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Suppressor of cytokine signaling 3 expression is diminished in sinonasal tissues from patients with chronic rhinosinusitis with nasal polyps

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To the Editor:

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nasal and paranasal sinuses that persists for at least 12 weeks and affects up to 30 million Americans.[1] CRS is often divided into 2 clinically and phenotypically distinct classifications: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP).[2] Despite the high prevalence of this disease, little is known regarding the mechanisms that underlie its pathogenesis, thus limiting effective treatment options. While CRSwNP and CRSsNP in Caucasian patients have classically been described as T_H2- and T_H1-associated diseases, respectively, it is becoming increasingly clear that the inflammatory responses within the affected sinus tissues of these patients are complex. For example, among other mediators, we have reported elevated levels of IL-6[3] and B-cell–activating factor of the TNF family[4] as well as increased numbers of B-lineage cells[5] in CRSwNP tissue than in controls.

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Many inflammatory cytokines mediate their effects via the JAK-STAT signaling pathways. Under normal conditions, these pathways have built-in negative feedback mechanisms to prevent chronic activation of inflammatory responses. One important group of regulatory molecules is the suppressor of cytokine signaling (SOCS) proteins, which negatively regulate JAK-STAT pathways by binding to JAK proteins and inhibiting the phosphorylation of STAT proteins.[6] Of the 7 SOCS proteins, SOCS3 is one of the best characterized and it is induced by STAT3 activation.[6] SOCS3 directly binds to and inhibits JAK1, JAK2, and TYK2, but not JAK3.[6] Previous reports have suggested that SOCS3 expression is elevated in T_H2 cells and that SOCS3 may play a role in T_H2 skewing in asthma and atopic diseases.[7] A recent study by Park et al[8] from South Korea found that levels of SOCS3 and SOCS1 were elevated in CRS ethmoid tissue compared with control tissue. Although SOCS proteins are known to negatively regulate inflammatory signaling pathways, Park et al suggested that elevated levels of SOCS1 and SOCS3 might contribute to disease pathogenesis in CRS.[8] Their report has prompted us to communicate contrasting results on SOCS3 expression in CRS from our studies in Chicago.

We used Western blots and densitometry to analyze the levels of total STAT3, activated STAT3 (pSTAT3), and total SOCS3 in uncinate tissue (UT) from 5 control patients, 18 patients with CRSsNP, and 24 patients with CRSwNP as well as 31 nasal polyp (NP) samples from patients with CRSwNP (see Table E1 in this article's Online Repository at www.jacionline.org). All patients were recruited from the Allergy-Immunology and Otolaryngology Clinics of the Northwestern Medical Faculty Foundation and the Northwestern Sinus Center at the Northwestern Medical Faculty Foundation. UT and NP tissues were obtained during routine functional endoscopic sinus surgery from patients with CRS. CRS was defined by the American Academy of Otolaryngology–Head and Neck Surgery Chronic Rhinosinusitis Task Force. Patients with an aspirin-exacerbated respiratory disease, established immunodeficiency, pregnancy, coagulation disorder, classic allergic fungal sinusitis, or cystic fibrosis were excluded from the study. Control tissues were obtained from subjects without a history of sinonasal inflammation during endoscopic skull-base tumor excisions, intranasal procedures for obstructive sleep apnea, and facial fracture repairs. Asthma was physician diagnosed, and atopy was defined by positive skin-prick testing results. Two of the 31 patients with CRSwNP were on nasal steroids. All subjects provided informed consent, and the study was approved by the Institutional Review Board of Northwestern University Feinberg School of Medicine. Western blots were performed as previously described[5] by using the following primary antibodies: mouse antihuman STAT3 (1:1000; Cell Signaling Technology, Danvers, Mass), rabbit antihuman pSTAT3 (1:1000; Cell Signaling Technology), mouse antihuman SOCS3 (1:250; BioLegend, San Diego, Calif), and mouse antihuman β-actin (1:10,000; Sigma-Aldrich, St Louis, Mo). The relative density of STAT3, pSTAT3, and SOCS3 bands was normalized to β-actin. STAT3 genomic sequencing was performed as previously described.[9] Comparisons between the groups were done by using 1-way ANOVA with Tukey's adjustment for multiple comparisons, and correlations were calculated by using Spearman *r* value with GraphPad Prism v5.0b (GraphPad Software, La Jolla, Calif); *P* value of less than .05 was considered significant.

We have previously reported that despite elevated levels of the STAT3-activating cytokine IL-6 and the soluble IL-6 receptor in NPs, levels of pSTAT3 were diminished in these same tissues compared to control tissues.[3] In the current study, we first confirmed and extended these results and found that pSTAT3 levels were also diminished in UT, as well as NPs, from both CRS groups compared to control UTs ($P < .001$, Fig 1, *A*), while levels of total STAT3 were not different among the groups (Fig 1, *B*). To exclude the possibility that germline or somatic mutations in STAT3, like those associated with decreased STAT3 signaling in hyper-IgE syndrome,[9] were not the cause of the decreased pSTAT3 in these patients, the *STAT3* gene was sequenced in a subset of 18 patients with CRSwNP. No

STAT3-coding mutations were identified (data not shown). Because SOCS3 is known to inhibit STAT3 phosphorylation, we investigated whether levels of SOCS3 was elevated in CRS tissues. Surprisingly, we found that levels of SOCS3 were significantly *decreased* in CRSwNP UTand NP tissues compared to controls (P <.01 and .001, respectively, Fig 1, *C*). In fact, levels of SOCS3 were significantly positively correlated with levels of pSTAT3 in our patient samples (Spearman $r = 0.5$, $P < .001$; Fig 1, *D*). Moreover, we found no association with asthmatic or atopic status on the levels of pSTAT3 (data not shown) or SOCS3 (Fig 1, *E* and *F*) in these tissues. Because many factors may play a role in diminishing pSTAT3 levels in CRS tissues, we also investigated whether soluble gp130 or eosinophilic cationic protein might play a role, but found no correlation between these factors and pSTAT3 levels (data not shown).

Previous work has suggested that elevated expression of SOCS3 is associated with T_H2-mediated diseases.[7] Given that CRSwNP is characterized by T_H2-associated inflammation and eosinophilia, we expected to find that SOCS3 levels were elevated in CRSwNP. Our results indicate that rather than being elevated, SOCS3 levels were diminished, along with pSTAT3, in CRSwNP sinonasal tissues regardless of asthmatic or atopic status. Together with the positive correlation between pSTAT3 and SOCS3 levels in our patient samples, these data suggest that factors other than SOCS3 are involved in the reduction of pSTAT3 levels in CRS tissues. Overall, low levels of SOCS3 in diseased tissue may suggest that there is an absence of an important anti-inflammatory regulatory mechanism that may promote chronic inflammatory signaling in CRS. In addition, our data indicate that there is a baseline level of pSTAT3 in control tissue that may be important for the maintenance of tissue homeostasis. Interestingly, however, our results are in direct contrast with the results of the study by Park et al.[8] It is worth noting that the study by Park et al did not include analysis of NP tissue, nor did it include any patients with asthma or atopy.[8] Similarly, while we have previously reported elevated expression of IL-6 in NP tissue,[3] Park et al[8] found no difference in IL-6 expression in their samples. One possible explanation for this discrepancy may be the use of ethmoid tissue by Park et al versus UT and NP in our studies. We have found that gene expression patterns vary widely within the sinus mucosa,[10] and it is possible that UT and ethmoid differ. Alternatively, although the study by Park et al found that the level of IL-13 was elevated in CRSwNP and the level of IFN-γ was elevated in CRSsNP, in line with phenotypes of Caucasian populations, it is possible that the discrepancy between our results could be due to differences in CRS phenotypes that have been previously noted between CRS in Asian and Caucasian populations.[11] Taken together, however, these data suggest that tissue sampling and patient demographics may be important factors to consider when comparing results of similar studies.

Our current work may provide a valuable insight into the mechanisms that drive chronic inflammation in CRSwNP, and ongoing studies are focused on further elucidating these mechanisms. A better understanding of CRS pathogenesis will hopefully uncover strategies for the development of improved therapeutic strategies for patients suffering from CRS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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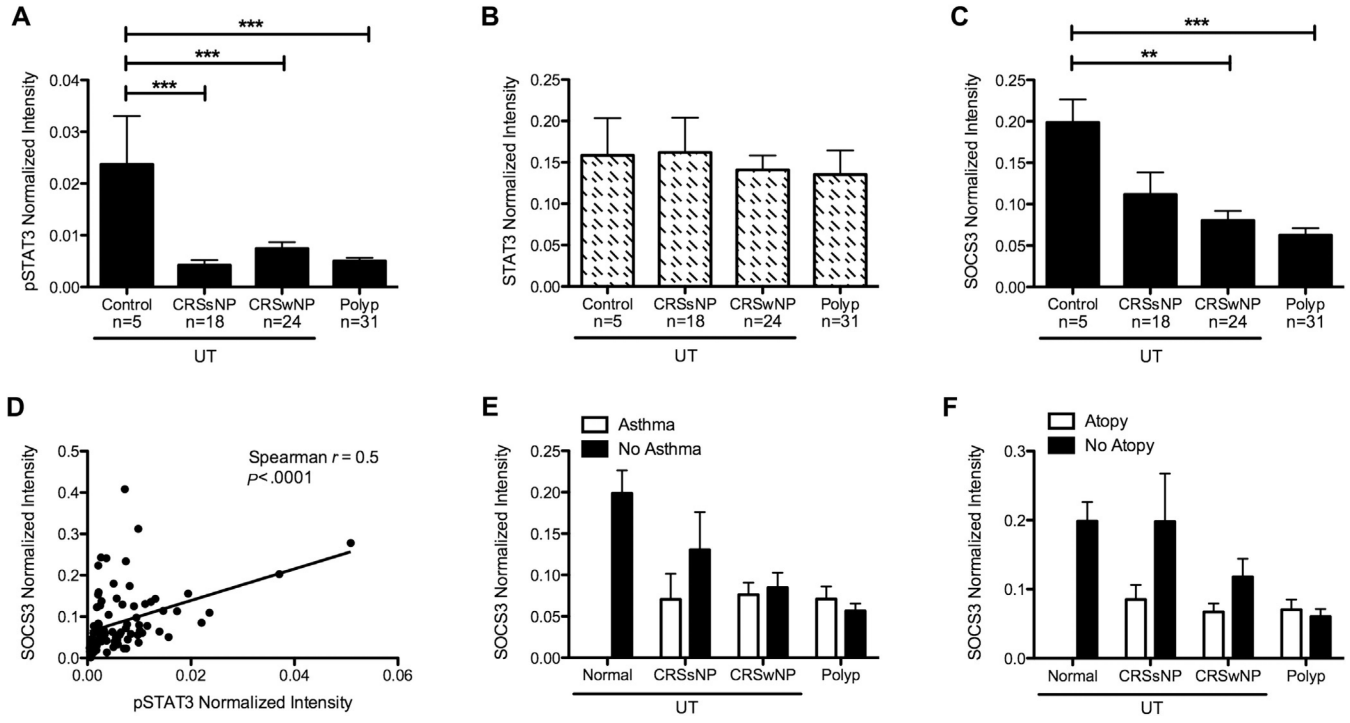

FIG 1.

pSTAT3 and SOCS3 are diminished in CRSwNP. Protein from UT or NP tissue was analyzed by using Western blotting and normalized to β-actin for pSTAT3 (**A**), total STAT3 (**B**), and SOCS3 (**C**). **D**, Correlation of pSTAT3 and SOCS3. Effect of asthmatic status (**E**) or atopic status (**F**) on SOCS3 levels. Data in Fig 1, *A*, *B*, *C*, *E*, and *F* represent mean ± SEM; $^{**}P < .01$ and $^{***}P < .001$ by 1-way ANOVA with Tukey's adjustment.