Mucin 1 downregulation associates with corticosteroid resistance in chronic rhinosinusitis with nasal polyps

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Background: A number of patients with chronic rhinosinusitis with nasal polyps (CRSwNP) are resistant to oral corticosteroids. Mucin 1 (MUC1) shows anti-inflammatory properties, and its cytoplasmic tail (CT) interacts with transcription factors, facilitating their nuclear translocation. Because glucocorticoid receptor (GR) nuclear translocation is key to the anti-inflammatory effect of corticosteroids, we hypothesized that MUC1 is involved in the effectiveness of corticosteroids.
Objective: To analyze the role of MUC1 in corticosteroid effectiveness in different cohorts of patients with CRSwNP and elucidate the possible mechanisms involved.
Methods: Seventy-three patients with CRSwNP took oral corticosteroids for 15 days. Corticosteroid resistance was evaluated by nasal endoscopy. The expression of MUC1 and MUC1 CT was evaluated by real-time PCR, Western blotting, and immunohistochemistry. Beas-2B knockdown with RNA interference for MUC1 (siRNA-MUC1) was used to analyze the role of MUC1 in the anti-inflammatory effects of dexamethasone.
Results: Nineteen patients had nasal polyps that were resistant to oral corticosteroids (NP-CR). MUC1 expression was downregulated in these patients. Primary epithelial cells from patients with NP-CR were insensitive to the anti-inflammatory effects of dexamethasone. In siRNA-MUC1 Beas-2B, dexamethasone showed weaker anti-inflammatory effects, a reduced inhibition of phospho-extracellular-signal-regulated kinases 1/2, a less severe mitogen-activated protein kinase phosphatase 1 increase, and a reduced GR nuclear translocation. Immunoprecipitation experiments revealed that MUC1-CT and GRα form protein complexes and translocate to the nucleus in response to dexamethasone. MUC1-CT–GRα complex was downregulated in NP-CR tissue.
Conclusion: MUC1-CT participates in the corticosteroid response that mediates GRα nuclear translocation. The low expression of MUC1 in patients with CRSwNP may participate in corticosteroid resistance. (J Allergy Clin Immunol 2015;135:470-6.)

Key words: *MUC1, corticosteroid resistance, nasal polyp, chronic rhinosinusitis, glucocorticoid receptor*

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a disease of the sinuses characterized by mucosal thickening and polyp formation. CRSwNP is present in approximately 10% to 15% of patients with asthma and more than 90% of patients with aspirin-intolerant asthma.[1] The etiology of nasal polyposis is currently unknown. However, 80% of white patients have major eosinophilic inflammation with a T_H2 cytokine profile, and bacterial colonization and superantigens might modulate the disease severity.[1]

Corticosteroids show strong anti-inflammatory activity and are the first-line therapy for patients with CRSwNP. Placebo-controlled studies have shown that topical corticosteroid therapy reduces polyp size,[2,3] nasal symptom severity,[4] and the incidence of recurrence after polypectomy.[5,6] However, topical corticosteroid therapy is not effective in all patients, leading to the use of systemic corticosteroids. Significant reductions in symptom severity and polyp size were demonstrated in a study involving a 2-week course of oral corticosteroids.[7] Thus, oral corticosteroids are now accepted as an effective therapy for the treatment of severe CRSwNP. However, a number of patients show resistance to the effects of systemic corticosteroids and must undergo sinus surgery to control the disease.

The epithelial mucus layer represents a protective barrier against pathogens and irritants. Mucins are involved in this protective function by participating in mucociliary clearance. Different roles have been described for mucins depending on whether they are secreted or membrane-bound. Mucin 1 (MUC1), which is a membrane-bound mucin, acts as a sensor receptor and participates in cellular signaling.[8] MUC1 has recently emerged as an anti-inflammatory molecule that inhibits bacteria- and virus-induced inflammation in airways.[9-11] The anti-inflammatory role of MUC1 emanates from its ability to inhibit the activation of several toll-like receptors (TLR2-9) by bacteria and viruses.[9-12]

The MUC1 protein comprises 2 polypeptide subunits: an N-terminal extracellular subunit and a C-terminal subunit. A highly conserved cytoplasmic tail (CT) in the C-terminal subunit modulates multiple intracellular signals.[13] The 72-amino-acid MUC1-CT contains 7 tyrosine residues, the phosphorylation status of which has been associated with intracellular signal transduction in cancer cells as well as with the inhibition of TLR5-MyD88 assembly and consequent inflammation in airway

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Abbreviations used
CRSwNP: Chronic rhinosinusitis with nasal polyps
CT: Cytoplasmic tail
ERK1/2: Extracellular-signal-regulated kinases 1/2
GR: Glucocorticoid receptor
hsp: Heat-shock protein
MKP1: Mitogen-activated protein kinase phosphatase 1
MIF: Macrophage migration inhibitory factor
NP: Nasal polyps
NP-AIA: Nasal polyps with aspirin-intolerant asthma
NP-ATA: Nasal polyps with aspirin-tolerant asthma
NP-CR: Nasal polyps resistant to oral corticosteroids
NPwA: Nasal polyps without asthma or aspirin intolerance
PGN: Peptidoglycan
TLR: Toll-like receptor

epithelial cells.[14,15] The interaction of MUC1-CT with signal transducers and its nuclear translocation and subsequent biological responses are believed to be regulated by specific protein-protein interactions, but the precise mechanisms by which this occurs remain poorly understood. It has been demonstrated that MUC1-CT may interact with estrogen receptor (ER)α by being translocated to the nucleus as a MUC1-CT–ERα transcription complex in response to 17β-estradiol.[16] Similar findings were observed for the β-catenin–MUC1-CT transcription complex as a modulator of morphogenesis.[17,18]

Corticosteroids exert their anti-inflammatory functions by binding to their intracellular receptor, the glucocorticoid receptor (GR), which is a ligand-inducible transcription factor. In the absence of its ligand, GR resides predominantly in the cytoplasm, where it is sequestered in a multimeric chaperone complex comprising heat-shock protein (hsp) 90, hsp70, hsp90-binding protein p23, immunophilins, and other factors to prevent its degradation and assist in its maturation.[19] Upon ligand binding, the GR complex translocates to the nucleus with the help of a number of proteins, such as nuclear localization signals and importins, where it exerts its anti-inflammatory effects.[19]

Recent evidence showed that corticosteroids increased MUC1 expression *in vitro*[20,21] as well as in human NP epithelium following a 2-week course of oral corticosteroids.[22] However, the association of oral corticosteroid efficacy with MUC1 expression as well as the possible interactions among corticosteroids, GR, and MUC1 are currently unknown. Interestingly, recent evidence has indicated that MUC1-CT binds to hsp90 to form protein complexes.[23] Because hsp90 participates in GR stability and nuclear translocation, MUC1-CT could also mediate GR activity.

Based on the findings in previous reports, we hypothesized that MUC1 may control corticosteroid efficacy and GR signaling. We thus investigated the expression of MUC1 in patients with CRSwNP that did and did not respond to oral corticosteroid therapy. Using NP tissue, NP epithelial cell cultures, and *in vitro* mechanistic analysis, we evaluated the link between MUC1-CT and the anti-inflammatory efficacy of corticosteroids.

METHODS

Study design, experimental protocols, and statistical analysis are provided in the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

Expression and distribution of TLRs and MUC1 are altered in patients with NP-CR

In this study, 73 patients with CRSwNP and no response to intranasal corticosteroids after 3 months were recruited to initiate a 15-day course of oral corticosteroids. At the end of oral therapy, 19 patients were classified as having NP-CR because their NP endoscopic score was not reduced by more than 1. In total, 7, 2, and 10 of the 19 patients with NP-CR had the NP without asthma or aspirin intolerance (NPwA), NP with aspirin-tolerant asthma (NP-ATA), and NP with aspirin-intolerant asthma (NP-AIA) phenotypes, respectively. The clinical characteristics of all patients before and after oral corticosteroid therapy are defined in Table E2 (in the Online Repository at www.jacionline.org), and the characteristics of the patients with NP-CR are shown in Table E3 (in the Online Repository at www.jacionline.org).

The TLR family plays a key role in pathogen recognition and induction/regulation of the innate and adaptive immune responses in the NP epithelium.[24] In this study, we observed deregulation of TLR expression in the NP epithelium in the different patient groups (see Fig E1 in this article's Online Repository at www.jacionline.org). In fact, TLR2 showed weak expression in the NPwA, NP-ATA, and NP-AIA groups before and after oral corticosteroid treatment, while it was significantly increased and distributed widely on the NP epithelium in the NP-CR group. In contrast, TLR4 did not show significant differences among the groups and was distributed homogeneously throughout the NP epithelium in all groups before and after treatment. TLR5 was expressed mainly in basal epithelial cells of the NPwA group and was strongly expressed throughout the whole NP epithelium in the NP-CR group and, to a lesser extent, in the NP-AIA group, showing no difference of expression before and after oral corticosteroid treatment.

Because the anti-inflammatory effect of MUC1-CT may involve inhibition of TLR activation, we next explored the expression and distribution of MUC1 in NP. MUC1 mRNA expression was downregulated before and after oral corticosteroid therapy in the NP tissue of patients with NP-CR (Table E2; Fig 1, *A*) compared with the other groups, while no differences were observed among responders in the NPwA, NP-ATA, and NP-AIA groups (Table E2; Fig 1, *A*). The same pattern of MUC1 expression was observed between patients with NP-CR showing NPwA, NP-ATA, and NP-AIA (see Table E3). In a similar manner, MUC1-CT protein expression was significantly decreased in patients with NP-CR, mainly in the NP epithelium before and after treatment (Fig 1, *B-D*).

Expression and distribution of corticosteroid-dependent targets in NP

Several of the anti-inflammatory properties of corticosteroids are by post-transcriptional mechanisms, including the upregulation of mitogen-activated protein kinase phosphatase 1 (MKP1), which inhibits the activation of several mitogen-activated protein kinases. In this study, mRNA and protein expression of MKP1 showed similar low expression in all groups of patients before treatment, and increased significantly in responders, but not in non-responders after oral corticosteroid treatment (see Fig E2, *A*, and Fig E3, *A* and *B*, in the Online Repository at www.jacionline.org). IL-8 was equally over-expressed in the NP of all patients