ORIGINAL CONTRIBUTION

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Chronic sinusitis and rhinitis: Clinical terminology "Chronic Rhinosinusitis" further supported*

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SUMMARY

Background: *Chronic rhinosinusitis (CRS) is a heterogeneous group of inflammatory diseases of the nasal and paranasal cavities, either accompanied by polyp formation (CRSwNP) or without polyps (CRSsNP). The etiology and pathophysiology is unknown; some specialists define rhinitis and sinusitis as one clinical entity, whereas others regard both as separate diseases. We therefore investigated the immunological background of the chronically inflamed nasal and sinusal mucosa.*

Methods: *Protein levels of the inflammatory mediators IL1-β, IL-5, ECP, INF-γ, and TGF-β were assessed in ethmoidal mucosal samples from CRSsNP and CRSwNP patients, and compared with the expression profile in nasal inferior turbinate (IT) mucosa of the same patient groups. Nasal tissue of patients without disease served as control.*

Results: *In CRSwNP, increased levels of IL-5 and ECP were not only observed in ethmoidal samples, but also in the nasal IT-mucosa. TGF-β levels were lower in polyps, reaching significance versus ethmoidal mucosa of CRSsNP. In CRSsNP, INF-γ levels are up-regulated in both ethmoidal and nasal mucosa compared to control tissue.*

Conclusion: *On the basis of similar inflammatory mediator profiles, we conclude that rhinitis accompanies chronic sinusitis, supporting the consensus term "rhinosinusitis". On the other hand, CRSsNP and CRSwNP should be regarded as distinct clinical entities.*

Key words: rhinitis, sinusitis, chronic rhinosinusitis, nasal polyps, inflammatory mediators

INTRODUCTION

Although often mistakenly regarded as trivial, inflammation of the nasal (rhinitis) and sinus mucosa (sinusitis) are prevalent [1] medical conditions of the upper airways associated with a substantial impaired quality-of-life [2], reduced workplace productivity, and serious medical treatment costs [3].

Although rhinitis may present without sinusitis (e.g. allergic rhinitis), there is consensus among specialists that acute and chronic sinusitis are usually associated to rhinitis [4-8]. Indeed, rhinitis and sinusitis often clinically coexist and are concurrent in many patients. According to the European Position Paper on Rhinosinusitis and Nasal Polyps (EP3OS)[5,8] and previous guidelines and consensus documents [4,6,7,9], the correct terminology is therefore "rhinosinusitis". Although being a valuable guideline to ENT specialists, general practitioners, and epidemiologists, the clinical and epidemiological definitions that relate rhinitis and sinusitis in these documents [5,8] are not yet confirmed by solid research evidence. As a consequence, there is still controversy over the use of the terms sinusitis and rhinosinusitis [6,7,10]. From a research point of view, the anatomical continuum of the nasal mucosa with the sinuses and the fact that it is clinically often difficult to differentially diagnose rhinitis and sinusitis, is indeed not a proof for a similar etiology and pathophysiology of both disease entities. Furthermore, the U.S. Food and Drug Administration (FDA) considers rhinitis and sinusitis as distinct disease entities with differences in pathophysiology, treatment, and risk-benefit assessment for drug development (Guidance for Industry: Sinusitis: Designing Clinical Development Programs of Nonantimicrobial Drugs for Treatment. Available at http://www.fda.gov/Cder/guidance/index.htm, accessed February 2009). This has major impact on the appropriate structure of clinical studies designed to grant introduction of new compounds to the market [7].

Moreover, according to the EP3OS guidelines, CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP) should, for research purposes, also be regarded as one entity with CRSsNP as the major finding and CRSwNP being a subgroup. However, the question then remains why polypoid tissue does develop in CRSwNP patients and not in all CRS patients. A part of the answer might be given by recent

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research evidence demonstrating that these pathologies can be immunologically separated into distinct groups on the basis of the expression of inflammatory and remodeling mediators [11-13]. Based on these findings, key mediators to differentiate CRS include interferon (IFN)-γ and transforming growth factor (TGF)-β1, being up-regulated in CRSsNP, while CRSwNP typically shows increased eosinophil cationic protein (ECP) and interleukin (IL)-5 and low TGF-β1 levels.

As the current consensus term "rhinosinusitis" is not intended to describe or suggest an underlying etiology, but just fits most accurately the anatomical, histological, and clinical presentation of the condition(s) to be defined [5], it is not excluded that the pathophysiological background of both diseases might be distinct, as postulated in the FDA's guidance document, and as is also reported to be the case for CRSsNP and CRSwNP [11-13]. In this study we therefore aimed to investigate the immunological background of the inflamed nasal and sinus mucosa in chronically inflamed upper airways. To do so, we assessed the expression pattern of several key inflammatory mediators in ethmoidal mucosa or polyp tissue from CRSsNP and CRSwNP patients respectively, in comparison to the expression profile in nasal mucosa (inferior turbinates; IT) of the same patient groups. IT's of an additional group of patients without disease to the upper and lower airways was incorporated for baseline measurements.

MATERIALS AND METHODS

Patients and sinonasal tissues

Tissues were obtained at the Department of Otorhinolaryngology, Ghent University Hospital, Belgium during routine endonasal sinus surgery and approved by the local ethical committee. All patients gave their written informed consent before collecting material. All patients stopped oral and topical application of corticosteroids for at least 1 month before surgery.

The diagnosis of chronic sinus disease was based on a documented medical history, clinical examination, nasal endoscopy, and computed tomography of the paranasal cavities according to the current European [5,8] and American Guidelines [6]. Shortly, CRSsNP and CRSwNP were clinically defined as inflammation of the nose and the paranasal sinuses characterized by two or more symptoms, one of which: 1) nasal congestion or nasal discharge, potentially accompanied by facial pain and reduction of smell, and 2) either endoscopic signs of polyps and/or mucopurulent discharge primarily from middle meatus, and/or oedema primarily in middle meatus, and/or CT changes showing mucosal changes within the ostiomeatal complex and/or sinuses. The rhinosinusitis with more than 12 weeks of symptoms, with no complete resolution of symptoms, was defined as being chronic. Clinical data of the patients are summarized in Table 1.

The tissues collected during surgery were immediately transported to the laboratory, snap-frozen in liquid nitrogen, and stored at –80°C until homogenization.

Tissue homogenates

Snap-frozen tissue specimens were weighed and suspended in a 10 times volume of 0.9% NaCl solution with protease inhibitor Complete (Roche, Mannheim, Germany). The tissue was homogenized with a mechanical homogenizer (B. Braun, Melsungen, Germany) at 1000 rpm for 5 minutes on ice. The homogenates were centrifuged at 3000 rpm for 10 minutes at 4°C, and the supernatants were stored at –20°C until analysis.

Tissue levels of the inflammatory mediators IL1-β, IL-5, ECP, INF-γ, and TGF-β

Tissue homogenates were assayed for IL1-β and IL-5 levels by means of Luminex xMAP technology using the Fluorokine MAP Multiplex Human Cytokine Panel A kit (R&D Systems, Minneapolis, Minn, USA) on a Bio-PlexTM 200 Array Reader (Bio-Rad, Hercules, CA, USA).

Total TGF-β1 and IFN-γ were measured by using commercially available ELISA kits (R&D Systems). For TGF-β, acid was added during the ELISA procedure, resulting in physicochemical activation of latent TGF-β. Total TGF-β concentrations are reported including both active and latent forms. ECP levels were measured by the UNICAP system (Pharmacia, Uppsala, Sweden). All data were expressed as picograms per gram tissue.

Statistical analysis

All results were expressed as Box-and-Whisker plots representing medians and interquartile ranges; n refers to tissues obtained from different patients. Statistical analysis was performed by a nonparametric Kruskal-Wallis test, taking the data of inferior turbinates from controls, CRSsNP and CRSwNP in one analysis, and of inferior turbinates from control, ethmoidal mucosa from CRSsNP, and polyps from CRSwNP in another analysis. Kruskal-Wallis was accompanied by a Dunns'

Table 1: Patients' clinical data.

	control (IT)	CRSsNP		CRSwNP	
		IT	ethmoidal mucosa	IT	ethmoidal polyps
Number of subjects	18	15	19	11	22
Median age (range; y)	27.6 (17.6-59.6)	39.7 (18.2-61.8)	39.0 (20.0-81.5)	41.3 (18.1-53.2)	48.4 (18.7-71.4)
Gender (male/female)	12/6	7/8	9/10	7/4	13/9
Athma in history	2	1	0	3	5
Skin prik test-positive	3	2	2	4	7
Aspirin intolerance reported	0	1	0	0	2

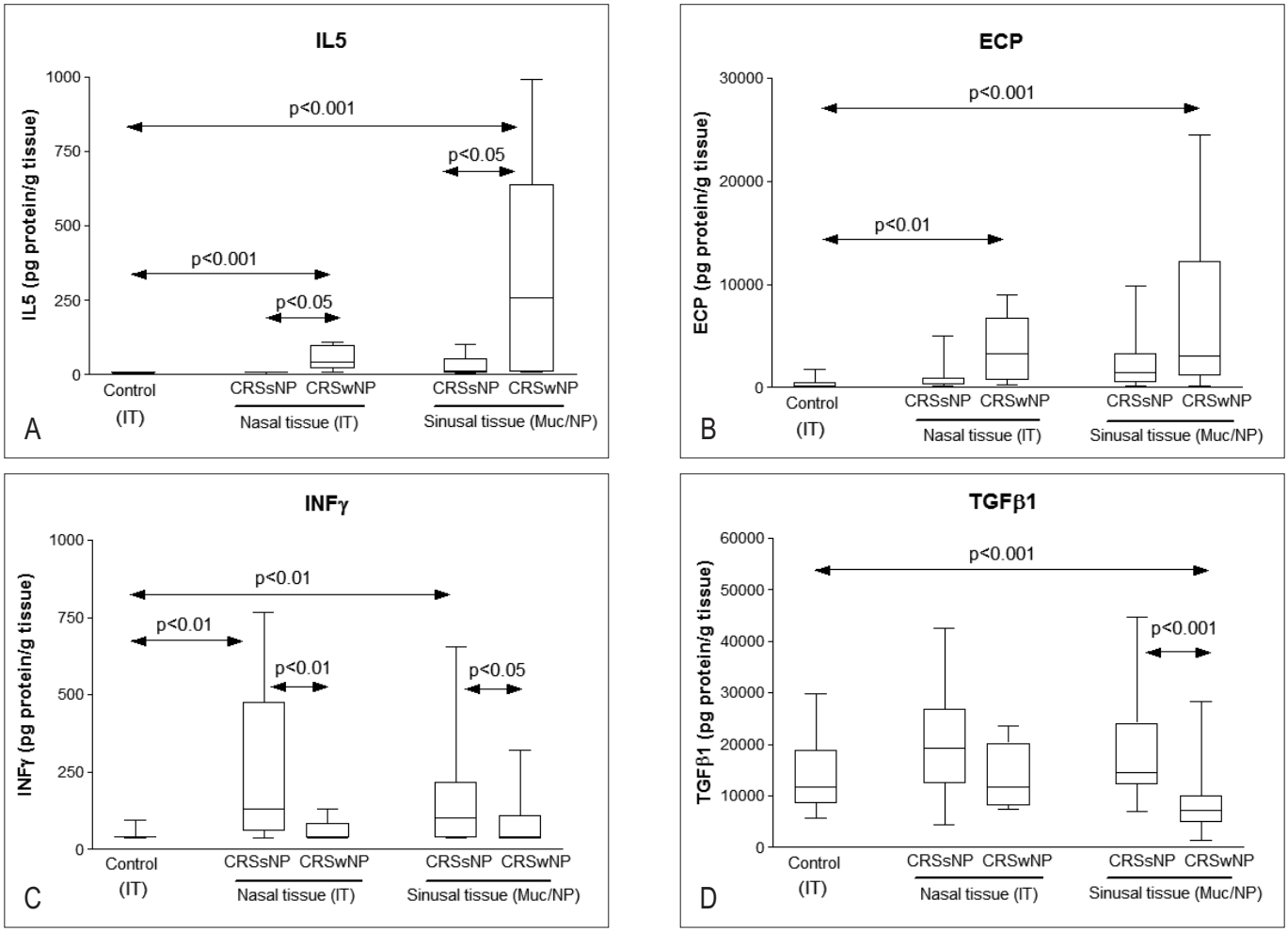

Figure 1. Quantitative tissue protein levels of key inflammatory mediators. Values are expressed as Box-and-Whisker plots showing the median, the lower and the upper quartile, and the minimum and the maximum value. CRSsNP: chronic rhinosinusitis without nasal polyps, CRSwNP: chronic rhinosinusitis with nasal polyps, ECP: eosinophil cationic protein, IFN-γ: interferon-γ, IL-5: interleukin-5, IT: inferior turbinate, NP: nasal polyps of ethmoidal region, Muc: mucosa of ethmoidal region, TGF-β1: transforming growth factor β1.

Multiple Comparison Test as a Post Test between all subgroups within each analysis. A p-value less than or equal to 0.05 was considered to be statistically significant (Graphpad, San Diego, CA, USA).

RESULTS

To assess the immunological background of rhinitis and sinusitis, and to evaluate the validity of nasal inferior turbinates as control tissues in the research to sinus diseases, the expression pattern of several key inflammatory mediators in ethmoidal mucosa from CRSsNP or polyp tissue from CRSwNP patients was measured and compared to the expression profile in the nasal mucosa of the same patient groups. The nasal mucosa of disease-free patients undergoing surgery for anatomical abnormalities was used for baseline measurements. The key inflammatory mediators, including the proinflammatory cytokine IL-1β, the eosinophilic granule protein ECP, Th1 and Th2-cell subset cytokines IFN-γ and IL-5 respectively, and the T-regulatory and remodeling marker TGF-β1, were measured in homogenized ethmoidal mucosal samples and inferior turbinates (IT).

Protein levels of the Th2 cytokine IL-5 and the eosinophilic marker ECP were significantly higher in ethmoidal samples ($p < 0.001$ for and ECP) and IT ($p < 0.001$ for IL-5; $p < 0.01$ for ECP) of CRSwNP compared to control IT, while the levels of both inflammatory markers were not significantly higher in CRSsNP (Figure 1A and 1B). In both ethmoidal tissue and IT, IL-5 and ECP levels were higher in CRSwNP compared to CRSsNP, reaching statistical significance for IL-5 (Figure 1A) but not for ECP (Figure 1B).

In CRSsNP, a clear-cut increased expression of the Th1 cytokine INF-γ was observed in both ethmoidal mucosa ($p < 0.01$) and IT ($p < 0.01$) versus control. INF-γ levels were significantly higher in CRSsNP compared to CRSwNP for both IT ($p < 0.01$) and ethmoidal tissue ($p < 0.05$), (Figure 1C).

The levels of the T-regulatory and remodeling marker TGF-β1 in IT from CRSsNP and CRSwNP and in ethmoidal mucosa from CRSsNP were similar to the levels measured in IT from controls (Figure 1D). However, TGF-β1 showed a sig-

nificantly lower expression level in ethmoidal tissue from CRSwNP compared to IT from controls ($p < 0.05$) and ethmoidal mucosa from CRSsNP ($p < 0.001$) (Figure 1D).
For IL1-β, no significant differences were observed among all groups (data not shown).

DISCUSSION

In this study we report that the distinct cytokine patterns found in sinusal tissue of CRSwNP and CRSsNP are reflected in the nasal inferior turbinate mucosa of these respective patient groups. These data suggest that chronic rhinitis and sinusitis might be regarded as one disease entity, supporting the use of the clinical term chronic 'rhinosinusitis'. The data also confirm our previous observation that within the rhinosinusitis group, CRSsNP and CRSwNP should be regarded as distinct clinical entities. Furthermore, the implication of the nasal mucosa in the disease process of sinusitis has some repercussion on the choice of the appropriate control tissue in research protocols.
Although rhinitis may present without sinusitis, both acute and chronic sinusitis are usually (except in dental maxillary sinusitis and other specific cases) associated to rhinitis. Therefore, various expert panels, task forces, and position papers proposed the integrated clinical term rhinosinusitis over the separate disease entities rhinitis and sinusitis. Indeed, from an anatomical and histological point of view, it seems unlikely to think of an isolated ethmoid sinus disease whereby the middle or superior turbinate mucosa is not involved [4-8]. However, this argumentation purely speculates on anatomical characteristics, clinical features, and histological similarities, and does not incorporate the pathophysiological mechanism involved in the disease status. It furthermore also does not take into account the different immunological features of CRSsNP and CRSwNP, and has not yet been fully supported by basic research evidence. After all, the exact pathogenesis and etiology of CRSsNP and CRSwNP still remain unknown.
In case chronic sinusitis is accompanied by rhinitis, pro-inflammatory parameters should be increased in both the sinus and nasal mucosa. Depending on the etiology of, and the catalyst relationship between rhinitis and sinusitis, the inflammatory cytokine pattern might be similar or distinct between the ethmoidal and nasal regions. In Caucasians, we previously showed that CRSwNP is typically associated with a Th2 skewed eosinophilic inflammation with high IL-5 and ECP concentrations in the ethmoidal mucosa, while CRSsNP is characterized by a Th1 milieu with increased levels of INF-γ. CRSwNP is furthermore accompanied by reduced levels of TGF-β, while CRSsNP shows increased TGF-β production [11-13].
IL-5 is known to activate eosinophils, to prolong eosinophil survival, and to promote the release of eosinophil granules [14]. In a previous study of our group [15], a strong correlation was found between the eosinophil activation marker ECP and IL-5 levels in ethmoidal mucosal tissue. Here we show that the increased levels of ECP and IL-5 in CRSwNP are also reflected in the inferior turbinate mucosa. TGF-β1 is implicated in extracellular matrix formation [16]. Consequently, low levels of TGF-β1 have been associated with edema formation in polyps [13]. The absence of edema in inferior turbinates from CRSwNP and CRSsNP, and ethmoidal mucosa from CRSsNP is in agreement with the control levels of TGF-β1 found in these tissues, while the TGF-β1 levels in mucosal tissue of CRSwNP were indeed significantly lower compared to ethmoidal mucosa of CRSsNP and IT from controls. IFN-γ is the hallmark cytokine of Th1 cells. It regulates a variety of physiological and cellular responses such as an increase in antigen presentation by macrophages and the suppression of Th2 cell activity. The Th1-nature of CRSsNP was confirmed by significantly higher levels of IFN-γ in CRSsNP ethmoidal mucosa and inferior turbinates vs tissue from controls and CRSwNP.
These data show that the distinct cytokine patterns found in the nasal mucosa of CRSwNP and CRSsNP patients are a reflection of the cytokine profile in the sinusal tissue of the respective patient groups, supporting the idea that chronic rhinitis and sinusitis can be regarded as one disease entity. These findings potentially have significant impact on the knowledge on the etiology and the clinical understanding of chronic nasal and paranasal diseases as well as implications for diagnosis and research perspectives on future drug development [7]. At present, the treatment options for rhinosinusitis are limited but there is interest within the pharmaceutical industry in the development of new drugs other than antibiotics for the treatment of rhinosinusitis. It is therefore of utmost importance that researchers can rely on a solid definition of the disease entity based on knowledge on the etiology. However, a major concern in regarding rhinitis and sinusitis as one disease entity is that it could hamper disease classification and drug development because the symptoms of these two diseases overlap, and treatment approaches would necessarily have to approach both nasal and sinusal symptoms. This rationale presumes a different etiology and/or pathophysiology in both nasal and paranasal inflammation. Up to date, such evidence is however lacking. It formerly has been demonstrated for allergic and/or viral rhinitis that the sinuses are involved in nasal disease [17-19].
Here we show, besides the fact that the nasal mucosa is involved in the sinusal disease status, that the pathophysilogical background of the inflamed nasal and paranasal regions is not different as indicated by the similar cytokine patterns in nasal and ethmoidal samples within either the CRSsNP or CRSwNP entity.
Further evidence for the involvement of the nose in sinus disease comes from observations on the middle turbinate in CRSwNP. Chen et al. [20] reported that IL-5 mRNA levels in CRSwNP were elevated the highest in the anterior ethmoidal mucosa, but also found an up-regulation in the lateral and medial parts of the middle turbinate (MT). The involvement of the MT in the disease process is not surprising as from a clini-

cal point of view, the middle turbinates in CRSwNP are commonly involved in the inflammatory process, also showing polypoidal changes. Here we found that the protein levels of IL-5 in IT of CRSwNP subjects are lower than in the ethmoidal samples, but they are still significantly higher than in IT from non-diseased patients, demonstrating their involvement in the disease. Similarly, in a study by Eweiss et al. [21] the number of eosinophils in the polyp tissue was significantly higher than the number in the IT mucosa from the same patients; again, eosinophil numbers were significantly higher than in IT from controls.

The involvement of the nasal turbinates in the sinus disease process can also be placed in the discussion of which tissue type could be regarded as a valid reference for control measurements. From the above we conclude that IT from healthy control patients, but not from CRS patients, can serve as reliable tissue for comparative measurements of inflammatory mediators in research setups related to upper airways diseases.

CONCLUSION

In conclusion, our data illustrate that the inflammatory mediator profile in nasal mucosa follows the profile found in ethmoidal mucosa from CRSsNP and CRWwNP patients, respectively. These data indicate that rhinitis and sinusitis can be regarded as one disease entity, supporting the use of the term 'rhinosinusitis' as proposed before (EPOS, Am guidelines). We confirm that CRSsNP and CRSwNP should be viewed as distinct disease entities.

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REFERENCES

1. Collins JG. Prevalence of selected chronic conditions: United States, 1990-1992. Vital Health Stat. 1997; 194: 1-89.
2. Alobid I, Bernal-Sprekelsen M, Mullol J. Chronic rhinosinusitis and nasal polyps: the role of generic and specific questionnaires on assessing its impact on patient's quality of life. Allergy 2008; 63: 1267-1279.
3. Anand VK. Epidemiology and economic impact of rhinosinusitis. Ann Otol Rhinol Laryngol Suppl 2004; 193: 3-5.
4. Anon JB, Jacobs MR, Poole MD, et al. Antimicrobial treatment guidelines for acute bacterial rhinosinusitis. Otolaryngol Head Neck Surg 2004; 130 (1 Suppl): 1-45.
5. Fokkens W, Lund V, Mullol J, et al. European position paper on rhinosinusitis and nasal polyps 2007. Rhinol Suppl 2007; (20): 1-136.
6. Meltzer EO, Hamilos DL, Hadley JA, et al. Rhinosinusitis: establishing definitions for clinical research and patient care. J Allergy Clin Immunol 2004; 114 (6 Suppl): 155-212.
7. Meltzer EO, Hamilos DL, Hadley JA, et al. Rhinosinusitis: developing guidance for clinical trials. J Allergy Clin Immunol 2006; 118 (5 Suppl): S17-S61.
8. Thomas M, Yawn BP, Price D, Lund V, Mullol J, Fokkens W. EPOS Primary Care Guidelines: European Position Paper on the Primary Care Diagnosis and Management of Rhinosinusitis and Nasal Polyps 2. Prim Care Respir J 2008; 17: 79-89.
9. Report of the Rhinosinusitis Task Force Committee Meeting. Alexandria, Virginia, August 17, 1996. Otolaryngol Head Neck Surg 1997; 117: S1-68.
10. Wallace DV, Dykewicz MS, Bernstein DI, et al. The diagnosis and management of rhinitis: an updated practice parameter. J Allergy Clin Immunol 2008; 122: S1-84.
11. Bachert C, Wagenmann M, Hauser U, Rudack C. IL-5 synthesis is upregulated in human nasal polyp tissue. J Allergy Clin Immunol 1997; 99: 837-842.
12. Van Bruaene N, Perez-Novo CA, Basinski TM, et al. T-cell regulation in chronic paranasal sinus disease. J Allergy Clin Immunol 2008; 121: 1435-1441.
13. Van Zele T, Claeys S, Gevaert P, et al. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. Allergy 2006; 61: 1280-1289.
14. Rothenberg ME, Hogan SP. The eosinophil. Ann Rev Immunol 2006; 24: 147-174.
15. Bachert C, Gevaert P, Holtappels G, Johansson SGO, van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. J Allergy Clin Immunol 2001; 107: 607-614.
16. Wells RG, Discher DE. Matrix elasticity, cytoskeletal tension, and TGF-beta: the insoluble and soluble meet. Sci Signal 2008; 1: e13.
17. Gwaltney JM, Jr., Phillips CD, Miller RD, Riker DK. Computed tomographic study of the common cold. N Engl J Med 1994; 330: 25-30.
18. Alho OP. Nasal airflow, mucociliary clearance, and sinus functioning during viral colds: effects of allergic rhinitis and susceptibility to recurrent sinusitis. Am J Rhinol 2004; 18: 349-355.
19. Berrettini S, Carabelli A, Sellari-Franceschini S, et al. Perennial allergic rhinitis and chronic sinusitis: correlation with rhinologic risk factors. Allergy 1999; 54: 242-248.
20. Chen YS, Arab SF, Westhofen M, Lorenzen J. Expression of interleukin-5, interleukin-8, and interleukin-10 mRNA in the osteomeatal complex in nasal polyposis. Am J Rhinol 2005; 19: 117-123.
21. Eweiss A, Dogheim Y, Hassab M, Tayel H, Hammad Z. VCAM-1 and eosinophilia in diffuse sino-nasal polyps. Eur Arch Otorhinolaryngol 2009; 266: 377-383.

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