cells) were stained for $\text{CD4}^+\text{CD25}^+$ FoxP3⁺ cells (Tregs) with the use of a human regulatory T-cell staining kit (eBioscience Inc., San Diego, CA) as per the manufacturer's instructions. Briefly, the samples were stained for extracellular CD4 and CD25 markers by use of a cocktail of anti-human CD4–fluorescein isothiocyanate (CD4-FITC) and anti-human CD25–allophycocyanin (CD25-APC). Following fixation and permeabilization (using the fixation/permeabilization buffers included in the kit), the cells were washed and blocked for nonspecific binding sites by use of normal rat serum and then stained with anti-human FoxP3–phycoerythrin (FoxP3-PE) or rat immunoglobulin G2a (IgG2a)-PE isotype negative control. Samples were acquired using the FACS Canto flow cytometer (BD Bioscience, San Jose, CA), running DiVA acquisition software. Ten thousand gated events were acquired from each sample. Lymphoid cells were selected based on forward-scatter and side-scatter properties. $\text{CD4}^+\text{CD25}^+$ cells were selected from the lymphoid population (Fig. 1A, gray box). Of these cells, the triple-positive ($\text{CD4}^+\text{CD25}^+\text{FoxP3}^+$) cells were selected (Fig. 1B). Appropriate isotype controls for FoxP3 (Fig. 1C), CD4, and CD25 were used for setting quadrant markers. The percentage of Tregs was analyzed and compared with the use of FloJo software (Tree Star, Inc., Ashland, OR).

Real-time polymerase chain reaction

Total RNA was extracted from PBMCs using the RNeasy Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. For complementary DNA (cDNA) synthesis, 1 μg total RNA was transcribed using SuperScript[®] VILOTM cDNA Synthesis Kit (Invitrogen). The reaction was carried out at 25°C for 10 minutes, followed by 42°C for 60 minutes, and 85°C for 5 minutes. The cDNA was further diluted 1:4. Gene expression was measured in real time on ABI PRISM 7900HT thermocycler (Life Technologies Corporation, Carlsbad, CA) using the 2 $\times$ QuantiFast SYBR Green PCR Master Mix (Qiagen Inc.), QuantiTect Primer Assays (Qiagen Inc.) and 1 μl cDNA as per the manufacturer's recommendations. The polymerase chain reaction (PCR) was carried out for 40 cycles for the target genes and 25 cycles for β -actin, each cycle consisting of 10 seconds at 95°C and 30 seconds at 60°C . All PCR assays were performed in triplicate. The target genes included: the T_H2 cell inducing transcription factor GATA-binding factor 3 (GATA-3), T_H2 cytokines IL-4 and IL-13, the proinflammatory T_H1 cytokine IFN- γ , the Treg cytokines IL-10 and

TGF β 1, the Treg-associated transcription factor FoxP3, and the T_H17 cytokine IL17A. These were selected as markers of T_H1, T_H2, T_H17, and Treg function. Data were presented as relative fold expression of the target gene (FoxP3, GATA-3, IFN- γ , IL-4, IL-10, IL-13, IL-17A, and TGF- β 1) to the expression of the housekeeping gene (β -actin). Briefly, relative expression was calculated using the formula: 2^{-Ct} , where $Ct = [Ct_{(target\ gene)} - Ct_{(\beta-actin)}]$.

Statistical analysis

All data have been reported as medians with their interquartile range (IQR) unless stated otherwise. Differences between groups were analyzed by use of Mann-Whitney's U test and by Wilcoxon signed rank test. Correlation efficiencies between the CT scores of CRS subjects and PB Treg counts were calculated and the r^2 values were reported. A p value 0.05 was considered statistically significant.

Results

Subject characteristics

Twenty CRS subjects were recruited for the study. The median age of the subjects was 50 years (range, 21–66) and 60% were male. Fourteen subjects (70%) were diagnosed with nasal polyposis (CRS_{NP}) and 7 (35%) were scheduled to undergo surgery for this diagnosis. Ten CRS subjects (50%) had been diagnosed with asthma and 14 had a positive allergy test to standard screening panel. In addition to these, 19 healthy controls were also recruited. The CRS subjects and controls were similar in terms of their demographics including race, sex, and age (Table 1; $p > 0.05$, all). Six control subjects had a positive allergy test, and there were no asthmatics in this group. The total dose of prednisone ranged from 100 to 400 mg (mean, 182 mg) over a course of 5 to 12 days (mean, 8.3 days) in the CRS subjects. The duration of symptoms for CRS subjects was 16.4 ± 13.5 years (mean $\pm$ standard deviation [SD]). These subjects had not been prescribed any systemic and/or local corticosteroid within 3 months prior to recruitment in the study. Of the 20 subjects, 5 had undergone revision surgery.

Nasal symptoms

The median total nasal symptom score for the CRS subjects at visit 1 (15.5; IQR [10.5, 20.5]) was significantly higher than the controls (0; IQR [0, 2.5]; $p < 0.001$). Additionally, the CRS subjects showed significant improvements in the nasal symptoms on administration of prednisone. The median total nasal symptom score decreased from 15.5 (IQR [10.5, 20.5]) at visit 1 to 6 (IQR [4.0, 12.5]) at visit 2 ($p = 0.005$).

Treg counts in PB

PBMCs were isolated from all subjects and the percentage of Tregs was determined by flow cytometry. There were no significant differences in PB Treg counts between CRS subjects before administration of prednisone (median, 0.395%; range, 0.206% to 0.676%) and controls (median, 0.320%; range, 0.124% to 0.59%) ($p = 0.55$; Fig. 1D). However, a significant decrease in the CD4⁺CD25⁺FoxP3⁺ Tregs was observed in CRS subjects after

prednisone administration (before: median, 0.395%; range [0.206% to 0.676%]; after: 0.261%; range [0.185% to 0.434%]; $p = 0.042$) (Fig. 1E).

Correlation between the CT scores of CRS subjects and PB Treg counts

The baseline median Lund-Mackay CT score was 14 (range, 4–24) for CRS subjects. No correlation was observed between the CT scores and the percentage PB Treg cells before ($r^2 = 0.005$, $p > 0.05$) or after ($r^2 = 0.091$, $p > 0.05$) treatment with prednisone.

Expression of cytokines and transcription factors in PB

There were no significant differences in the transcript levels of FoxP3, TGF- β 1, IL-10, IL-13, IFN- γ , and IL-17A between controls and CRS subjects before corticosteroid administration (Table 2, Fig. 2; $p > 0.05$). However, the transcript levels of the T_H2 cytokine IL-4 and the T_H2 cells inducing transcription factor GATA-3 were significantly higher in the CRS subjects at their first visit when compared to controls (Table 2; $p < 0.05$). As CRSwNP has typically been associated with an increased T_H2 milieu, we divided our CRS subjects into CRSwNP ($n = 16$) and CRSsNP ($n = 6$) despite the limited power for subgroup analysis. We observed significantly higher transcript levels of IL-4 and GATA-3 in subjects with CRSwNP (median IL-4 levels: 60.02, IQR [36.69, 122.97], and median GATA-3 levels: 8.65, IQR [4.87, 21.64]) subjects when compared to the controls (median IL-4 levels: 22.26, IQR [4.24, 64.38]; and median GATA-3 levels: 4.92, IQR [1.82, 6.91]) ($p = 0.015$ and 0.05 for IL-4 and GATA-3, respectively). However, there were no significant differences in the levels of these mediators in subjects with CRSsNP compared to subjects with CRwNP and controls ($p > 0.05$; data not shown).

The administration of prednisone was associated with a significant reduction in the transcript levels of FoxP3 ($p = 0.048$; Table 2) in CRS subjects. In addition to this, the transcript levels of GATA-3 and IFN- γ also showed significant reduction post-prednisone treatment ($p < 0.05$; Table 2). However, no effect was observed on the transcript levels of TGF- β 1, IL-10, IL-4, IL-13 and IL-17A (Table 2; $p > 0.05$) in CRS subjects treated with prednisone. Similarly, no effect was observed when the subjects were stratified into CRSwNP and CRSsNP groups ($p > 0.05$; data not shown).

Discussion

Even though topical and short courses of systemic corticosteroids have been the mainstay of treatment for alleviating inflammation associated with CRS,^{17,18} the exact mechanism by which they exert their role in CRS is not fully understood. A few studies have attempted to objectively study the effect of systemic and topical corticosteroids on nasal symptoms, CT scores, the quality of life of CRS subjects posttreatment,^{17,18} and on the immune mechanisms that mediate these effects.⁹ Most of the earlier studies associate CRS with defects in T-cell function.

Because Treg cells play a pivotal role in balancing immune responses and maintaining peripheral tolerance, we undertook this study to understand the effect of corticosteroid therapy on symptoms and peripheral immune function in subjects with CRS. Short courses of systemic corticosteroids were administered to CRS subjects and the effects on symptoms

and PB Treg cells (CD4⁺CD25⁺ FoxP3⁺ cells) were assessed. Similar to earlier studies,^{16–18} we observed a significant improvement in symptoms post–prednisone administration in CRS subjects. The symptom improvement was associated with a reduction in Treg counts and FoxP3 transcripts in the PB. Additionally, there was no effect of prednisone administration on the transcript levels of the Treg cytokines, IL-10 and TGF- β 1. Thus, the efficacy of systemic corticosteroids observed post–prednisone treatment in our subjects could not be attributed to increased PB Treg number and function.

Our results gain support from a recent study that reports reduced absolute Treg numbers in PB of immunocompetent mice and human subjects after treatment with systemic corticosteroids.²⁵ Two other studies conducted in mouse models of asthma and autoimmune encephalomyelitis also report a similar reduction in the proportion of Tregs post–corticosteroid treatment.^{26,27} On the other hand, our results are in contrast with several *in vivo* studies conducted in asthmatic subjects, in subjects with autoimmune diseases including systemic lupus erythematosus, multiple sclerosis, and myasthenia gravis, and in mouse models that show a positive correlation between treatment with systemic corticosteroids and an increase in Tregs in peripheral and secondary lymphoid organs.^{6, 21, 22, 28,29} This may suggest differential effects in the PB and local tissues.

Classically, corticosteroids are thought to exert their anti-inflammatory and immunosuppressive actions by regulating the expression of genes encoding a variety of inflammatory proteins, either acting on/as transcription factors or by modulating the chromatin structure of these target genes.^{30,31} Several reports suggest that T cells, in general, are the major targets of corticosteroids, which induce their apoptosis in peripheral lymphoid organs.²⁶ In addition to inducing apoptosis, corticosteroid treatment also limits the development of IL-10 secreting dendritic cells, which in turn are required for the induction of Treg cells.²⁷ Also, corticosteroids could potentially affect the migration of Tregs; for example, inducing their migration to the affected sinuses. It has been recently reported that the migration potential of Tregs is impaired in CRSwNP subjects¹⁰ and this might explain the decreased Tregs observed in the nasal polyps from CRSwNP subjects in several studies.^{3, 4,10–13} Treatment with topical steroids was shown to enhance the accumulation of Tregs in nasal polyps of CRSwNP subjects with reduced FoxP3 levels prior to treatment.⁹ We regret that our study was not designed to evaluate Tregs in the sinus tissue of CRS subjects, something that remains for future work. However, our earlier observations in nasal biopsies of CRS subjects treated with systemic and topical corticosteroids prior to surgery showed an increased accumulation of Tregs in CRSwNP subjects.¹⁴ This highlights the differences in the effects of corticosteroids in the systemic vs the local milieu in CRS.

Naive T cells differentiate toward different T-cell subtypes on the basis of expression of transcription factors. GATA-3 is critical for commitment toward T_H2 cells and controls the expression of IL-4 and IL-5.^{3,12} Most previous studies associate CRSwNP with T_H2-skewed inflammation. Consistent with these observations, we observed significantly increased transcript levels of GATA-3 and subsequently increased IL-4 transcript levels in CRSwNP subjects when compared to CRSsNP subjects and controls.^{3, 4,12} Additionally, treatment with prednisone was associated with a significant reduction in the transcript levels of GATA-3. Corticosteroids have been shown to inhibit the induction of GATA-3 and T-bet (a

T_H1-specific transcription factor).³² Consistent with these observations, we also observed a reduction in IFN- γ levels (a T_H1 cytokine) in our CRS subjects after treatment with prednisone.

We also compared the number of Tregs in the PB of CRS subjects (before administration of corticosteroids) and normal controls. We failed to detect any significant differences in the percentage of CD4⁺CD25⁺FoxP3⁺ Tregs and in FoxP3 transcript level when comparing the CRS subjects and normal controls. This contrasts with our earlier observation, which demonstrated fewer CD4⁺CD25⁺FoxP3⁺ Tregs in the PB of CRS subjects when compared with controls.¹⁴ The discrepancy could be explained by the fact that CRS subjects in our earlier study were on systemic and/or topical corticosteroids prior to the recruitment in the study. Another possibility is the heterogeneity of disease manifestations in CRS. Consistent with our current results, a study of Chinese subjects failed to detect a difference in PB CD4⁺CD25^{high}CD127^{low} cells (defined as Tregs) in CRSwNP subjects compared with control subjects.¹⁰ These investigators also found an increased number of PB CD4⁺CD25^{high}CD127^{low} cells in the CRSsNP subjects when compared to controls, which indicated that the chronic and persistent inflammatory state associated with CRSsNP may affect the number of peripheral Tregs.¹⁰ It is, however, difficult for us to comment on the differences in the PB Treg counts between the CRSsNP subjects and CRSwNP subjects because of the small number of subjects used in our study (n = 6 for CRSsNP). Therefore, we have combined the 2 groups for all our analyses. We note that there is little evidence to date to support differences between these groups in Treg response to steroids. Because of the variation in phenotype, treatment, timing, and disease status, in addition to small sample sizes, our study does not support the use of circulating Tregs as an accessible biomarker of CRS.

Our study has several limitations. First, we analyzed the Treg counts and transcript levels of the cytokines and transcription factors only before and after the complete course of systemic steroids was administered. In addition to this, the CRS subjects recruited for the study received varying doses of prednisone, with dose and length of treatment determined by the treating physician. Further stratification of CRS subjects on the basis of dose and time of treatment divided our study population into much smaller groups and did not make any significant influence on the reported results (data not shown). We note, however, that all our subjects received high enough doses to affect PBMCs, consistent with previous reports in literature.³³ It thus remains an open question whether the effects we observed in our study are transient and could be different after shorter or longer time periods of corticosteroid treatment. Second, we used CD4, CD25, and FoxP3 as markers of Tregs. Even though there is still no consensus for the markers used for Tregs, several other markers, including CD127, activation-inducible tumor necrosis factor receptor family receptor (AITR), and cytotoxic T-lymphocyte antigen 4 (CTLA4) were subsequently reported to be useful in characterizing these cells. Third, we used PBMCs to evaluate transcript levels of Treg-associated cytokines, IL-10 and TGF- β 1, and FoxP3. Analysis of purified CD4⁺CD25⁺FoxP3⁺ Tregs for these studies might have provided more useful information on the effects on the Tregs themselves. Next, the study is underpowered with small sample sizes used for the subgroup analysis. Adding more samples to the subgroups would have strengthened our results further. Last, we can make no conclusions about the local effects in the sinonasal tissue itself due to the

nature of our design (that mimics normal clinical practice in which tissue samples are not taken after systemic steroid therapy).

Conclusion

Our results indicate a decrease in PB Tregs after treatment with prednisone with evidence of decreased T_H1 as well as T_H2 function. These data indicate that corticosteroid effects on Tregs in CRS are complex involving local signals in the tissue that differ from their effects on circulating cells.

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FIGURE 1.

Representative 2-dimensional scatter diagram of (A) CD4⁺ (FITC) CD25⁺ (APC) cells from the lymphocyte population of PBMCs analyzed in (B, C); (B) CD4⁺CD25⁺ cells stained with anti-human FoxP3 (PE) antibody (CD4⁺CD25⁺FoxP3⁺ Tregs); and (C) CD4⁺CD25⁺ cells stained with the isotype control antibody (rat IgG2a-PE); (D) percentage of CD4⁺CD25⁺FoxP3⁺ Tregs in PBMCs of healthy controls (circles) and CRS subjects (visit 1: open squares, CRSsNP subjects; filled squares, CRSwNP subjects); and (E) in PBMCs of CRS subjects (open circles: CRSsNP subjects; closed circles: CRSwNP subjects) before (visit 1) and after (visit 2) administration of prednisone. The dark bars represent the medians. APC = allophycocyanin; CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; FITC = fluorescein isothiocyanate; FoxP3 = forkhead box P3; IgG2a = immunoglobulin G2a; PBMC = peripheral blood mononuclear cell; PE = phycoerythrin; Treg = T regulatory cell.

FIGURE 2.

Transcript levels (normalized to the levels of β -actin) of (A) FoxP3, (B) IL-10, (C) TGF- β 1, (D) GATA-3, (E) IL-4, (F) IL-13, (G) IFN- γ , and (H) IL-17A from PBMCs of CRS subjects before and after administration of prednisone. CRS = chronic rhinosinusitis; FoxP3 = forkhead box P3; GATA-3 = GATA-binding factor 3; IFN- γ = interferon γ ; IL = interleukin; mRNA = messenger RNA; TGF- β 1 = transforming growth factor β 1; PBMC = peripheral blood mononuclear cell.

TABLE 1

Demographics and clinical characteristics of the subjects

	CRS^a	Controls
n	20	19
Sex (male:female)	12:8	11:8
Age, years (range)	21–66	22–64
Allergy (RAST-positive)	14/20	6/19
Asthma	10/20	–
Nasal polyposis	14/20	–
Lund-Mackay CT score, median (interquartile range)	14 (4–24)	–
Surgery required	7/20	–
Revision surgery	5/20	–

^aInformation on CRS subjects reflects the time-point before steroid treatment.

CRS = chronic rhinosinusitis; CT = computed tomography; RAST = radioallergen sorbent test.

TABLE 2

Transcript levels of cytokines and transcription factors from PBMCs^a

	Controls	CRS (before steroids)	<i>p</i> ^b	CRS (after steroids)	<i>p</i> ^b
FoxP3	42.69 (3.75, 134.18)	79.57 (28.49, 213.95)	0.13	51.04 (12.8, 160.58)	0.048
IL-10	5.88 (1.52, 16.11)	2.80 (1.60, 10.60)	0.72	6.37 (2.55, 12.33)	0.55
TGF- β 1	10.82 (6.17, 14.37)	9.13 (7.80, 14.26)	0.88	10.36 (8.86, 15.21)	0.50
GATA-3	4.93 (1.82, 6.91)	7.48 (4.70, 18.33)	0.05	5.79 (2.37, 8.52)	0.012
IL-4	22.26 (4.24, 64.38)	58.39 (31.14, 116.7)	0.019	50.05 (17.28, 70.91)	0.11
IL-13	7.45 (3.37, 31.97)	8.24 (4.63, 13.86)	0.92	9.22 (6.33, 16.33)	0.84
IFN- γ	17.01 (6.52, 37.49)	22.22 (13.87, 111.7)	0.13	22.21 (10.11, 38.5)	0.036
IL-17A	12.2 (4.73, 18.88)	11.12 (13.48, 20.46)	0.54	13.48 (5.71, 21.74)	0.62

^aValues are median (interquartile range) except where indicated.

^bSignificant *p* values are in bold.

CRS = chronic rhinosinusitis; FoxP3 = forkhead box P3; GATA-3 = GATA-binding factor 3; IFN- γ = interferon γ ; IL = interleukin; PBMC = peripheral blood mononuclear cell; TGF- β 1 = transforming growth factor β 1.