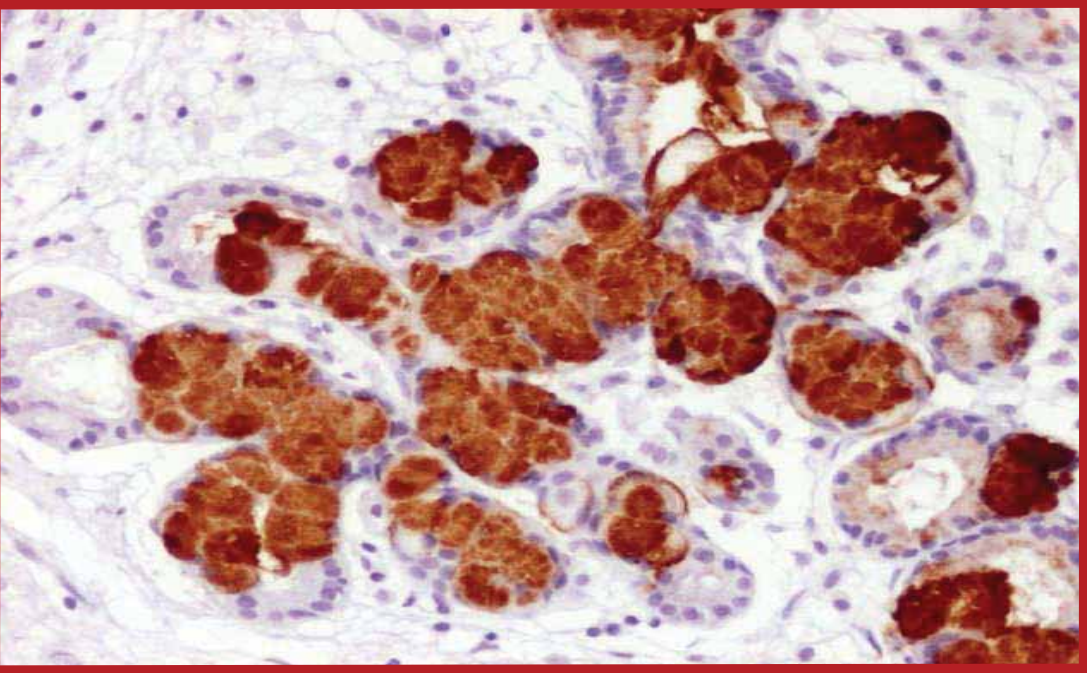
DOCTORAL THESIS

Mucus Hypersecretion, MUC genes and Mucins in Inflammatory Nasosinusal Diseases. Regulation by Proinflammatory and Antiinflammatory Agents



Mª Asunción Martínez Antón

July 2008

UNIVERSITAT DE BARCELONA

Facultat de Medicina

Departament de Medicina

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List of Publications

Study 1. Martínez-Antón A, Debolós C, Garrido M, Roca-Ferrer J, Barranco C, Alobid I, Xaubet A, Picado C, Mullol J. Mucin genes have different expression patterns in healthy and diseased upper airway mucosa. *Clin Exp Allergy* 2006, 36:448-57.

Study 2. Martínez-Antón A, De bolós C, Alobid I, Benítez P, Roca-Ferrer J, Picado C, Mullol J. Corticosteroid therapy increases membrane-tethered while decreases secreted mucin expression in nasal polyps. *Allergy* 2008 DOI: 10.1111/j.1398-9995.2008.01678.x *(in press)*.

Study 3. Martínez-Antón A, Callejas FB, Fuentes M, de Bolós C, Roca-Ferrer J, Picado C, Mullol J. Dexamethasone decreases basal and IL-1β-induced MUC5AC expression and secretion in A549 cells *(in preparation)*.

Study 4. Martínez-Antón A, Roca-Ferrer J, Mullol J. Mucin gene expression in rhinitis syndromes. *Curr Allergy Asthma Rep* 2006, 6:189-97 [Review].

Abbreviations

A549, human lung carcinoma cell line

Acetylcholine, Ach

ACP, antrochoanal polyps

ADAM10, A disintegrin and metalloprotease

AIA, aspirin-intolerant asthma

AMOP, adhesion-associated domain in MUC4 and other proteins

AR, allergic rhinitis

asialoGM1, asialoganglioside tetraosylceramide

ATA, aspirin-tolerant asthma

ATP, adenoside 5'-triphosphate

BEAS-2B, human bronchial epithelia cell line

CF, cystic fibrosis

CFP, nasl polyps from cystic fibrosis patients

CFTR, CF transmembrane conductance regulator

CK, Cys Knot

CLCA, calcium-activated chloride chanel

CNS, central nervous system

COPD, chronic obstructive pulmonary disease

COX-2, cyclooxygenase-2

CRE, cAMP-response element

CREB, cAMP-response element binding protein

CRS, chronic rhinosinusitis

CS, corticosteroids

CS-domain, Cys-rich domain

CytMix, cytokine mixture

DEX, dexamethasone

ECP, eosinophil cationic protein

EGF, epidermal growth factor

EGFR, epidermal growth factor receptor

EMCN, endomucin

ERK, extracellular signal-regulated kinase

ESS, endoscopic sinus surgery

FCS, fetal calf serum

GalNAc, N-acetylglactosamine

GCH, goblet cell hyperplasia

GCs, glucocorticoids

GRE, glucocorticoid response elements

GRP, gastrin release peptide

HB-EGF, heparin binging-EGF

hCLCA, human calcium-activated chloride channel

HETEs, hydroxyeicosatetraeinoic acids

I3P, inositol triphosphate

Ig, immunoglobulin

IL, interleukin

IκKβ/γ, IκB kinase

JAK, Janus kinase

LPS, lipopolysaccharide

LTA, lipotheichoic acid

MAPK, mitogen-activated protein kinase

MARCKS, myristolated alanine-rich C kinase substrate

MBP, major basic protein

MCAM, melanoma cell adhesion molecule

MEK, MAPK/ERK kinase

MSK1, mitogen-and-stress-activated protein kinase 1

MyD88, myeloid differentiation primary response gene

NA, noradrenaline

NANC, non-adrenergic non-cholinergic

NCI-H292, human lung mucoepidermoid carcinoma cell line

NE, neutrophil elastase

NFκB, nuclear factor κB

NHBE, normal human bronchial epithelial cells

$NHBE_{ALI}$, NHBE cells grown in air-liquid interface culture system

NHNE, normal human nasal epithelial cells

NIDO, Nidogen homology region

NIK, NFκB inducing kinase

NKA, neurokinin A

NKCC, Na^+-K^+-Cl^- cotransporter

NM, nasal mucosa

NP, nasal polyp

NPY, neuropeptide Y

P2Y2, purinoceptor 2Y2

PAF, platelet activating factor

PAFR, platelet activating factor receptor

PBS, phosphate buffered saline

PGE_2, prostaglandin E_2

PI3K, phosphoinositide 3-Kinase

PKC, protein kinase C

PL, phospholipase

pp90rsk, 90-KDa ribosomal S6 kinase

Qol, quality of life

RAR, retinoic acid receptor

ROS, reactive oxygen species

RSK1, p90 ribosomal S6 protein kinase 1

SEA, sea urchin sperm protein, enterokinase and agrin

SP, substance P

TACE, TNF-α-converting enzyme

TACE, TNF-α converting enzyme

TAK, TGF activated kinase

TFFs, trefoil factors

TGF, transforming growth factor

TLR, toll-like receptor.

TNF-α, tumor necrosis factor-α.

TR, tandem repeats

UTP, uridine triphosphate

VIP, vasoactive intestinal peptide

VNTR, variable number of tandem repeats

vWF, von Willebrand factor

1. INTRODUCTION

Chapter 1. Respiratory system

Chapter 1. Respiratory system

1. The nose.

The major function of the respiratory system is gas exchange between our body and the external environment. It is subdivided into an upper and a lower respiratory tract. The nose, included in the upper respiratory tract, participates in several functions related to respiration, providing the necessary air resistance for the proper function of the lung and preparing inhaled air by filtering, warming, and moistening it before reaching the lungs. The nose is a double organ composed of two nasal cavities divided by a septum. In these cavities there are three prominent structures called upper, middle, and lower turbinates. Both nasal septum and turbinates are covered by a respiratory mucosa (1). This organization is essential for the functions in which the nose is involved: respiration, inhaled air humidification, inhaled air clearance by mucociliary transport, immunological response, and voice resonance and modulation. The vascularisation of the nose comes from the external and internal carotid arteries while innervations can be sensitive, parasympathetic, sympathetic, and non-adrenergic non-cholinergic.

2. Nasal Mucosa.

2.1. Histology. Nasal mucosa is composed of a respiratory epithelium, a basement membrane, and a submucosa (Fig. 1).

- *Epithelium.* This is a pseudostratified columnar ciliated epithelium containing goblet, basal, ciliated, and non-ciliated cells. In addition, immune cells, inflammatory cells, and phagocytic cells migrate to, remain within, or transit through it to the lumen.

- *Basement membrane.* The basement membrane consists predominantly of types III, IV, and V collagen, type V laminin, and fibronectin, produced by epithelial cells and subepithelial fibroblasts (2).

- *Submucosa.* Under the epithelium and separated by the basement membrane, there is the submucosa containing: a) an external area rich in blood fenestrated capillaries (3, 4), b) submucosal glands, composed of both serous and mucous cells and glandular ducts which drain secretions to the nasal lumen, and c) venous sinusoids that form the erectile tissue. In the connective tissue around submucosal glands, there is a blood vessels net responsible for the nasal congestion and decongestion. Finally, there exists a bone structure in which nasal mucosa is attached.

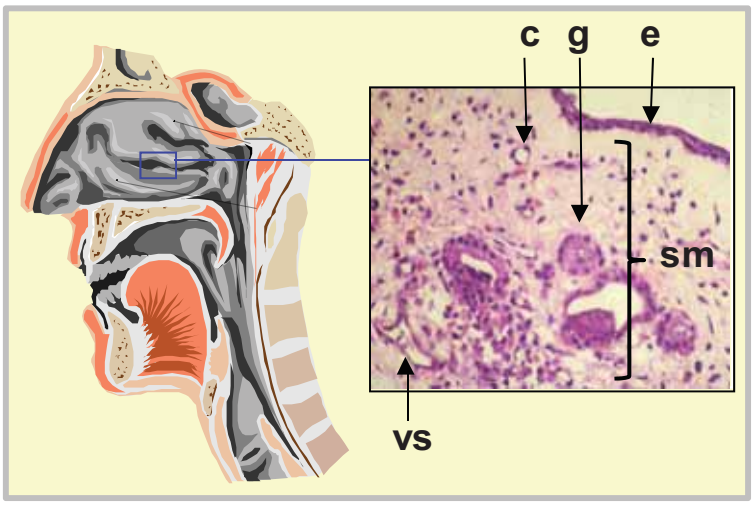


Figure 1. Nasal mucosa histology. The epithelium (e), the submucosa (sm), submucosal glands (g), venous sinusoids (vs) and capillars (c) are shown in this tissue section. Hematoxilin-eosine staining. Magnification 100X.

2.2. Physiology. In physiological situations, the respiratory epithelium is covered by a mucus layer, containing an upper *gel* layer that traps inhaled particles, and a lower *sol* layer in which epithelial cell cilia are embedded. The main role of this mucus is to cover and protect the respiratory tract by trapping pathogens and irritant substances and to facilitate their removal by mucociliary clearance. This function is carried out by the action of epithelial-cell cilia that are embedded in the mucus gel phase and sweep it along with a coordinated "beat". Chronic increases in volume and viscosity of the mucus layer impair clearance and contribute to the pathophysiology of hypersecretory conditions of the airways, for instance asthma and chronic rhinosinusitis.

In addition, the nasal mucosa through its epithelium plays other functions such as physical barrier, transport, secretion, and inflammatory modulation (Table 1).

Table 1. Functions of sinunasal epithelium.

Functions	Mechanisms	Final result
1. Physical protection	· Via intercellular adhesion complexes (tight junctions, desmosomes) · Via mucin secretion	· Selective absorption · Humidification and warming of inhaled air · Entrapping of noxious agents
2. Transport	· Via cilia beats	· Transport of mucus from the lung to the throat
3. Secretion	· Via its cellular types	· Mucins, cytokines, adhesion molecules, growth factors
4. Target of proinflammatory and antiimflammatory agents	· Via specific receptors	· Response to cytokines, glucocorticoids, chromones, antihistamines

3. Nasal secretions.

The mucus layer consist of nasal secretions mainly composed of water, ions, serum protein transudates, antimicrobial proteins and mucus glycoproteins (mucins) (5). These secretions come from different origins (6):

a. Capillary vessels: these are the source of albumin, immunoglobulin (Ig) G, IgM, fibrinogen, complement, and other plasma proteins.

b. Inflammatory mediators: histamine, leukotrienes, prostaglandins, tryptase, major basic protein (MBP), eosinophil cationic protein (ECP), neutrophil elastase, and many others.

c. Submucosa gland cells:

c.1. Serous cells: produce antimicrobial proteins such as secretory IgA, lactoferrin, lysozime, and peroxidase.

c.2. Mucous cells: they mainly produce mucus glycoproteins (mucins).

d. Epithelial goblet cells: mucins.

From all these elements, mucins are the major component of mucus, and they are responsible for its rheological properties, being this way the main molecules involved in the physiology of nasal mucosa secretions.

4. Physiologic regulation of mucus secretion.

In physiologic conditions, the airway mucus secretion is under the control of a variety of mechanisms, but the nervous system seems to have a prominent role. Three main neural pathways are responsible for the innervations of the airways: sympathetic (adrenergic), parasympathetic (cholinergic), and sensory nerves. A fourth system integrated in the other three is the non-adrenergic non-cholinergic (NANC)(7). The dominant neural control of human airway mucus secretion is cholinergic (8), although some adrenergic and sensory mediators have shown to stimulate nasal glandular secretion in different experimental models depending on the animal species studied. The peptides relevant in the regulation of mucus secretion are (Fig. 2):

- ***Cholinergic nerves***: acetylcholine (Ach), by acting on specific muscarinic M1 and M3 receptors, regulates glandular secretion in human nasal mucosa (9-11); and vasoactive intestinal peptide (VIP) stimulates and inhibits glandular secretion in human nasal and bronchial mucosa, respectively (11, 12).

- ***Adrenergic nerves***: the regulation of mucus secretion by noradrenaline (NA) and neuropeptide Y (NPY) is specie-specific and has not been demonstrated in human airways (13).

- ***Sensory nerves***: gastrin release peptide (GRP), substance P (SP), and neurokinin A (NKA) stimulate nasal glandular secretion from human nasal mucosa (14).

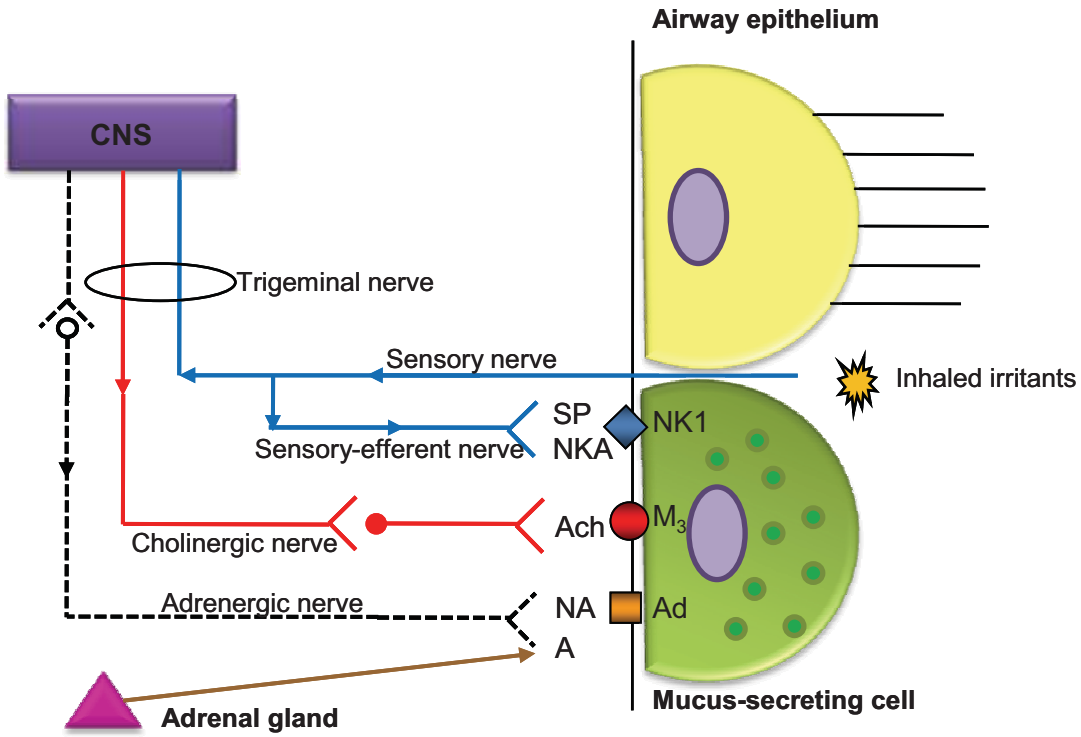


Figure 2. Innervation of airway mucus-secreting cells. This simplified diagram shows the principal neuronal pathways that induce secretion. Cholinergic (parasympathetic) nerves constitute the dominant pathway (red), whereby acetylcholine (Ach) interacts with muscarinic M3 receptors to increase mucus output. Adrenergic (sympathetic) neural control of airway secretion (broken black lines and noradrenaline [NA]) has not been demonstrated in human airways. Cathecholamines like adrenaline (A) from the adrenal medulla interact with adenoreceptors (Ad) on the secretory cells to increase mucus output. Sensory nerve endings (blue) in the epithelium detect inhaled irritants and relay impulses via sensory (afferent) pathways to the central nervous system (CNS) to initiate reflex secretion. Axonal neurotransmission via collateral sensory-efferent pathways leads to release of sensory neuropeptides including substance P (SP) and neurokinin (NK) A, which interact with NK1 receptors to increase secretion.

Chapter 1 summary.

Among all the structures that compose the respiratory tract, the nose by means of the nasal mucosa is the organ involved in the preparation of the inhaled air by filtering, warming and humidifying it before reaching the lungs, this way protecting the airways from external irritants and pathogens. To develop this function, the nasal mucosa secretes mucus through its epithelial and submucosal gland cells and promotes mucocilliary clearance through its ciliated epithelial cells embedded in nasal secretions.

In physiologic conditions, the nervous system has a prominent role in the regulation of nasal secretions, and although sympathetic, parasympathetic, and sensory nerves have been found in the nasal mucosa, the parasympathetic pathway seems to be the predominant. On the other hand, in pathologic conditions, the nervous system loose control on nasal secretions and this control is mainly replaced by inflammatory mediators.

Nasal polyposis represents a disease in which mucus secretion, especially mucin secretion, is under control of several cytokines, chemokines, and growth factors, in turn found increased in this pathology. In order to understand the basic physiology of this inflammatory disease showing mucus hypersecretion, a review on nasal polyp classification, associated diseases, histophysiology and treatment is done in chapter 2.

Chapter 2. Nasal polyposis

Chapter 2. Nasal polyposis

1. Definition and classification.

Chronic rhinosinusitis (CRS), including nasal polyps, is defined as an inflammation of the nose and paranasal sinuses characterized by two or more symptoms, one of which should be either nasal blockage/obstruction/ congestion or nasal discharge, and with/out facial pain/pressure, with/out reduction or loss of smell (15).

Chronic rhinosinusitis with or without nasal polyps is often considered one disease entity, because it seems impossible to clearly differentiate both entities (16-18). Chronic rhinosinusitis with nasal polyps is considered a subgroup of chronic rhinosinusitis.

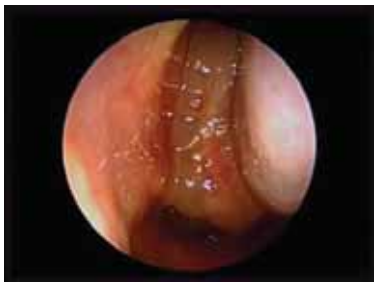


Figure 3. Endoscopic image of a nasal polyp

Nasal polyps are edematous masses originated from the middle meatus that cause long-term symptoms, in particular nasal obstruction, sense of smell reduction (hyposmia) or even anosmia, rhinorrea, and facial pain. The typical history of patients suffering from nasal polyposis is similar to perennial rhinitis (19) (Fig. 3).

Nasal polyposis is not a consistent disease, and it may be divided into different subgroups based on clinical aspects, etiology, histopathology (20) and mediators' content (21). The more general classification is the following (22):

1. Unilateral nasal polyps:

    a. Antrochoanal polyps, a commonly large isolated unilateral cyst-like non-eosinophilic formation.

2. Bilateral nasal polyps:

   a. Idiopathic unilateral or bilateral, mostly eosinophilic polyps without involvement of the lower airways.

   b. Bilateral eosinophilic polyps with concomitant asthma and/ or aspirin sensitivity

   c. Polyposis associated to other diseases such as cystic fibrosis, Churg-Strauss syndrome, Kartagener syndrome, etc.

2. Epidemiology and co-morbidities.

The exact prevalence on nasal polyposis in the general population is not known, because there are few epidemiological studies and their results depend on the study population selected and the diagnostic methods used. Data published by the American General Health Survey show that patients seeking for medical advice owing to chronic rhinosinusitis-related symptoms represent the 14.7% of the American population, although nasal polyposis affect 2 to 5% of the general population (23). Other relevant publications mention nasal polyposis prevalence of 4.3% in the general population (24). The incidence is higher in men than in women and significantly increases after the age of 40 years (25, 26). Nasal polyps occur more frequently in subgroups of patients such as asthmatics, aspirin sensitive and cystic fibrosis patients (27).

2.1 Asthma. Asthma is a chronic inflammatory condition of the lower airways clinically characterized by variable airflow limitation that is at least partially reversible, both spontaneously and after treatment. The common histopathologic features in asthmatic patients are mucus hypersecretion, epithelial damage, inflammatory cells infiltration, and enlargement of basement membrane (28).

Asthma is clearly associated to nasal polyposis, fact supported by its prevalence, being this of 7 % in the Spanish and Catalan general population

and increasing up to 30% in a population of patients with nasal polyposis (29).

2.2. Aspirin Sensitivity. Aspirin-induced asthma is a distinct clinical syndrome characterized by the triad apirin sensitivity, asthma and nasal polyposis (30), and starts with a prolonged episode of nasal congestion, rhinorrea, and hyposmia with persistent mucosal inflammation. Physical examination often reveals nasal polyps. Bronchial asthma and aspirin intolerance develop subsequently. The intolerance appears after ingestion of aspirin when an acute asthma attack occurs, often accompanied by rhinorrhea and conjunctival irritation (31).

Aspirin induced-asthma affects about 10% of asthmatic patients, this percentage increasing to 20% in severe asthmatic patients (32). In addition, in patients with chronic rhinosinusitis undergoing endoscopy surgery reveals that 11-20% of them have aspirin sensitivity, being this fact an indirect marker of the severity of polyposis in this group of patients (33). The prevalence of nasal polyps in aspirin sensitive asthmatics may be over 60-70%, as compared to less than 10% in the population of aspirin-tolerant asthmatics (34).

2.3. Cystic fibrosis. Cystic fibrosis (CF) is the most common severe genetic disease, with an incidence rate varying from 1 per 2000 to 1 per 6500 living newborn babies. Defective expression of the CF transmembrane conductance regulator (CFTR) in CF epithelial cells is associated with mucus hypersecretion, inflammation, and infection that begin in early life and lead to a marked cyclical airway obstruction and infection responsible for the morbidity and mortality in patients with CF (35).

In patients suffering from CF, nasal polyposis is associated with a higher prevalence than in the general population, this prevalence ranging from 6 to 48%. In addition, 92 to 100% of CF patients present radiologic signs of sinonasal disease (36). Fifty percent of the children between 4 and 16 years of age suffering nasal polyposis present CF (37).

2.4. Allergic rhinitis. Allergic rhinitis (AR) is a heterogeneous disorder characterized by the presence of one or more of the following nasal symptoms: sneezing, itching, rhinorrhea, and nasal congestion. The incidence of this pathology is 18-29% in the general European population (38), and 21.5% in Spanish population. These clinical signs are similar to those of nasal polyposis, and for this reason nasal polyposis was suggested to be an atopic manifestation, even when nasal polyps and atopy association was rare. Between 0.5 and 1.5% of patients with allergic rhinitis present nasal polyps while in patients with non-allergic rhinitis this percentage increases up to 5% (39, 40). Accordingly, allergy does not seem to be a predisposing factor for nasal polyposis.

3. Histopathology of nasal polyps.

Histomorphologically, polyp tissue reveals frequent epithelial damage, a thickened basement membrane, edematous stromal tissue, a reduced number of glands and vessels, and no visible neural structures (41). It is therefore assumed that denervation of nasal polyps causes a decrease in secretory activity of the glands and induces an abnormal vascular permeability, leading to an irreversible tissue oedema.

Nasal polyps contain a great amount of inflammatory cells, specially eosinophils, lymphocytes, and mast cells (42, 43). These cells, together with structural cells (fibroblasts and epithelial cells), release molecules such as histamine, cytokines, chemokines, transcription factors, and eicosanoids that act as inflammatory mediators playing a crucial role in the persistent eosinophilic inflammation observed in nasal polyps (44). Additionally, these inflammatory mediators are involved in the stimulation of mucus hypersecretion, specifically increasing mucin expression and secretion (45, 46). The final presentation of this mucus hypersecretion is rhinorrhea, a common symptom in patients suffering from nasal polyposis.

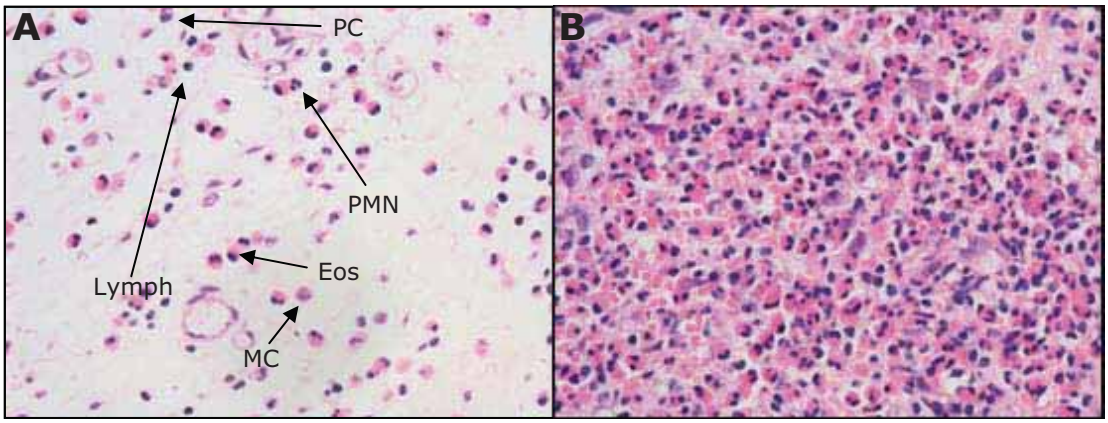
Nasal polyps have been divided in 4 subclasses attending to their different histology, and the wrong-called "allergic" polyp is the one that characterize the nasosinusal polyposis (47). This is oedematous, eosinophillic, and the most common type. The presence of edema in the stroma, goblet cell hyperplasia, increased eosinophil and plasma cell content in the stroma, and a thick basement membrane are common features of this polyp subtype. In addition, they are bilateral and represent around 80% of the nasosinusal polyps (48).

3.1. Inflammatory cells in nasal polyps. (Fig. 4)

- *Mast cells*. Nasal polyps contain a great number of mast cells (49). After mast cell activation, degranulation occurs in the form of histamine, serotonin, platelet activating factor (PAF), leukotrienes, and prostaglandins. Mast cells also produce cytokines (IL-1β, TNF-α, IFN-γ, IL-4, IL-5, and IL-6) that activate adhesion molecules, induce eosinophilic infiltration, and perpetuate inflammation.

- *Eosinophils*. The infiltration of the nasal polyp mucosa by eosinophils is a hallmark of nasal polyposis (50). The eosinophillic infiltrate is due to several causes: a) increased production of eosinophils in the bone marrow induced by growth factors; b) eosinophil chemotaxis induced by cytokines, adhesion molecules, and chemoattractants; c) *in situ* eosinophil activation; and d) increased eosinophil survival. Eosinophil migration occurs from the submucosa to the basement membrane and to the epithelium. Once in the tissue, the activation and survival of eosinophils will be increased by cytokines and other mediators (51, 52). Glucocorticoid efficacy in nasal polyps is mainly due to their inducing effect of eosinopil apoptosis/ cell programmed death (53).

Figure 3. Inflammatory cell infiltrate in nasal polyps. A) Poorly infiltrated nasal polyp. Eos: eosinophils, Lymph: Lymphocytes, PMN: polimorphonuclears, MC: mast cells, and PC: plasma cells. B) Massive infiltration mainly by eosinophils in nasal polyp tissue. Hematoxiline-eosine staining. 400X magnification.



- *T cells Lymphocytes*. These cells, found activated in nasal polyps, promote eosinophilic inflammation (54) together with other inflammatory cells. They represent a mixed population, consisting in CD4+ and CD8+ cells, and show a mixed Th1/Th2 profile. In nasal polyps, T lymphocytes prevail over B lymphocytes, while T suppressors (CD8+) prevail over the T helper cells (CD4+)(55).

- ***B cells Lymphocytes.*** These cells are responsible for the production of the immunoglobulin E (IgE) involved in the early allergic reaction through activation and degranulation of mast cells.

3.2. Inflammatory mediators in nasal polyps.

- *Histamine.* This inflammatory mediator, released after mast cell activation, and degranulation, has strong effects on smooth muscle constriction, and increases vascular permeability and edema (56). Curiously, even being found in great amount in nasal polyps, antihistamines have not shown clinical efficacy in their treatment.

- *Cytokines.* They are responsible for the induction of intercellular signaling, by activation of membrane specific receptors, that

leads to cellular proliferation, cellular differentiation, cellular chemotaxis, growing, and Ig secretion modulation (21, 57). In nasal polyps, the anti-inflammatory effects of glucocorticoids are due to both, induction of eosinophil apoptosis and inhibition of eosinophil regulatory cytokines.

- ***Chemokines***. They promote the chemotaxis of inflammatory cells such as lymphocytes, monocytes, and eosinophils (58, 59). RANTES, and eotaxin-1, -2, -3, and -4 are the main chemokines found in nasal polyps (60, 61).

- ***Eicosanoids***. They are products of arachidonic acid metabolism, which include 2 large mediator families, leukotrienes and prostanoids. Due to hyperproduction or failure in the degradation processes, eicosanoids can accumulate and become involved in the pathogenesis of nasal polyposis (62). The enzyme cyclooxigenase (COX) metabolizes arachidonic acid into prostaglandin H_2, source of other prostaglandins, prostacyclins, and tromboxans while lipoxigenases metabolize AA into leukotrienes, lipoxins, and hydroxyeicosatetraeinoic acids (HETEs) (63, 64).

4. Clinical aspects and diagnosis

Symptoms in acute and chronic rhinosinusitis as well as in CRS with nasal polyps are similar, although the symptom pattern and their intensity may vary. The general symptoms are: nasal blockage, congestion or stuffiness, nasal discharge or postnasal drip, facial pain or pressure, and reduction/loss of sense of smell (15).

Nasal polyps may cause nasal congestion, which can be a feeling of pressure and fullness in the nose and paranasal cavities. This is typical for ethmoidal polyposis, which in severe cases can cause widening of the nasal and paranasal cavities demonstrated radiologically. Disorders of smell are more prevalent in patients with nasal polyps than in other chronic rhinosinusitis patients (65).

Clinically, nasal polyp diagnosis is based on clinical symptoms and on endoscopy and CT-scan of the paranasal sinuses showing the presence of endoscopically visible bilateral polyps growing from the middle meatus into nasal cavities, and affecting etmoidal and maxillary sinuses (15, 66). During the last decade more attention has been paid not only to symptoms but also to their effect on patient's quality of life (QoL) (67, 68).

5. Management of nasal polyposis.

The goals of nasal polyposis treatment, both clinically and surgically, is aimed to reduce or eliminate nasal polyps, to restore respiration and sense of smell, to relieve rhinosinusitis symptoms, and finally to prevent nasal polyp recurrence. There are different treatment recommendations depending on the severity of symptoms, but topical and oral corticosteroids are the basis for an optimal treatment of nasal polyposis (Table 2) (Fig. 5)(15).

5.1. Glucocorticoids. Glucocorticoids (GCs) are the most effective drugs in the prevention and suppression of inflammation originated by mechanical, chemical, infectious, and immunologic stimuli. GCs inhibit different inflammatory aspects by inducing or reducing gene transcription and expression of mediators, receptors, adhesion molecules, and cytokines (69, 70).

The main anti-inflammatory effects of GCs are based on their ability to reduce the synthesis of several cytokines (IL-1, -2, -3, -4, -5, -6, -8, TNF-α, IFN-γ, GM-CSF) from many cells (macrophages, monocytes, lymphocytes, and epithelial and endothelial cells), either by inhibiting their transcription interacting with gene glucocorticoid response elements (GRE), by transrepression with transcription factors or avoiding protein translation by eliminating the mRNAs (71, 72). This affects recruitment, localization, protein synthesis, and survival of inflammatory cells such as eosinophils. The recruitment of inflammatory cells is also diminished by an inhibited

expression of adhesion molecules such as ICAM-1 and VCAM-1 (73), which affects the influx of basophiles and mast cells in the epithelial layers of nasal mucosa. GCs also act by suppressing the arachidonic acid pathways, directly inhibiting phospholipase A_2 (PLA_2) and COX-2 gene expression (74-76). Additionally, GCs reduce eosinophil survival, either by inhibiting the expression of cytokines from other inflammatory cells or by blocking the action of cytokines on eosinophils by activating endonucleases and apoptosis (51, 53).

Table 2. Treatment evidence and recommendations for adults with chronic rhinosinusitis with nasal polyps, according to EP^{3}OS (from reference 15)*

Therapy	*Level*	*Grade of recommendation*	*Relevance*
Oral antibiotics < 2 weeks	no data	D	no
Oral antibiotic > 12 weeks	no data	D	yes, for late relapse
Topical antibiotics	Ib	D	no
Topical steroids	Ib	A	yes
Oral steroids	Ib	A	yes
Nasal douche	Ib no data in single use	A	yes, for symptomatic relief
Decongestant topical/ oral	no data in single use	D	no
Mucolytics	no data	D	no
Antimycotics-systemic	Ib (-)#	D	no
Antimycotics-topical	Ib (-)#	A	no
Oral antihistamine	Ib (1)#	A	no, in allergy only
Capsaicin	II	B	no
Proton pump inhibitors	II	C	no
Immunomodulators	no data	D	no
Phytotherapy	no data	D	no
Anti leukotrienes	III	C	no

*Some of these studies also included patients with CRS without nasal polyps. #: (Ib) study with a negative outcome.

A combination of topical and systemic glucocorticoids is the recommended therapy for nasal polyposis. The use of topical GCs taken on a daily basis for several months to years is considered the first line therapy in mild to moderate nasal polyps, to reduce symptoms and avoid surgery. Systemic oral GCs are indicated to start off or enforce topical treatment. In addition, other drugs such as long term antibiotics, nasal vasoconstrictors, antihistamines, and antileukotriens can be occasionaly used in combination with GCs (15).

Glucocorticoids have a proven therapeutic effect on nasal polyposis symptoms and they can reduce the underlying cause of nasal polyposis, the mucosal inflammation. Symptoms such as nasal obstruction, rhinorrhea, and occasionally hyposmia are reduced during the period of treatment, especially in obstructive polyposis (77-79). Additionally, glucocorticoids delay the recurrence after surgery (80). However, surgery needs to be considered in case of failure, side effects or unwillingness of the patient to accomplish the drug treatment (22).

5.2. Endoscopic polypectomy and sinus surgery. The introduction of nasal endoscopy has promoted a revolution in the rhinology clinical daily practice. The endoscopic sinus surgery (ESS) has as main objectives the restoration of nasal ventilation and the unblocking of the natural drain of paranasal sinuses, in order to reestablish the physiologic purge of its secretions. The restoration of ventilation and the recovery of mucocilliary function are priorities for the cure of the disease and the maintenance of a healthy sinunasal mucosa (81).

Extensive postoperative care and follow-up is required to preserve the postoperative results and prevent relapse of polyps. Nevertheless, nasal polyposis is a chronic disease with a high rate of recurrences (40% after 5 years) even after careful medical and surgical treatment (65).

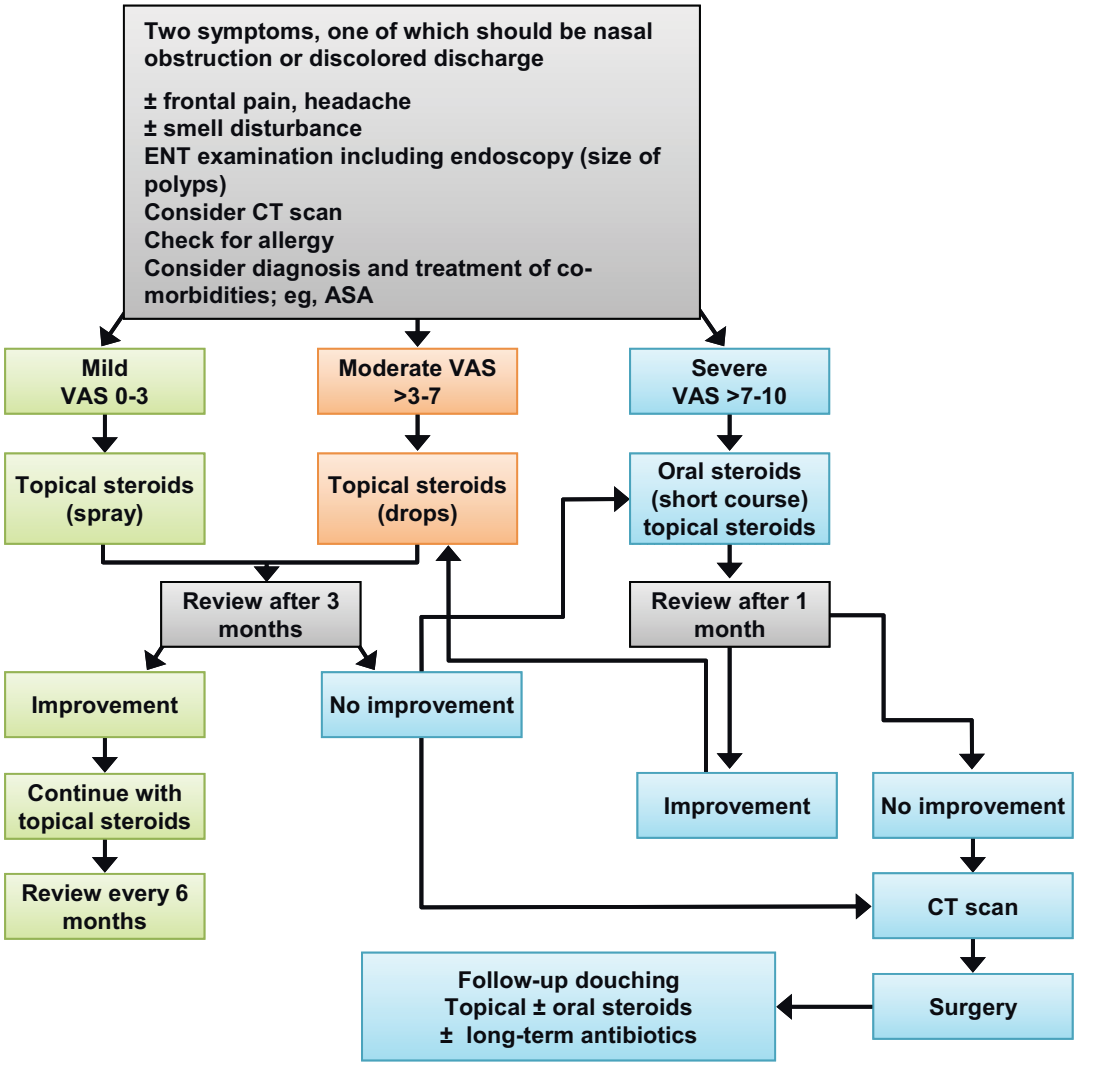


Figure 5. Treatment scheme for adults with chronic rhinosinusitis with nasal polyps, according to EP^{3}OS (from reference 15).

Chapter 2 summary.

Nasal polyposis is a common upper airways disease, frequently associated to asthma, and aspirin sensitivity. These diseases share, among others, airways obstruction and mucus hypersecretion symptoms. Nasal polyposis is also characterized by a high inflammatory cell infiltration, and consequently an elevated number of inflammatory mediators reside within NP. These inflammatory mediators, apart from causing the persistent inflammation of the tissue, are involved in the mucus overproduction/ hypersecretion present in NP.

Glucocorticoids are first-choice therapy in the management of nasal polyposis and other inflammatory respiratory diseases, and although they have demonstrated being effective in reducing polyp size and NP inflammatory component, their efficacy on the mucus hypersecretion found in inflammatory respiratory diseases have always been controversial. Further studies are needed in order to elucidate GC effects on mucus overproduction, specially centered on the main component of mucus (mucins).

In chapters 3 and 4, studies dealing with the expression and regulation of mucins in health and disease are reviewed.

Chapter 3. Mucins

Chapter 3. Mucins

1. Overview.

The apical epithelial surfaces of mammalian respiratory, gastrointestinal, and reproductive tracts are coated by mucus, a mixture of water, ions, glycoproteins, and lipids. Mucosal components are secreted apically by goblet cells in polarized epithelium and by secretory cells in submucosal glands. The main function of this mucus is to provide a protective barrier against pathogens and toxins and contribute to the innate defensive system in mucosal immunology (82), although it has another functions tissue-specific. In this regard, in the gastric mucosa mucus secretion prevents the auto-digestion due to the elevated concentration of chlorhydric acid, in the respiratory mucosa mucus is produced to avoid the entrance of external agents into the airways, while in cervical mucosa in addition to protecting and lubricating, viscoelastic properties of mucus are changed in a controlled way in order to allow fecundation.

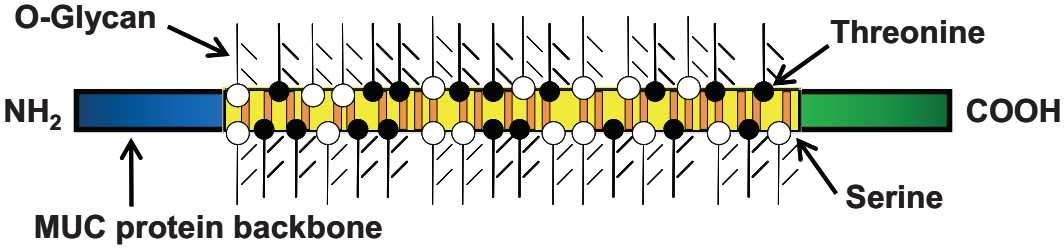


Fig.6. Schematic representation of a secreted mucin glycoprotein depicting a MUC protein backbone and its O-glycans. A MUC protein backbone typically consist of an NH_2-terminal domain (blue), a central domain (yellow) with a high number of tandem repeats (TR)(orange), and a COOH-terminal domain (green). Numerous O-glycans are attached to threonine (●) or serine (○) residues in the TR domains.

Mucins are high-molecular-weight proteins (average weight of glycosylated mucins: 1 MDa) extensively O-glycosylated (up to 80% of the total mass) and represent the main macromolecular component of mucus. A common feature of mucins is their main core, composed of variable number of tandemly repeated (VNTR) amino acid sequences that are rich in serine,

threonine, and proline. It is in these tandem repeats (TR) where the O-glycosylation occurs (Fig. 6). Goblet cells in the surface epithelium and mucous cells in submucosal glands are the main cells involved in the synthesis and secretion of mucins.

2. Classification.

Mucins have been given the acronym MUC, followed by a number. More than 20 human mucin genes are deposited in the genebank, but the definition of what determines a mucin gene has not always been consistent (46). If macromolecules rich in serine/threonine residues, but without TR in its protein backbone are considered serine/threonine-rich glycoproteins rather than mucins, then the present list of human mucins would have 18 members all of which having tandem repeats.

Table 3. Human MUC genes: classification and genomic localization.

Human Mucin	Locus	Mucin type	References
Membrane-tethered with Tandem Repeats			
MUC1	1q21	Pan-epithelial	110
MUC3A	7q22	Intestinal	111, 112
MUC3B	7q22	Intestinal	88
MUC4	3q29	Airway	90
MUC11	7q22	Colonic	100
MUC12	7q22	Colonic	100
MUC13	3q13.3	Colonic	101
MUC16	19q13.2	Reproductive	104
MUC17	7q22	Intestinal	105
MUC20	3q29	Renal	109
Secreted, cys-rich with Tandem Repeats			
MUC2	11p15.5	Intestinal	113, 114
MUC5AC	11p15.5	Airway	92, 114, 115
MUC5B	11p15.5	Airway	114, 116
MUC6	11p15.5	Gastric	114, 94
MUC19	12q12	Salivary	108
Secreted, cys-poor with Tandem Repeats			
MUC7	4q13.3	Salivary	117
MUC8	12q24.3	Airway	118
MUC9	1p13	Reproductive	99
Mucins without Tandem Repeats			
MUC14	4q22.1	Endothelial	102
MUC15	11p14.3	Colonic	103
MUC18	11q23.3	Airway	119

In addition to TR, additional motifs are present in the amino and carboxi terminal domains of mucin backbone. These allowed classification as membrane-tethered or secretory mucins, the latter being subdivided into those that are cysteine rich or cysteine poor. MUC1, MUC3A, MUC3B, MUC4, MUC11, MUC12, MUC13, MUC15, MUC16, MUC17, MUC18, and MUC20 mucins have transmembrane domains in their carboxi terminal and thus are membrane-tethered mucins. MUC2, MUC5AC, MUC5B, MUC6, and MUC19 are polymeric secreted mucins while MUC7 and MUC8 are non-polymeric mucins (Table 3).

Although mucins are produced by all epithelial cells in the mucosa, they have distinctive histologic expression patterns, and combinations and relative amount of individual mucins may vary among cell and tissue types (Table 3).

3. Structure.

Because of their complex structure, mucins are difficult to study by classical biochemical procedures. With the application of recombinant technology, structures of the mucin core peptides are being elucidated. Besides their complex structure, many MUC genes are polymorphic, with alleles having a variable number of TRs (VNTR)(120). Additional variations occur with minor changes in the length of the repeat unit (length polymorphisms) and repeating sequences (sequence polymorphisms). In addition, MUC genes can undergo alternative splicing (121).

Both secreted and membrane-tethered mucins are characterized by a serine/threonine-rich central domain (mucin domain) typically encoded by a single central exon of the MUC gene (Table 4). Serine and threonine hydroxyl groups provide sites for the O-glycosylation found on mature mucins, which consists primarily of simple core sugars with a high percentage of sialyl groups (122-124).

Table 4. Tandem repeat (TR) sequences of MUC proteins

Mucin	Amino acids/TR	Number of TR/MUC*	References
MUC1	20	21-125; 41 and 85 are most common	83, 84
MUC2	23 16	21 51-115; 100-115 are most common	85, 86
MUC3A	17 375	20 Unknown	87, 88
MUC3B	17 375	At least 33[#] Unknown	87, 88
MUC4	16	145-395	89, 90
MUC5AC[$]	8 5	(124, 17, 34, 66)[$]	91, 92
MUC5B[$]	29	(11, 11, 17, 11, 22)[$]	93
MUC6	169	15-26	94, 95
MUC7	23	5-6	96, 97
MUC8	13 41	6[&] 3[&]	98
MUC9	15	6	99
MUC11	28	At least 36[#]	100
MUC12	28	At least 22[#]	100
MUC13	15	10	101
MUC14[£]	0	No TR	102
MUC15[£]	0	No TR	103
MUC16	156	9	104
MUC17	59	5	105, 106
MUC18[£]	0	No TR	107
MUC19	7 7 15 16 19 8 5	At least 6[#] At least 3 At least 4 At least 2 At least 2 At least 4 At least 3	108
MUC20	19	2-6	109

* For MUC genes that exhibit variable number of TR, this number is reported as a range. [$] The number *n* of TR is different in specific regions of MUC5AC and MUC5B. [#] The full DNA sequence for some mucins is not yet reported. [&] Repeats are degenerated. [£] Mucins that lack TR.

While *gel*-forming (secreted) mucins are entirely extracellular, membrane-tethered mucins have a single membrane-spanning domain and a short cytoplasmic tail in addition to the extensive extracellular domain.

3.1. Secreted mucins. Among all the secreted mucins currently identified, the most well-known are the so-called *gel*-forming mucins. The non-polymeric group, has not been fully characterize and its capacity as mucins is already questioned. Notwithstanding that, they have been classified as secreted, cys-poor mucins or non-polymeric mucins.

- ***Gel-forming mucins.*** This family of mucins is composed of MUC2, MUC5AC, MUC5B, MUC6, and MUC19. The first four are clustered together in chromosome 11p15.5 (114) (Fig. 7). The exon-intron boundaries and translated amino acid sequences of the regions upstream and downstream of the central domain are highly conserved across the four genes, indicating that the complex arose by a series of duplications (125, 126). The MUC19 gene is located on chormosome 12q12 (108). All these polymeric mucins share a similar structure composed of a large central exon and flanking 5' and 3' regions.

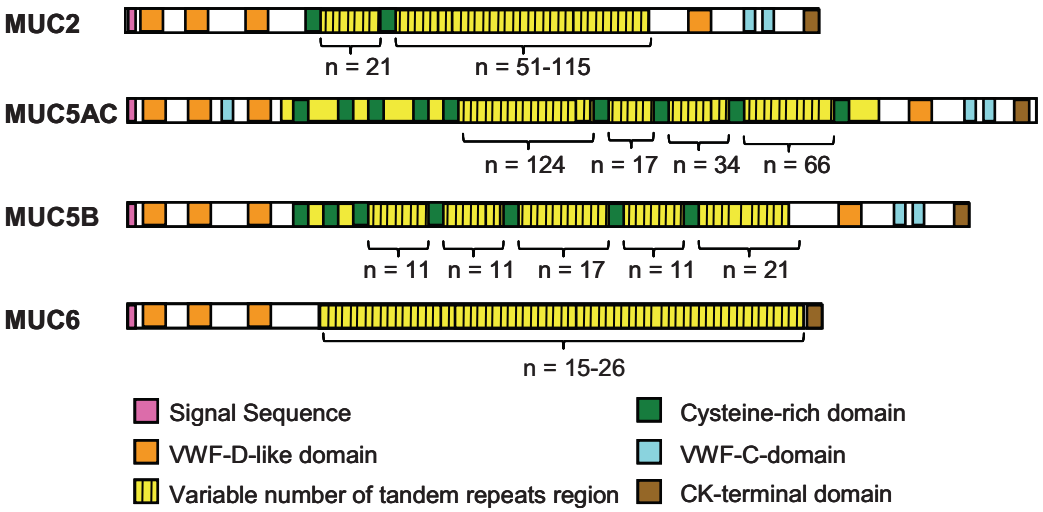


Figure 7. Modular motifs in gel-forming mucins. Key domains include N-terminal signal sequence, variable number of tandem repeats (VNTR) sequence, von Willebrand factor (VWF)-C and -D domains, cysteine-rich domains, and carboxi-terminal Cysteine-Knot (CK) domain. n = number of tandem repeats in a given region. The length of each polypeptide is not drawn in scale.

In all *gel*-forming mucins, but MUC6 and MUC19, the mucin domains are interrupted by several copies of a 90-100 amino acid residues cysteine-rich domain (91, 93), called CS-domain (Cys-rich subdomain). All mucins have signal peptides at their N-termini followed by 3 homologous cysteine-rich domains known as the D1, D2, and D3 (disulfide-rich) domains (127), similar to structural domains found within von Willebrand factor (vWF). A fourth D-domain (D4) is located after the corresponding mucin

domain in all mucins except for MUC6 and MUC19 (108, 126). Other cysteine-rich vWF-like domains, which include the C (Cysteine-rich) and CK (Cysteine Knot) domains are found at the C-termini region (127, 128). The 5' of MUC19 gene is predicted to code for similar vWF D domains, whereas the 3' region differs and codes only for C and CK domains (108) (Fig. 7).

- ***Non-polymeric mucins.*** Three genes fall into this class: MUC7, MUC8, and MUC9, in the chromosome locations 4q13.3, 12q24.3, and 1p13, respectively. MUC8 encodes a mucin domain with a 42-bp tandem repeat unit, but the cDNA and gen sequences remain incomplete. In contrast, MUC7 is fully sequenced and the mucin domain shows variation in length, with two major alleles containing five or six 69-bp repeats (96). Neither of these genes encodes vWF-like domains. MUC9, also called oviductin, received the name of MUC9 because it contains Ser/Thr rich repeated units, clustered in their carboxy-terminal portions (99).

3.2. Membrane-tethered mucins. The membrane-tethered mucins share several properties, as to be expressed by distinct cellular types, epithelial or not. They can be expressed in four different forms: membrane-anchored, soluble (proteolytic cleavage of the membrane-bund form), secreted (alternative splicing variants), and lacking the tandem repeat array (alternative splicing variants) (121, 129-131). Besides the serine/threonine region that differs in size and sequence, all membrane-tethered mucins contain EGF (epidermal growth factor) modules and/or SEA (sea urchin sperm protein, Enterokinase and Agrin) domains (132) (Fig. 8).

Membrane-tethered mucins can be subdivided in two groups: small (MUC1, MUC13, MUC14, MUC15, MUC18, and MUC20) and large (MUC3A, MUC3B, MUC4, MUC11, MUC12, MUC16, and MUC17) mucins. From the small mucins only MUC1 has been fully characterized and is considered, without question, a membrane-tethered mucin. MUC3A, MUC3B, MUC11, MUC12, and MUC17 are clustered in the gene location 7q22.1.

Cell surface mucins are typically composed of dimers of two dissimilar subunits, held together by non-covalent sodium dodecyl sulfate (SDS)-labile bonds. The larger subunit is wholly extracellular and heavily glycosilated. The smaller subunit of tethered mucins consists of a short extracellular region, the single-pass transmembrane domain, and the CT.

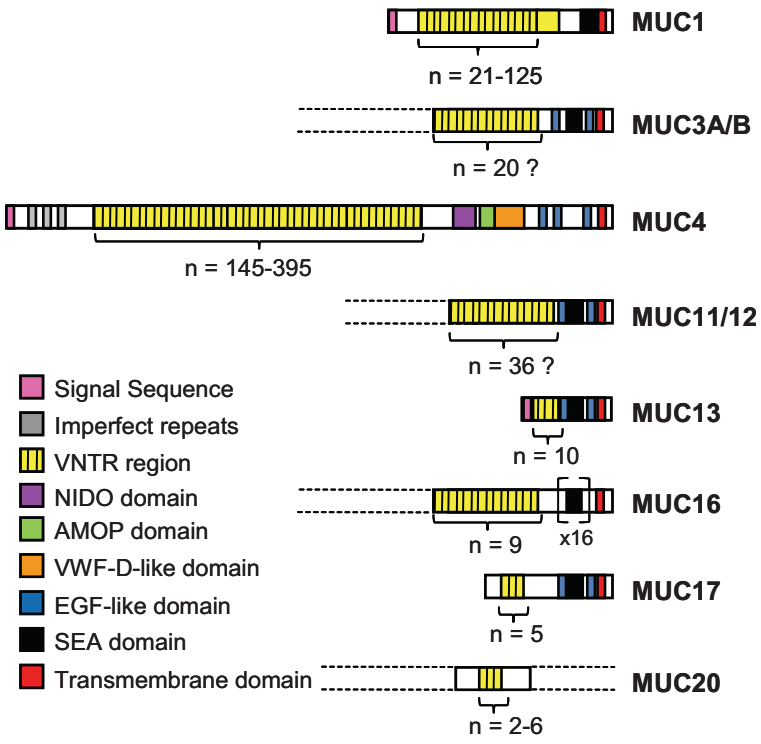


Figure 8. Modular motifs in membrane-tethered mucins. Key do-mains include N-terminal signal sequence, variable number of tandem re-peats (VNTR) sequence, sperm protein, entero-kinase, and agrin (SEA) domain, transmembrane domain, nidogen homo-logy sequence (NIDO), adhesion-associated do-main in MUC4 and other proteins (AMOP), von Willebrand factor (VWF) domain, and epidermal growth factor (EGF)-like regions. n = number of tandem repeats in a given region. The length of each polypeptide is not drawn in scale.

- ***MUC3A, MUC3B, MUC11, MUC12, and MUC17***. All these mucins are clustered in the chromosome locus 7q22.1. They have a repetitive serine/threonine/proline-rich domain. The Ser/Thr/Pro region is followed by two EGF modules flanking a SEA domain, a putative transmembrane motif, and an intracytoplasmic tail (Fig. 8). BLASTing the two genomic sequences of MUC3A and MUC3B against the human genome sequence predicted that the two sequences belonged to the same genomic unit, suggesting that MUC3A and -3B may arise from a unique gene. The same situation is found when dealing with MUC11 and MUC12, and now both genes are referred as MUC12 according to the HUGO Gene Nomenclature Committee (132).

- ***MUC1, MUC4, MUC16.*** These three mucins are composed of two dissimilar subunits dimer. The larger subunit of MUC1 and MUC4 is almost entirely composed of the VNTR domain, and the one of MUC16 is also predominated by tandem repeats (133, 134) (Fig. 8). The smaller subunit of these mucins consists of a short extracellular region, the single-pass transmembrane domain, and the CT. Both MUC1 and MUC4 N-termini contain a signal peptide that directs localization of the mature protein to the apical membrane in polarized epithelial cells (135-137). The main differences between these mucins, besides the number of tandem repeats and their size that differs in all them, are: a) MUC1 has one SEA domain, MUC4 none, and MUC16 has 16 SEA domains, only the second resembling the one found in MUC1 (Fig amb functions dels dominis) (134), and b) MUC4 has imperfect repeats at the beginning of the N-termini region, and contains NIDO (Nidogen homology region), AMOP (adhesion-associated domain in MUC4 and other proteins), vWF and EGF domains in the C region (138, 139) (Table 5).

- ***MUC13, MUC14, MUC15, MUC18, MUC20.*** This group of mucins has been more recently identified as mucin family members, and less information on their regard is reported. MUC13, whose gene is located at locus 3q13.3, shares some homology with the 7q22 mucins, in particular in the SEA, EGF and transmembrane domains (101). MUC14 (also EMCN, endomucin), MUC15, and MUC18 (MCAM, melanoma cell adhesion molecule) encode numerous serine and threonine residues, but do not encode TR domains in their protein backbones (102, 103, 107). MUC20 is located close to MUC4 on chromosome 3q29. It contains at least four exons and its protein backbone contains a mucin tandem repeat of 19 amino acids consisting of many Ser, Thr, and Pro residues (109).

Table 5. Function of mucin structural domains.

Domain	Function	In which Mucin
Signal sequence	Directs localization of the mature protein	MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC19
N- and C-terminal cleavage regions	Involved in mucin packaging within secretory granules	MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC16
Central domain (VNTR)	Provides multiples anchorage sites for glycosylation	All mucins except for MUC14, MUC15, MUC18
vWF-like domains	Allow polymerization of mucins by interchain disulfide bond formation	MUC2, MUC4, MUC5AC, MUC5B, MUC6, MUC19
Intra-VNTR region Cys domains	Involved in folding or intracellular packaging, or in lectin-type interactions	MUC2, MUC5AC, MUC5B
EGF-like domains	Mediate interactions with other transmembrane proteins	MUC3A, MUC3B, MUC4, MUC11, MUC12, MUC13, MUC17
Sea domain	Cleavage site for transmembrane mucins. Might also be involved in binding to neighboring carbohydrates.	MUC1, MUC3A, MUC3B, MUC4, MUC11, MUC12, MUC13, MUC16, MUC17
Transmembrane domain	Allows anchorage to cellular membrane	MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17
Cytoplasmic tail	Involved in intracellular signaling	MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17
NIDO domain	Unknown	MUC4
AMOP	Might be implicated in adhesion process	MUC4

N-, amino; C-, carboxi; VNTR, variable numbe of tandem repeats; vWF, von willebrand factor; EGF, epidermal growth factor; SEA, sperm protein, enterokinase, and agrin; NIDO, nidogen homology sequence; AMOP, adhesion-associated domain in MUC4 and other proteins.

4. Biosynthesis and secretion.

Mucin biosynthesis and intracellular trafficking involve three major cellular compartments: the endoplasmic reticulum, the Golgi complex and the mucin granules. In the endoplasmic reticulum mucins are folded, N-glycosylated and likely C-mannosylated, and form disulfide-bond dimers (127, 140). The bulk of O-glycosylation occurs once the mucin precursors reach the Golgi complex, where sulfation, formation of mucin disulfide-bond oligomers/mutimers and proteolysis also take place (141-144). Inside mucin granules, mucins are accumulated and stored until proper regulatory signals triggers granule exocytosis (145) (Fig. 9).

Mucin assembly is a sequential process which begins with the formation in the endoplasmic reticulum of interchain disulfide bonds that

link the CK-domains obtaining mucin dimmers, and ends in the acidic compartments of the Golgi complex, where additional disulfide bonds are established among the N-terminal D-domains obtaining multidimers.

While N-glycosylation occurs cotranslationally in the RE (146), O-glycosylation is initiated in the *cis*-Golgi by addition of N-acetylglactosamine (GalNAc) to serine and threonine residues in central TR rich regions. O-glycan is then elongated by the addition of hexoses [galactose (Gal), N-acetylglucosamine (GlcNAc), fucose] or sialic acid by specific glycosyl tranferases (GT)(147, 148).

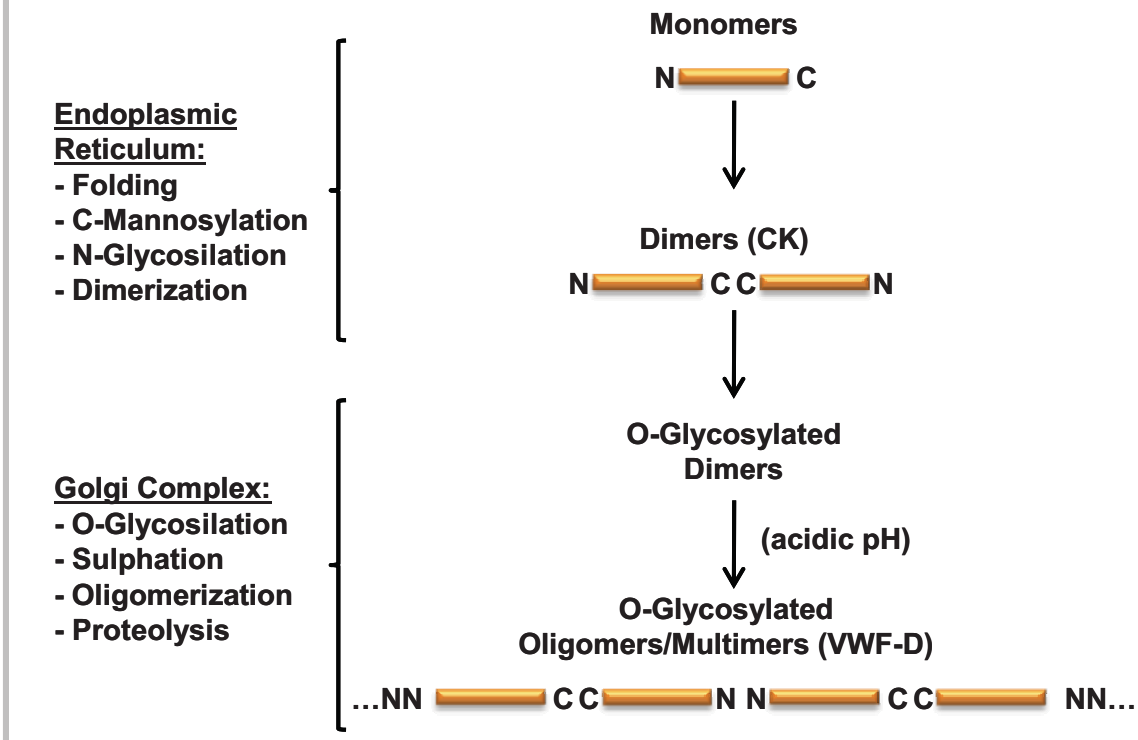


Figure 9. Schematic representation of gel-forming mucin assembly. Immediately after its synthesis and translocation into the endoplasmic reticulum, the polypeptide chain is N-glycosylated and forms disulfide-bonded dimers through its COOH-terminal Cysteine-Knot (CK) domains. In parallel, C-mannosylation occurs in the Cysteine-rich domains. The dimers are then transported to the Golgi complex and the tandem repeats are O-glycosylated. In the *trans*-Golgi compartments O-glycosylated dimers are assembled into disulfide-bonded multimers though their NH2-terminal von Willebrand factor D-like domains (VWF-D).

Once disulfide-bond oligomers/multimers are assembled in the trans-Golgi compartments, they are sorted and accumulated into condensing granules. During the transition from condensing granules to mature storage granules, an increase in electron density occurs (149), which suggest that mucins become more concentrated. The Ca^{2+} and the H^{+} content of the mature granules is high (150, 151). These ions would bind to sialic and sulfate groups in mucins O-glycans, promoting strand cross-linking and further entanglement (152).

The exocytosis process by which mucins are secreted to the extracellular media is not completely known. What seems to be clear is that all the mucin is delivered to the exterior via storage granules and no vesicular non-storage constitutive way occurs (153). Probably the formation of storage granules before escaping from the cell is essential to concentrate mucins to an optimal physical state. Granules in mucin storage granules seem to have two locations: peripheral and central. It seems that mucins stored in central granules are released by compound exocytosis, a regulated event that requires secretagogue stimulation such as cholinergic agonists, proteases, arachidonic acid metabolites, secreted inflammatory cell products and pathogens (154, 155), while single granules located at the periphery are released without stimulation in a constitutive manner (156).

After secretion, the expansion of the polymers is an important factor in determining mucus *gel* properties (157). The influence of the extracellular environment, mainly water availability and ionic composition, at the point of mucin secretion is increasingly recognized as a major factor in determining the physical properties of mucus.

5. Distribution and functions in healthy tissues.

Mucins exhibit a highly ordered tissue distribution, indicating a tight regulation of their expression. Some, such as the membrane-tethered mucins, MUC1 and MUC4, are present in multiple tissues. Others have more limited expression, such as MUC2 predominantly in the intestine. The airway

appears to produce the largest variety of mucins (46) and provides examples of cell-specific expression. MUC5AC is produced and secreted by goblet cells of the airway luminal epithelium, while MUC5B is secreted by airway submucosal glands (46) (Table 6). Carcinomas, which are derived from epithelial cells, frequently show an altered expression of mucins compared to their normal counterparts (158). Disregulation of mucin expression also frequently accompanies inflammatory responses (46).

Although all mucins have a major role in the innate defense, of the systems that they cover, against infective agents, particles and toxins, many other functions are carried out by these macromolecules through both, their structural protein backbone domains and their multiple carbohydrate chains. The dazzling display of diverse carbohydrate chains suggest that mucins may bind most of the bacteria, viruses, and inhaled particles, thus mediating the clearance of almost all inhaled substances from airways (122, 160). In addition, a network of mucins would also provide a strongly negatively charged milieu around the epithelium and thus a repulsive force to aid in the expulsion of bacteria. While secreted mucins are mainly involved in the protection role of the mucus, membrane-tethered mucin functions are related to both protection and cell signaling pathways.

5.1. Secreted mucins. In normal physiology, the secreted mucins, in particular the polymeric mucins MUC5AC and MUC5B provide the organizing framework of the airways mucus *gel* and are major contributors to its rheological properties (161). In this way, MUC2, MUC5AC and MUC5B are directly involved in the barrier and protective functions of mucus. In the stomach there is a clear example of how mucin glycans protect the epithelium from pathogens. The dual role of MUC5AC and MUC6 mucins defend the gastric epithelium from *Helycobacter pylori* combining specific glycan structures (Le^b and syalyl Le^x) on MUC5AC that act as ligands for the bacterium (162), and the terminal α1-4-linked GlcNAc residues of MUC6 which function as an antibiotic (163).

Table 6. Human mucin genes: tissue expression (158, 159).

Human Mucin	Main tissue Expression	Tissue Distribution
MUC1	Breast, pancreas	Cornea, salivary glands, esophagus, stomach, pancreas, large intestine, lung, breast, prostate, ovary, kidney, uterus, cervix, dendritic cells, nose
MUC2	Jejunum, ileon, colon	Conjunctiva, middle ear, stomach, small intestine, colon, nasopharynx, lung, prostate, nose
MUC3A	Colon, small intestine	Thymus, small intestine, colon, kidney
MUC3B	Colon, small intestine	Small intestine, colon
MUC4	Airway, colon	Cornea, lung, salivary glands, esophagus, small intestine, kidney, endocervix, nose
MUC5AC	Airway, stomach	Conjunctiva, middle ear, stomach, gall bladder, nasopharynx, lung, nose
MUC5B	Airway, submandibular glands	Middle ear, sublingual gland, laryngeal submucosa glands, esophageal glands, stomach, duodenum, gall bladder, nasopharynx, lung, nose
MUC6	Stomach, ilium	Stomach, duodenum, gall bladder, pancreas, kidney
MUC7	Sublingual and submandibular glands	Lacrimal glands, salivary glands, lung
MUC8	Airways	Nose, lung
MUC9	Faloppian tubes	Oviduct
MUC11	Colon, airway, reproductive trat	Middle ear, thymus, lung, small intestine, pancreas, colon, liver, kidney, uterus, prostate
MUC12	Pancreas, colon, uterus, prostate	Middle ear, pancreas, colon, uterus, prostate
MUC13	Colon, trachea	Conjunctiva, stomach, small intestine, colon, lung, kidney
MUC14	Endothelium	Endothelium
MUC15	Colon, airway, small intestine, prostate	Conjunctiva, tonsils, thymus, lymph node, breast, small intestine, colon, liver, spleen, prostate, testis, ovary, leukocytes, bone marrow
MUC16	Ovarian epithelial cells	Conjunctiva, ovary
MUC17	Duodenum, colon	Intestinal cells, conjunctiva epithelium
MUC18	Lung, breast	Prostate, airway, breast
MUC19	Salivary gland, trachea	Salivary gland, lung, kidney, liver, colon, placenta, prostate
MUC20	Kidney	Lung, liver, kidney, colon, placenta, prostate

5.2. Membrane-tethered mucins. Just like secreted mucins, membrane-tethered mucins are involved in the epithelium protection. For example, the bacterial protein flagellin interacts with the Muc1 extracellular domain (164) and stimulates cellular signaling pathways that may represent the initial stages of host response to infection (165). Membrane-tethered

mucins, particularly MUC1, MUC4, and MUC16 seem to have both anti- and proadhesion capacities. In this regard, MUC1 bind intracellular adhesion molecule-1 (ICAM-1) (166) through its extracellular domain peptide core and develops adhesion and motility functions (167). In addition, MUC1-CT domain affects multiple signaling pathways through interactions largely regulated by phosphorylation (168-171). A proadhesive function has been attributed to MUC16 due to its ability to bind the tumor marker mesothelin (172). On the other hand, MUC4 interacts with ErbB2 via EGF-like domains, inducing ErbB2 phosphorylation. The ErbB2-MUC4 complex then stimulates phosphorylation of p38 MAPK to promote survival and differentiation (173, 174). In addition, ErbB2 receptor has been found to modulate epithelial cell proliferation following damage in airways of asthmatics (175).

5.3. Non-mucin components. Although the mucins are major molecular constituents of mucus, mucus contains many other proteins (176) and non-proteins components (177). One of the important roles for mucus is the retention of these non-mucin secreted molecules in the immediate extracellular environment. There is evidence that some non-mucin proteins are either covalently or non-covalently associated with the extracellular complex (178-180). Some of these molecules are trefoil factors (TFFs), which have been described to interact to the cysteine-rich vWF C domains at the C termini of both MUC2 and MUC5AC (181), interactions probably involved in the modulation of the rheological properties of the mucus *gel*.

Molecules involved in host defense from infection, including secretory IgA (sIgA), collectins, defensins, and histatins are also present in respiratory mucus. These molecules may be retained simply by the biophysical properties of mucus, although direct interaction between them and mucins has been demonstrated. For instance, MUC5B appears to form noncovalent complexes with sIgA, amylase, proline-rich proteins, and histatins in salivary and bronchial secretions (179, 182-184).

Chapter 3 summary

Mucins are major component of the mucus that covers and protects the respiratory, gastrointestinal, and urogenital tracts. They are high-molecular-weight glycoproteins mainly produced and secreted by goblet cells in the epithelium and mucous cells in submucosal glands of the tissue mucosa. To date, more than 20 MUC genes encoding for mucins have been described. They have been subdivided in membrane-tethered (10 mucins) and secreted mucins (8 mucins), and there are some others not yet classified.

Although all mucins share a common structure with a variable number of tandem repeats of regions rich in serine and threonine residues and a highly glycosylated central domain, they have structural differences closely related to their functions. In this manner secreted mucins seem to be involved in mucosa protective functions while membrane-tethered mucins play a role in cell signaling cascades related to adhesion, migration, and proliferation.

Although mucins have been found to be expressed in a great variety of tissues, some of them show tissue- and even cell-specificity. Moreover, their cellular and tissular distribution appears to be altered in pathologic conditions. In this way, future goals for research in the biology of mucins must not only be focused on what goes wrong in chronic inflammatory diseases associated to mucus hypersecretion but also in the normal innate immune defense role that mucins play in healthy tissues.

In the next chapter a review on airways mucin expression in health and disease, pathologic regulation of mucins, and treatment of mucus hypersecretion is done.

Chapter 4. Airways mucins

Chapter 4. Airways Mucins

1. Mucin gene expression in the respiratory tract.

Among all the identified mucin genes, at least 12 human mucin genes (MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC7, MUC8, MUC11, MUC13, MUC15, MUC19, and MUC20) have been found to be expressed in the respiratory tract of healthy individuals (101, 103, 108, 109, 185, 186). Notwithstanding that, only MUC5AC and MUC5B have been convincingly demonstrated to be major components of human airways secretions (187, 188), representing 90% of the mucin content in the sputum. Nearly all the remaining 10% is made up of three membrane-tethered mucins, MUC1, MUC4, and MUC16 (189) (Fig. 10).

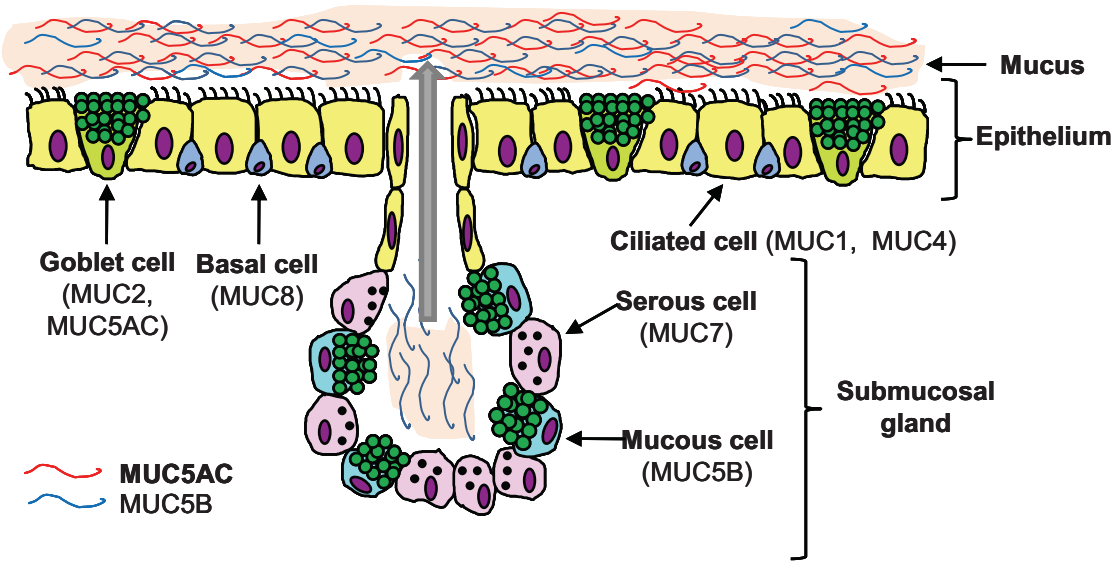


Figure 10. Mucins on the respiratory epithelium. A schematic representation of the respiratory epithelium showing basal, ciliated, and goblet cells in the surface epithelium and mucous and serous cells in a submucosal gland. MUC2 and MUC5AC (red) are produced mainly in epithelial goblet cells while MUC5B (blue) and MUC7 are produced mainly in mucous and serous cells of submucosal gland, respectively. Ciliated cells produce MUC1 and MUC4 mucins while basal cells produce MUC8. Only MUC5AC and MUC5B are demonstrated to be major secreted mucins in airways. Mucins are secreted directly into the epithelial surface or are secreted into the gland ducts and released onto the surface (grey arrow). The rest of mucins known to be expressed in the airways are omitted since their amount and/or cellular localization is unclear.

MUC1 is a pan-epithelial membrane-tethered mucin expressed at the cell surface in numerous epithelial tissues, including the respiratory tract, as well as in hematopoietic tissues (190). In airways tissue from healthy individuals, goblet cells typically express MUC5AC mRNA and protein, while mucous cells in submucosal glands express MUC5B, MUC8, and MUC19 (108, 191). In addition, MUC2 and MUC4 are mainly expressed in epithelial cells while MUC7 is typically expressed in glands (186). Current thinking is that the expression of MUC5B in goblet cells in human airways epithelium is atypical and may be a marker of airways diseases (192).

2. Mucins in airway diseases.

In hypersecretory diseases of the airways, the overproduction of mucus with abnormal mucin composition is a pathological feature that affects the rheological properties of mucus. Hypersecretion of mucus contribute to the innate mucosal defense system against allergens, infectious pathogens, and environmental toxins. Paradoxically, these hypersecretory responses are a major contributor to the pathology of diseases such as cystic fibrosis (CF), asthma, and COPD. In some circumstances, tethering of the mucus to the epithelium has been observed, and rather than maintaining and sterile airway, mucus provides and environment within which bacteria in particular can flourish. Such chronic inflammatory exposure exacerbated the problem and is and important aspect of the morbidity and mortality in these common airways disorders. In patients with chronic airways inflammatory diseases, increased mucin production in the absence of exacerbation likely reflects goblet cell hyperplasia (GCH) in the airway epithelium, characteristic feature of these diseases (193-195) (Fig. 11), which increases the baseline level of mucin production. On the other hand, in exacerbations episodes induced by exposure to allergens or viral infections, a massive mucin hypersecretion occurs, probably due to the high number of goblet and/or mucus cells ready to respond to the sudden increase of inflammatory mediators. In addition,

increased mucin biosynthesis is likewise easily maintained because of the high number of MUC templates accessible to inflammatory mediators.

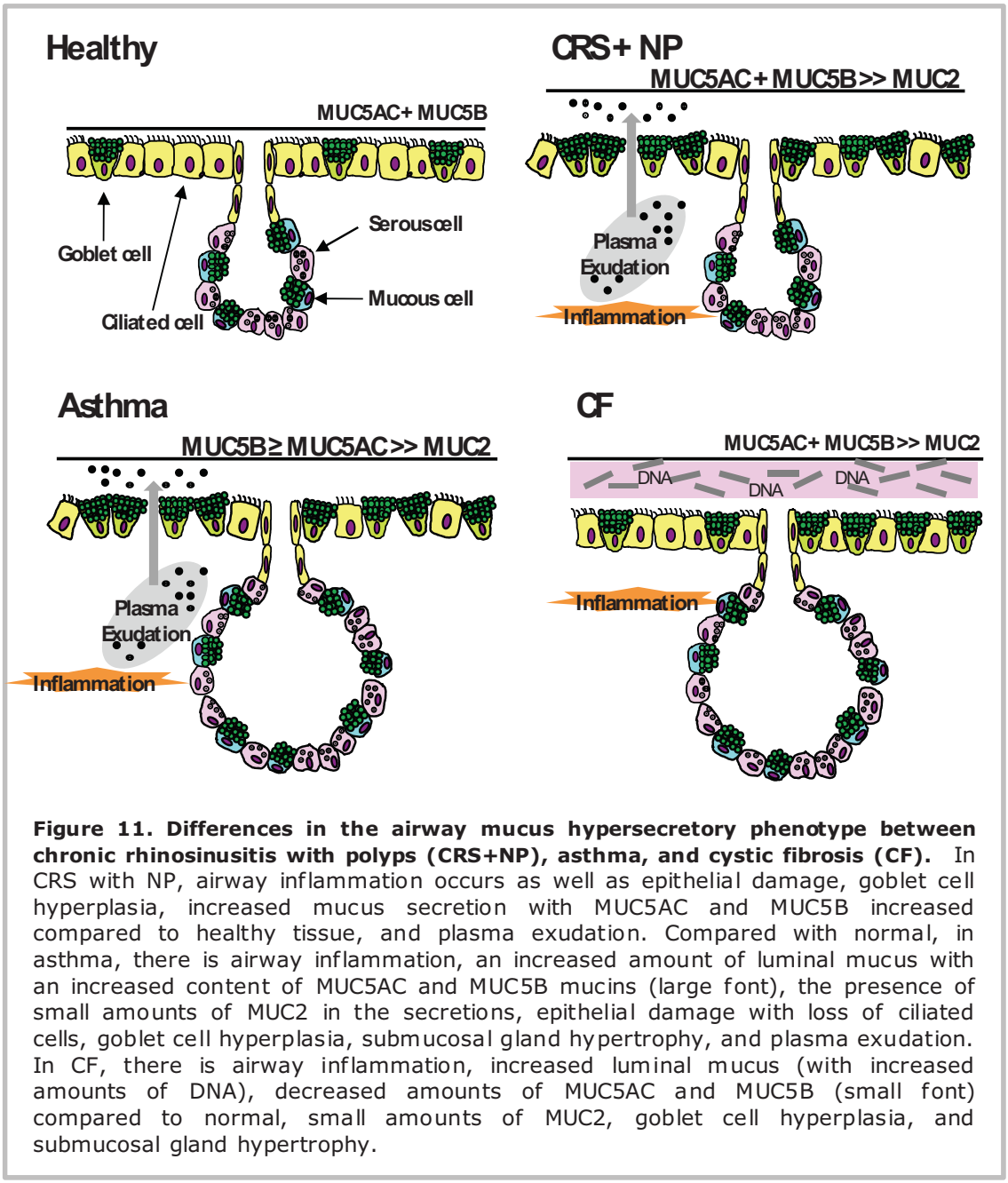


Figure 11. Differences in the airway mucus hypersecretory phenotype between chronic rhinosinusitis with polyps (CRS+NP), asthma, and cystic fibrosis (CF). In CRS with NP, airway inflammation occurs as well as epithelial damage, goblet cell hyperplasia, increased mucus secretion with MUC5AC and MUC5B increased compared to healthy tissue, and plasma exudation. Compared with normal, in asthma, there is airway inflammation, an increased amount of luminal mucus with an increased content of MUC5AC and MUC5B mucins (large font), the presence of small amounts of MUC2 in the secretions, epithelial damage with loss of ciliated cells, goblet cell hyperplasia, submucosal gland hypertrophy, and plasma exudation. In CF, there is airway inflammation, increased luminal mucus (with increased amounts of DNA), decreased amounts of MUC5AC and MUC5B (small font) compared to normal, small amounts of MUC2, goblet cell hyperplasia, and submucosal gland hypertrophy.

In chronic rhinosinusitis with/ without nasal polyps, the composition of mucus secretions may also be altered by the inflammatory process, and mucus viscosity usually increases, leading to mechanical obstruction of the sinus ostia and impaired mucociliary transport. Mucostasis caused by the

altered viscoelastic properties of mucus secretions may aggravate the already present mucositis. On the other hand, aspiration of infected sinus secretions into the lungs during sleep as well as the production in the infected sinus of cytokines and bronchoconstrictive mediators have been implicated in the development of bronchial asthma associated with chronic sinusitis (158).

Regarding the production of the three gel-forming mucins expressed in the upper airways, several studies have demonstrated that upper airway epithelial goblet cells express MUC2 and MUC5AC, and that mucous cells in submucosal glands express MUC5B (24, 280, 303). A similar distribution seems to be found in nasal polyps (24, 300, 303) although the healthy nasal mucosa and the pathologic tissue differ in mucin amounts. For instance, MUC8 mRNA expression has been found increased and MUC5AC mRNA decreased in bilateral nasal polyps compared to normal inferior turbinates (299). MUC8 was also found increased, at both mRNA and protein levels, in chronic rhinosinusitis mucosa (284). By contrast, several studies have described an increased expression of MUC5AC in bilateral nasal polyps (300, 328). These discrepancies may be due to the different samples used in the mentioned studies.

The distribution and differential expression of mucins in airways diseases such as chronic rhinosinusitis with/out nasal polyps, cystic fibrosis, asthma, and allergic rhinitis is thoroughly discussed in the review entitled "Mucin gene expression in rhinitis syndromes" by Martínez-Antón et al., attached to this thesis in *chapter 5* (196). Regulation of mucin expression related to each of these pathologies is also showed in the mentioned review.

3. Mucin regulation in diseased airways.

Respiratory tract infection and inflammation are characteristic features of patients with asthma, COPD, CF, and CRS. Bacterial or viral interactions with host trigger the activation of signal transduction pathways

that modulate host responses and increase expression of inflammatory genes. Many inflammatory mediators such as TNF-α, IL-1β, IL-6, and bacterial and inflammatory cell products are elevated in chronic airway diseases (197-201). These mediators have been shown to stimulate mucin gene expression by: 1) selectively controlling MUC gene steady-state equilibrium, and/or 2) regulating expression of MUC genes at the transcriptional and/or postranscriptional level.

The stimuli that upregulate mucin gene expression can be broadly categorized into: a) inflammatory cytokines, b) bacterial products, c) growth factors, d) environmental chemicals or pollutants (smoke, ozone), and e) miscellaneous chemical agents. Bacterial products and cytokines have been the most extensively studied because of their secretion into pathologic airways (46) (Tables 7-9).

The main regulatory regions found in MUC2, and MUC5AC which are involved in their regulation pathways are shown in figure 12 (202-206).

3.1. Bacterial products and viruses. Since a major function of mucins is to protect epithelia from infection, its not surprising that bacterial products can alter mucin gene expression. *Pseudomomas aeuroginosa* and *Staphylococcus aureus* (*S. aureus*) are the primary pathogenic bacteria found in the airways of CF patients, while *S. aureus* and *Haemophilus influenza* are common pathogens that exacerbate bronchitis in patients with COPD. The lipopolysaccharide (LPS) and flagellin of *P. aeuroginosa*, the lipotheichoic acid (LTA) in the *S. aureus* cell wall, and *H. influenzae* are capable to upregulate mucin expression in airway epithelial cells, especially for MUC2 and MUC5AC mRNA by different signaling pathways (Table 7). LPS MUC2-activation involves MAPK signaling pathway with downstream activation of nuclear factor κB (NFκB) (204). MUC5AC activation by LPS involved also a MAPK pathway via a previous epidermal growth factor receptor (EGFR) signaling cascade (207).

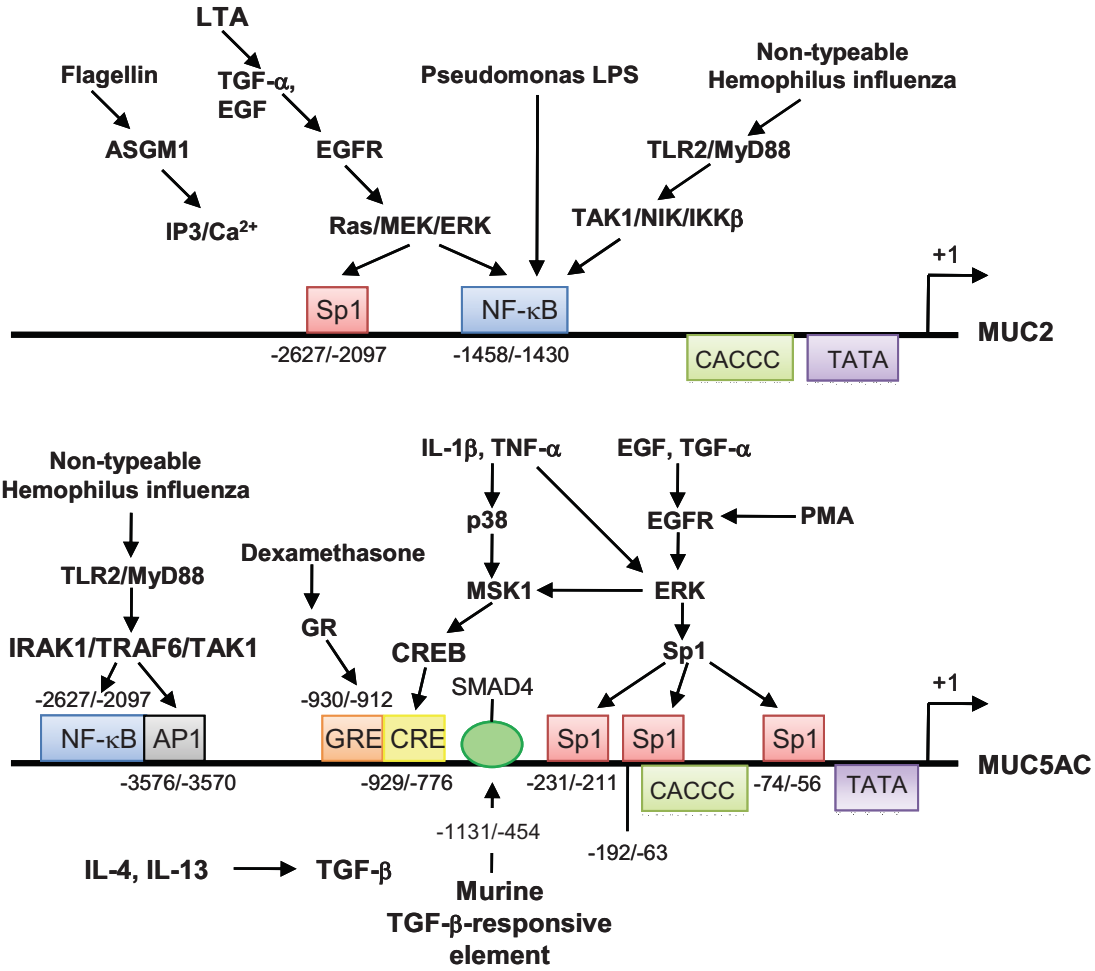


Figure 12. An outline of 5' promoter regulatory regions for MUC2 and MUC5AC. Although this picture is not comprehensive by itself, some specific pathways are provided to illustrate the stimulus and signal transduction pathways involved in MUC gene regulation that are explained in the text and in tables 7, 8, 9.

LTA can activate MUC2 expression by a downstream cascade similar to the one activated by LPS, but the first steps involve platelet activating factor receptor (PAFR) and ADAM10 (a matrix metalloprotease) and EGFR activation, followed by the MAPK cascade (208) (Table 7).

H. influenzae regulates MUC2 transcription by activating transforming growth factor (TGF)-β receptor, inducing Smad3/4 complex activation and downstream NFκB activation. Alternatively, *H. influenzae* can also bind the toll-like receptor (TLR)2, and activate MyD88, TAK1, NIK1, and IκKβ/δ. This

signal cascade results in NFκB-mediated transcription of the MUC2 gene (209). *H. influenzae* also upregulates MUC5AC expression by a pathway involving TLR2 and MyD88, followed by a p38 MAPK pathway (210) (Table 7).

Table 7. Regulation of human MUC gene expression by bacterial extracts and exoproducts in airways cells.

Stimuli	Mucin	Effect and via	Cell type	References
Pseudomonas aeuroginosa	2	Upregulation via Src-dependent Ras-MEK1/2-ERK1/2-pp90rsk-NFκB	NCI-H292	204
	5AC*	Upregulation via TACE mediated TGFα release and EGFR activation	NCI-H292	213
Staphyloccocus aureus LTA	2	Upregulation via PAFR→ G protein→ ADAM10→release of EGF from HB-EGF →EGFR→Ras→ MEK1/2→ NFκB	NCI-H292	208
Haemophilus influenzae	2	Upregulation via TLR2→MyD88→TAK1 →NIK→IκKβ/γ→IκBα→ NFκB	NHBE	209
	2	Upregulation via TGFβ→RI/II→ Smad3/4→ NFκB	NHBE	209
	5AC	Upregulation via TLR2→MyD88→p38 MAPK	A549	210
	5AC	Negatively regulated by PI3K→Akt	A549	210
Flagellin	2	Upregulation via asialoGM1→ATP release→autocrine/paracrine binding to G protein-coupled P2Y2 R→PLC activation→I3P production→Ca2+ mobilization →MEK1/2→ ERK1/2→ transcription factor activation	NCI-H292	211
Bordatella pertusis	2 5AC	Transcriptional upregulation	BEAS2B	214
ds RNA	2	Transcriptionl upregulation: ATP release→P2Y receptor→PLC→PKC→ p38 MAPK→NFκB	NCI-H292	212

All changes refer to mRNA level except for marked mucins (*), which refers to both mRNA and protein levels. A549, human lung carcinoma cell line; ADAM10, A disintegrin and metalloprotease; asialoGM1, asialoganglioside tetraosylceramide; ATP, adenoside 5'-triphosphate; BEAS-2B, human bronchial epithelia cell line; EGF, epidermal growth factor; EGFR, EGF receptor; ERK, extracellular signal-regulated kinase; HB-EGF, heparin binging-EGF; I3P, inositol triphosphate; IκKβ/γ, IκB kinase; LTA, lipoteichoic acid; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MyD88, myeloid differentiation primary response gene; NCI-H292, human lung mucoepidermoid carcinoma cell line; NFkB, nuclear factor κB; NHBE, normal human bronchial epithelial; NIK, NFκB inducing kinase; P2Y2, purinoceptor 2Y2; PAFR, platelet activating factor receptor; PI3K, phosphoinositide 3-Kinase; PKC, protein kinase C; PLC, phospholipase C; pp90rsk, 90-KDa ribosomal S6 kinase; TACE, TNF-α converting enzyme; TAK, TGF activated kinase; TGF, transforming growth factor; TLR, toll-like receptor.

P. aeuroginosa flagelin binds to the epithelial cell surface ganglioside, ASMG1, causing release of ATP and subsequent activation of P_2Y (a G protein-coupled receptor), with downstream activation of phospholipase C (PLC), formation of inositol triphosphate, and calcium mobilization. This triggers a MAPK pathway which results in MUC2 transcription (211) (Table 7).

Table 8. Regulation of human MUC gene expression by inflammatory cytokines in airways cells.

Stimuli	Mucin	Effect and via	Cell type	References
TNF-α	2	Increased expression. Regulation inhibited by PKC and Tyrosine Kinase inhibitors	NCI-H292	219
	5AC	Posttranscriptional regulation	NCI-H292	220
	2, 5AC	Regulation inhibited by RARα antagonist	NCI-H292	221
	5AC	Upregulation via ERK and p38 MAPK → MSK1→CREB →CRE	NHNE, NCI-H292	205
	1	Upregulation	NHNE	223
Cytokine-activated eosinophils	5AC*	TGF-α→EGFR activation	NCI-H292	224
IL-1β	2, 5AC	Upregulation via ERK and p38MAPK→COX2 →PGE_2	NCI-H292	222
	2, 5AC	Regulation inhibited by RARα antagonist	NCI-H292	221
	5AC	Upregulation via ERK and p38MAPK→ MSK1→CREB→CRE	NHNE, NCI-H292[#]	205
	8	Upregulation via ERK→RSK1→CREB→CRE	NHNE, NCI-H292[#]	225
IL-4	5AC, 5B	Decreased mRNA expression	$NHBE_{ALI}$	226
	5AC, 5B	No effect	$NHBE_{ALI}$	227
IL-5	5AC, 5B	No effect	$NHBE_{ALI}$	227
IL-9	5AC	Upregulation	NHBE, NCI-H292	228
	2, 5AC	Upregulation	NCI-H292	229
	5AC, 5B	No effect	$NHBE_{ALI}$	227
IL-13	5AC	No effect	A549, NCI-H292	230
	5AC, 5B	No effect	$NHBE_{ALI}$	227
	5AC	Decreased expression	NHNE	231
	2, 8	Increase expression	NHNE	231
IL-6/IL-17	5B	IL-6 activates cells in autocrine/paracrine fashion→ERK; IL-17→JAK2→IL-6 secreted	$NHBE_{ALI}$	227

All changes refer to mRNA level except for marked mucins (*), which refers to protein level.[#] Pathway studied in NCI-H292. A549, human lung carcinoma cell line; COX-2, cyclooxygenase-2; CRE, cAMP-response element; CREB, cAMP-response element binding protein; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; IL, interleukin; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; MSK1, mitogen-and-stress-activated protein kinase 1; NCI-H292, human lung mucoepidermoid carcinoma cell line; NHBE, normal human bronchial epithelial cells; $NHBE_{ALI}$, NHBE cells grown in air-liquid interface culture system; NHNE, normal human nasal epithelial cells; PGE_2, prostaglandin E_2; PKC, protein kinase C; RAR, retinoic acid receptor; RSK1, p90 ribosomal S6 protein kinase 1; TGF, transforming growth factor; TNF-α, tumor necrosis factor-α.

Respiratory viruses also induce mucin overproduction during infection and additionally trigger exacerbations in patients with asthma, bronchitis, or CF. Double-stranded (ds) RNA is a biologically active component of many respiratory viruses. In this way, a synthetic ds RNA is able to upregulate MUC2 expression in NCI-H292 cells through inducing ATP release and PLC

stimulation, resulting in PKC activation and finally NFκB activation via the p38 MAPK (212) (Taula 7).

3.2. Inflammatory cytokines. Like bacterial products, inflammatory cytokines induce MUC2 and MUC5AC expression in airway epithelial cells. IL-1β and TNF-α are common proinflammatory cytokines that are elevated in both airway and non-airway related disorders. In this regard, airways diseases with mucus cell hyperplasia have increased levels of these cytokines in the blood as well as in the airway tissue (45). IL-1β and TNF-α induce mucous cell hyperplasia in vivo in mouse models (215, 216). Both cytokines can also activate NFκB signaling in airway epithelial cells (217, 218), which can lead to the activation of MUC2 and MUC5AC. TNF-α was shown to increase MUC2 mRNA steady-state expression in NCI-H292 cells (219). TNF-α also increases the abundance of MUC5AC, but not MUC5B, mRNA and does so by increasing the stability of MUC5AC mRNA at the postranscriptional level (220), although this cytokine also can regulate MUC5AC expression at the transcriptional level (205, 221) (Table 8).

IL-1β, induces MUC2 and MUC5AC in H292 cells and normal human nasal epithelial (NHNE) cells via MAPK activation of both ERK1/2 and p38 pathways. Three different downstream mechanisms have been described for this activation (205, 221, 222) (Table 8).

Th2 cytokines such as IL-4, IL-9, IL-13 have controversial effects on mucin regulation. Steady-state analyses of mucin mRNA expression following exposure to Th2 cytokines, has shown that neither IL-4 nor IL-13 upregulates MUC5AC mRNA expression in human airway epithelial cancer cell lines (230) or in differentiated normal human bronchial epithelial (NHBE) cells (197). Another study shows that IL-4 decreases MUC5AC expression in differentiated NHBE cells (226). However a different situation is found regarding murine Muc5ac gene, as IL-13 has been reported to increase the promoter activity of Muc5ac in murin clara cells (232). While IL-9 do not alter human MUC5AC or MUC5B mRNA expression in differentiated NHBE cells (227), this cytokine increases MUC5AC expression

in NHBE cells cultured under submerged conditions and in NCI-H292 cells (228). It also increases MUC2 and MUC5AC mRNA steady-state expression in H292 and NHBE cells (229). Steady-state analyses of mucin mRNA expression following exposure to Th2 cytokines, has shown that neither IL-4 nor IL-13 upregulates MUC5AC mRNA expression in human airway epithelial cancer cell lines (230) or in differentiated normal human bronchial epithelial (NHBE) cells (197) (Table 8).

The non-Th2 cytokines IL-6 and IL-17 also increase MUC5AC and MUC5B mRNA steady-state expression in differentiated NHBE cells. IL-17 upregulates MUC5B expression through a paracrine/autocrine loop mediated by ERK signaling via JAK2-dependent signaling (227) (Table 8).

3.3. Others stimuli. Ligand-dependent activation of EGFR increases transcription of MUC2 (208) and MUC5AC (233, 234) genes. In addition to the above mentioned studies demonstrating NFκB-mediated regulation, other mediators such as TGF-α, EGF, and TNF-α, transcriptionally regulate MUC2 and MUC5AC and do so via the Sp1 transcription factor following activation of the EGFR/Ras/RAF/ ERK1/2 pathways (233). (Table 9).

Neutrophils are the predominant inflammatory cells in the airways of patients with CF, chronic bronchitis, and in acute, severe exacerbations of asthma. Neutrophil elastase (NE), increases expression of MUC4 and MUC5AC (235-237). NE can regulate MUC5AC expression by at least two different mechanisms: 1) inducing an oxidant stress in A549 and NHBE cells, which results in MUC5AC as well as MUC4 posttranscriptional regulation (238); and 2) inducing the release of TGF-α resulting in EGFR activation and transcriptional regulation of MUC5AC in H292 cells (236) (Table 9).

Reactive oxygen species (ROS), which can be released by airway inflammatory cells, also regulate MUC5AC expression and do so by EGFR activation (234) and by TNF-α-converting enzyme (TACE) (213) (Table 9).

Acrolein, an aldehyde component of tobacco smoke, also increases MUC5AC expression (220). Tobacco smoke transcriptionally regulates MUC5AC by two different pathways: 1) ROS activation of EGFR via TACE-mediated release of amphiregulin or 2) EGFR-independent Sc-Jnk activation of JunD/Fra-2 binding to AP-1 cis-elements in promoters (239) (Table 9).

Table 9. Regulation of human MUC gene expression by miscellaneous stimuli in airways cells.

Stimuli	Mucin	Effect and via	Cell type	References
Neutrophil elastase	2, 5AC	Regulation inhibited by RARα antagonist	NCI-H292	221
	4*	Posttranscriptional regulation	NHBE	237
	5AC	Proteolytic activity required, posttranscriptional regulation	A549, $NHBE_{ALI}$	235
	5AC*	Upregulation via ROS	A549, $NHBE_{ALI}$	240
	5AC*	Upregulation via TGF-α→EGFR→MEK1/2→ERK	NHNE	236
PGE_2	5AC	Upregulation	NCI-H292	220
	5B	No effect		
	8	Upregulation via ERK→RSK1→CREB	NCI-H292	241
EGF family of ligands	5AC*	Augmented by TNF-α-mediated upregulation of EGFR surface expression→ERK/MAPK	NCI-H292	207, 233, 234
	2, 5AC	Upregulation via EGFR→Ras→Raf→ERK→SP1	NCI-H292	242
	5B	No effect	NCI-H292	242
Uridine 5-triphosphate	5AC, 5B	Pertussin toxin-sensitive G protein and MEK1/2-MAPK dependent; PKC and PLC independent	$NHBE_{ALI}$	243
Tobacco smoke	5AC*	TACE mediated-TGFα release→EGFR activation	NCI-H292	244
	5AC	Upregulation via ROS→Src-dependent JNK activation→JunD→AP-1 and/or EGFR→Ras/Raf-MEK→ERK→Fra2→AP-1	NCI-H292	239
Acrolein	5AC	Increased expression	NCI-H292	220
	5B	No effect		

A549, human lung carcinoma cell line; AP1, activator protein 1; CRE, cAMP-response element; CREB, cAMP-response element binding protein; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; JNK, Jun N-terminal Kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; NCI-H292, human lung mucoepidermoid carcinoma cell line; NHBE, normal human bronchial epithelial cells; $NHBE_{ALI}$, NHBE cells grown in air-liquid interface culture system; NHNE, normal human nasal epithelial cells; PGE_2, prostaglandin E_2; PKC, protein kinase C; PLC, phospholipase C; RAR, retinoic acid receptor; ROS, reactive oxygen species; RSK1, p90 ribosomal S6 protein kinase 1; SP1, specificity protein 1; TACE, TNF-α-converting enzyme; TGF, transforming growth factor; TNF-α, tumor necrosis factor-α.

3.4. Post-transcriptional regulation. Few studies have dealt with post-transcriptional regulation of MUC genes. Nevertheless, several inflammatory mediators have now been shown to regulate mucin genes at the post-transcriptional level. TNF-α, NE, and IL-8 increase MUC5AC

expression in human epithelial cells by increasing mRNA stability (220, 235). The mechanisms involved in this post-transcriptional regulation in airway cells remain to be determined.

4. Treatment of mucus hypersecretion.

The prevalence of presentation of cough and sputum production in asthma, CF, COPD patients, and the impact in quality of life of a chronically runny nose in patients with allergic rhinitis, indicates an important role for mucus in the pathophysiology of these conditions (245). Consequently, treatments are being developed to treat airway hypersecretion.

There are two objectives in the treatment of mucus hypersecretion, namely short-term relief of symptoms and long-term benefit. The first of these involves facilitating mucus clearance and entails changing the viscoelasticity of mucus, increasing ciliary function, and encouraging cough. Theoretically, cough clearance is optimized when there is high viscosity and low tenacity (product of adhesivity and cohesivity). Decreasing viscosity may not markedly change mucus clearance. Of greater importance is the degree of adhesion on the mucus to the epithelium: decreased adhesion is linked to increased clearance. Long-term benefit involves reversal of the hypersecretory phenotype and entails reducing the number of goblet cells and the size of submucosal glands. In addition, treatment of the airway inflammation would be expected to be associated with treatment of hypersecretion (245).

Although mucus hypersecretion is associated with morbidity and mortality in several airway diseases, there is some controversy concerning the therapeutic value of drugs that affect mucus properties. Nevertheless, numerous compounds have been developed aimed at alleviating mucus hypersecretion. These compounds can be classified as expectorants, mucolytics, mucokinetics, or mucoregulators (Table 10).

The conventional pharmacotherapy used currently in clinical management of respiratory diseases, namely bronchodilators

(anticholinergics, β_2-adrenoceptor agonists and methylxanthines) and anti-inflammatories (primarily glucocorticoids), are not administered necessarily to target airway hypersecretion, but may exert some of their beneficial effects via actions on mucus (246). Additionally, novel medications are being developed for inhibition of airway mucus hypersecretion, including inhibitors of mucin exocytosis and inhibitors of goblet cell hyperplasia (Fig. 13).

Table 10. Mucoactive agents and their mechanism of action (195)

Mucoactive agent	Putative mechanism of action
Expectorant	Increases volume and /or hydration secretions. May also induce cough (eg. hypertonic saline)
Mucolytic	Reduces viscosity of mucus. · Non-peptide ("classical") mucolytics cleave disulphide bonds. · Low-molecular-weight- saccharide mucolytics interfere with non-covalent interactions in mucus, and may osmotically pull water into airway lumen · Peptide mucolytics degrade deoxyribonucleic acid (DNA) or actin
Mucokinetic	Increases "kinesis" of mucus and facilitates cough transport of mucus · β_2-adrenoceptor agonists increases airflow, ciliary beat, Cl^-/water secretion, and mucin secretion (small effect). · Surfactant reduces mucus adherence to the epithelium
Mucoregulator	Reduces process of chronic mucus hypersecretion (eg. glucocorticoids, anticholinergics, macrolide antibiotics).

4.1. Glucocorticoids. Glucocorticoids are highly effective in the treatment of nasal polyposis (15) and asthma (245). Although glucocorticoids inhibit plasma exudation (247) and have been reported to inhibit spontaneous and cytokine-induced glandular secretion in respiratory mucosa explants (248, 249), it is unclear whether or not glucocorticoids have direct inhibitory effects on mucin secretion. They effectively suppress expression of inflammatory genes, including those coding for cytokines (71, 72), so the inhibition of GCs of mucus hypersecretion is likely to be via indirect effects.

4.2. Inflammatory mediator antagonists (antihistamines and antileukotrienes). Histamine and cysteinyl leukotrienes markedly contribute to pathophysiology and clinical symptoms in rhinitis and asthma. Histamine H_1 receptor antagonists are extremely effective in rhinitis (250), likely due to inhibition of endogenous histamine-induced nasal secretion (251). Cysteinyl leukotriene (Cys-LT) receptor antagonists have also been found to be effective in management of asthma and rhinitis (252) partly due to inhibition of mucus secretion (253).

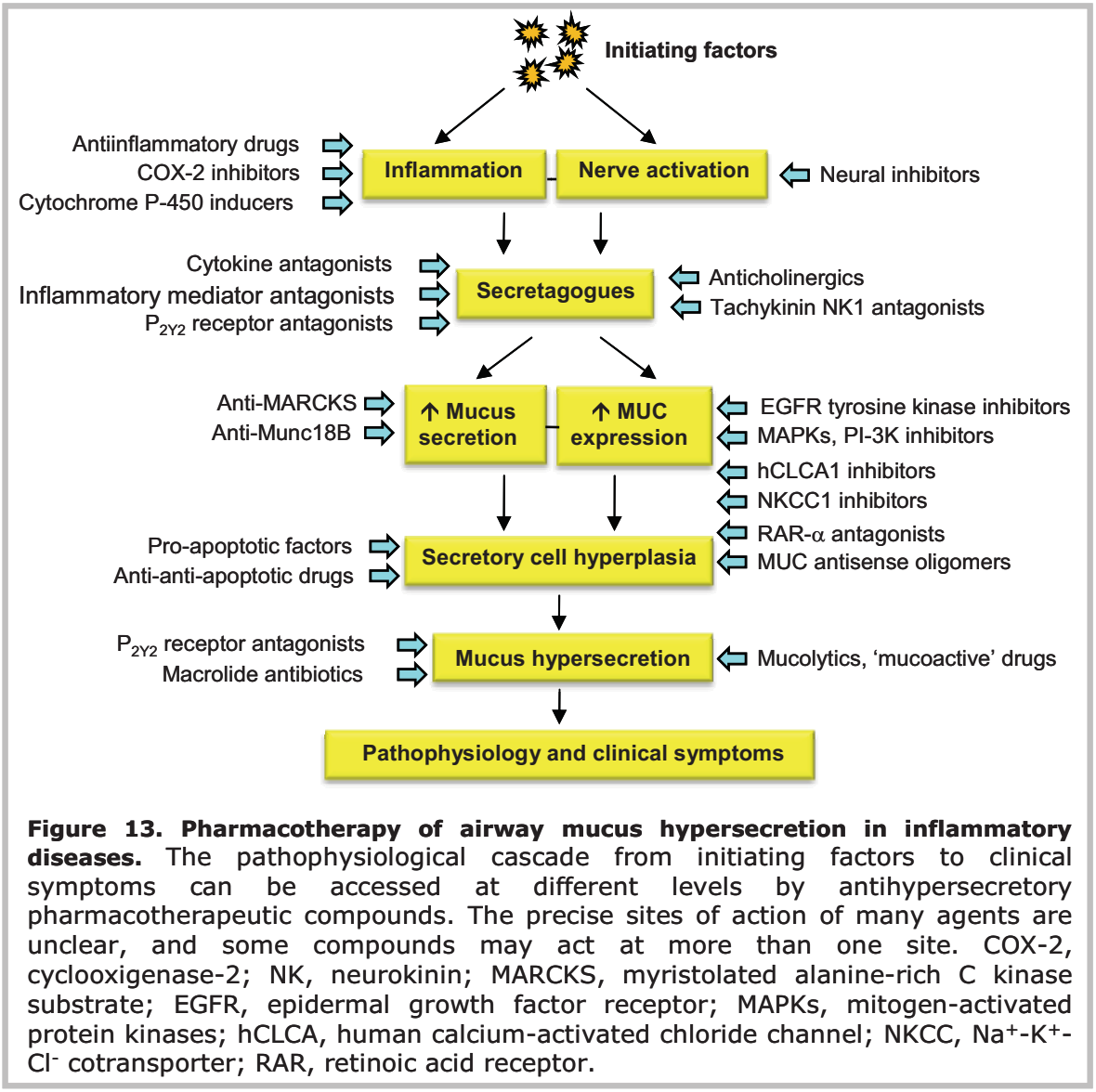


Figure 13. Pharmacotherapy of airway mucus hypersecretion in inflammatory diseases. The pathophysiological cascade from initiating factors to clinical symptoms can be accessed at different levels by antihypersecretory pharmacotherapeutic compounds. The precise sites of action of many agents are unclear, and some compounds may act at more than one site. COX-2, cyclooxigenase-2; NK, neurokinin; MARCKS, myristolated alanine-rich C kinase substrate; EGFR, epidermal growth factor receptor; MAPKs, mitogen-activated protein kinases; hCLCA, human calcium-activated chloride channel; NKCC, Na^+-K^+-Cl^- cotransporter; RAR, retinoic acid receptor.

4.3. Anticholinergics. The cholinergic nervous pathway represents the dominant neural stimulant to mucin secretion in the nose (254) and the lower airways (255). The mucus secretory response of submucosal glands to cholinergic stimulation is via muscarinic M_3 receptors, with water secretion mediated via M_1 receptors (10, 256-258). Glandular lactoferrin secretion has been reported to be enhanced by methacholine in bronchial and nasal mucosa, this effect being completely abrogated by the addition of atropine (248). However, it is unclear whether or not inhaled nonselective anticholinergics decrease mucus secretion or alter mucus viscosity (258).

4.4. N-acetylcysteine. N-acetylcysteine is a mucolytic compound with antioxidants properties. Reactive oxygen and nitrogen species are potent stimulants of mucus secretion (259). Consequently, antioxidants such as N-acetylcysteine as well as having some beneficial effects in inflammatory airway disease (260) might have clinical benefit for airway mucus hypersecretion.

4.5. Sensory efferent nerve inhibitors. The sensory neuropeptides facilitate several biologic activities, including effects on secretion. The tachykinins SP and NKA increase plasma exudation, an effect potentiated by the potent vasodilator activity of CGRP, and also increase mucin secretion via interaction to NK_1 receptors (254, 255). Numerous tachykinin receptor antagonists are in development, including antagonists selective for the NK_1, NK_2, or NK_3 receptor, but clinical trials are required to test their effectiveness on mucus hypersecretion.

4.6. Exocytosis inhibitors. Myristolated alanine-rich C kinase substrate (MARCKS) is a key signaling molecule in the intracellular pathways involved in mucus exocytosis (145). Blockade of MARCKS by a synthetic peptide inhibited mucin secretion by normal human bronchial epithelial cells in vitro (145) and by mouse airway epithelium in vivo (261). *Clostridium botulinum* neurotoxins (BoNT) could be targeted to airway secretory cells via a fusion ligand to selectively inhibit mucin exocytosis, and thereby reduce mucus output (262).

4.7. Mucin synthesis and goblet cell hyperplasia inhibitors. The EGFR and its tyrosine kinase appear to comprise a fundamental pathway involved in upregulation of mucin synthesis and goblet cell hyperplasia (263, 264). Inhibitors of EGFR tyrosine kinase block these responses and are in clinical trials for cancer, but not yet for airway hypersecretory diseases.

The p38 MAPK, the MEK/ERK, and the phosphatidylinositol 3-kinase pathways are all involved in extracellular events leading to mucin synthesis and goblet cell hyperplasia (210, 265-267). Inhibitors of these pathways inhibit mucus hypersecretory endpoints in experimental systems.

Calcium-activated chloride (CLCA) channels appear to be critically involved in development of and airway hypersecretory phenotype (268). In mice, suppression of mCLCA expression inhibits goblet cell hyperplasia, whereas overexpression increases goblet cell number (269). Talniflumate is a small molecule putative inhibitor of hCLCA which is currently being developed as a mucoregulatory treatment for asthma and COPD (270).

Hyperplastic airway goblet cells in COPD models express the antiapoptotic factor Bcl-2 and the proportion of Bcl-2 positive cells is reduced prior to resolution of the hyperplasia (271).

4.8. Protease inhibitors. Mast cell tryptase induces marked increases in mucus secretion (272). Tryptase inhibitors suppress airway inflammation in allergic sheep although effects on mucus were not evaluated (273).

Neutrophil elastase, cathepsin G, and proteinase-3 are potent stimulants of airway secretion (274-276). This links neutrophilic inflammation in the airways with mucus hypersecretion and suggests that inhibitors of neutrophil proteases would be effective in reducing mucus hypersecretion.

4.9. Purine nucleotide inhibitors. Adenosine 5'-triphosphate (ATP) and uridine triphosphate (UTP) increase airway mucin and water secretion via interaction with P_{2Y2} purinoceptors (155, 277). Consequently, P_{2Y2}

antagonists might inhibit airway hypersecretion. However, mucus hydration is associated with improved mucociliary clearance, and stimulation of water secretion may have greater therapeutic potential than inhibition of P_{2Y2}–mediated mucin secretion (160). Hence, there is considerable interest in development of P_{2Y2} agonists (277).

4.10. MUC gene suppressors. Although inhibition of MUC gene expression is a promising possibility for the treatment of mucus hypersecretion, few studies have dealt with the downregulation of MUC genes. For this reason few therapies have been described for the direct inhibition of MUC gene expression. Notwithstanding that, an 18-mer mucin antisense oligonucleotide has been found to suppress MUC gene expression induced by wood smoke in rabbit airway epithelial cells, as well as to inhibit mucous metaplasia of these cells (278).

Chapter 4 summary

Among the more than 20 MUC genes described, at least 12 human mucin genes have been found expressed in healthy airways. Notwithstanding that, only MUC5AC and MUC5B mucins have been consistently found in airways secretion. For this reason, most of the studies regarding mucin expression and/or regulation in healthy and diseased airways have been focused in these two *gel*-forming mucins.

In general, increased amount of mucins have been found in pathologic compared to healthy tissues. This could be explained by several factors, but the three most important are 1) the goblet cell hyperplasia found to occur in inflammatory airway diseases such as nasal polyposis and asthma; 2) the increased number of inflammatory cells and mediators present in pathologic respiratory tissues; and 3) the presence of pathogens in inflamed airways. All these factors would account for an upregulation of mucin production/secretion.

Although conventional and many new therapies have been suggested for the treatment of mucus hypersecretion, this is a quite poor studied area lacking rigorous clinical trials that guarantee the use of mucoactive agents in the daily clinical practice. Moreover, in order to develop more efficacious pharmacological agents, a better understanding of the mucin composition of secretions, their physiological properties, and the interactions that occur between mucins and other components both in health and disease should be achieved.

2. HYPOTHESIS AND OBJECTIVES

Hypothesis of Work

Mucin expression pattern has been found to be altered in upper and lower airways diseases compared to healthy tissues. Although this altered expression has been related to increased mucus secretion and changes in the viscoelasticity of mucus, the significance of this pathologic mucus regarding disease evolution and treatment has not been clearly elucidated.

We hypothesize that nasal polyps present a different mucin expression pattern compared to healthy nasal mucosa, and that this expression also differs among nasal polyps depending on their associated diseases (cystic fibrosis, asthma, and aspirin-sensitivity) or even in antrochoanal polyps. Disease-related changes in mucin expression might explain differences regarding composition and rheological properties of mucus, determining the appropriated treatment for each pathology.

Additionally, regarding mucin expression, nasal polyp patients show a differential response to glucocorticoid therapy in relation to their associated co-morbidities. Glucocorticoid effect on mucin expression can be directly exerted over mucin production and/or secretion, or indirectly through their anti-inflammatory role.

General objectives

1) To characterize mucin expression at baseline in healthy and diseased (nasal polyps) human upper airway mucosa.

2) To analyze mucin expression and its regulation by glucocorticoid therapy in nasal polyp patients (*in vivo*) and in a respiratory cell line (*in vitro*).

Specific objectives

Study 1. To study mucin expression at baseline in human healthy (nasal mucosa) and inflamed (nasal polyps) upper airway mucosa.

1. To characterize and compare the epithelial and glandular mucin expression patterns (MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC6, MUC7, and MUC8) in healthy nasal mucosa and in nasal polyps from different origin, at gene (*in situ* hybridization) and protein (immunohistochemistry) levels.

2. To analyze the inflammatory cell infiltrate in the studied tissues and search for possible correlations with mucin expression.

Study 2. To analyze mucin expression and its regulation by glucocorticoids in inflamed nasal mucosa *in vivo*.

1. To evaluate and compare epithelial and glandular mucin expression (MUC1, MUC4, MUC5AC, MUC5B, and MUC8) in nasal polyps from non-asthmatic and asthmatic patients with/out aspirin-sensitivity, and to evaluate the effect of oral and intranasal glucocorticoids on this expression.

2. To investigate the effect of glucocorticoid treatment on the number of goblet cells in the epithelium and mucous cells in submucosal glands in the studied groups of patients and search for correlations with mucin expression.

3. To evaluate symptoms (rhinorrhea and nasal obstruction) score in the studied groups of patients before and after glucocorticoid treatment and search for correlations with mucin expression.

Study 3. To analyze basal and cytokine-induced mucin expression and its regulation by glucocorticoids in a respiratory epithelial cell line.

1. To study MUC5AC and MUC5B gene and protein expression at baseline and after the proinflammatory cytokine IL-1β induction, and to analyze dexamethasone effect on these basal and IL1β-induced expression in a human lung mucoepidermoid cell line (A549).

3. RESEARCH WORK

Study 1. Mucin genes have different expression patterns in healthy and diseased upper airway mucosa. *Clin Exp Allergy* 2006; 36(4):448-57.

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ORIGINAL PAPER

Mucin genes have different expression patterns in healthy and diseased upper airway mucosa

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Clinical and Experimental Allergy

Summary

Background Mucus hyper-secretion is a feature of several airways diseases such as chronic rhinosinusitis, asthma, and cystic fibrosis (CF). Since mucins are major components of mucus, the knowledge of their distribution and regulation in nasal tissues is likely to improve mucus hyper-secretion therapy.

Objective The aim of this study was to evaluate and compare mucin gene expression at epithelial and glandular levels, and to identify potential mucin expression patterns for specific upper airways pathologies.

Methods Immunohistochemistry for MUC1, MUC2, and MUC4–MUC8 mucins was performed on healthy nasal mucosa (NM; $n = 12$), bilateral nasal polyps (NP; $n = 38$), NP from CF patients ($n = 10$), and antrochoanal (AC) polyps ($n = 11$). MUC2, MUC4, MUC5AC, and MUC6 mRNA expression were also analysed by *in situ* hybridization.

Results MUC1, MUC4, and MUC5AC mucins were highly expressed in the epithelium and their expression pattern was similar in all NP types, MUC1 and MUC4 being increased and MUC5AC decreased compared with NM. MUC8 was highly detected at both epithelial and glandular levels with marked variability between groups. MUC5B was mainly detected in glands and the expression in all polyp types was higher than in NM. Moreover, MUC5B expression was higher in NP epithelia from CF patients than in bilateral NP and healthy NM. Although MUC2 expression was low, especially in AC polyps, it was detected in most samples. In NM, MUC6 and MUC7 were scarcely detected and MUC7 expression was restricted to glands.

Conclusions These results suggest that NP have a different pattern of mucin expression than healthy NM and that CF polyps (increased MUC5B) and AC polyps (decreased MUC2) have a different mucin expression pattern than bilateral NP.

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Introduction

Mucus is the layer that covers, protects, and lubricates the luminal surfaces of epithelial respiratory, gastrointestinal, and reproductive tracts. Airway mucus is composed of water, ions, lung secretions, serum protein transudates, anti-microbial proteins, and mucus glycoproteins (mucins). The viscoelastic properties of mucus are mainly determined by the presence of mucins [1] that are high-molecular weight proteins extensively glycosylated, synthesized, and secreted by epithelial globlet cells and submucosal glands [2].

Genes encoding for 18 human apomucins (mucin protein backbones) have been cloned [3, 4]. Although eight of them, MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC7, MUC8, and MUC13 [5, 6], are normally expressed in the human respiratory tract, only MUC5AC and MUC5B have been convincingly demonstrated to be major components of human airway secretion [7, 8]. There are two major classes of mucins: secreted mucins (MUC2, MUC5AC, MUC5B, MUC6–MUC10, and MUC13–MUC16, MUC19) and membrane-bound mucins (MUC1, MUC3, MUC4, MUC11, MUC12, MUC17), which contain a transmembrane domain and a short cytoplasmic tail. From the

secreted mucins, *MUC2*, *MUC5AC*, *MUC5B*, and *MUC6* genes, are clustered in the 11p15 chromosomal region [9], and contain cysteine-rich domains homologous to von Willebrand factor D domains that are oligomerization sites. A common feature of both kinds of mucins is the presence of tandem repeats rich in Thr, Ser, and Pro, where the *O*-glycosylation occurs [10].

In chronic inflammatory airway diseases such as asthma, chronic bronchitis, chronic rhinosinusitis and nasal polyposis, and cystic fibrosis (CF), submucosal glands become enlarged, and the number of globlet cells increases in the airway surface epithelium, appearing in the distal airways where they are not normally present [11]. These cellular changes often result in mucus hyper-secretion usually with altered physiological properties. Different studies have demonstrated that mucus-secreting cells of nasal polyp (NP) epithelium contribute to increase mucus secretion [12, 13]. Bronchial globlet cells hyperplasia also accounts for mucin hypersecretion in asthmatics subjects [14]. In addition to increased mucus secretion, the distributional pattern of mucin gene expression in respiratory tissues also seems to be altered in these airways pathologies [14, 15]. Because mucus overproduction is commonly accompanied by globlet cells hyperplasia, it is important to identify which mucins are expressed in the globlet cells of the nasal mucosa (NM) epithelium. Recent studies have demonstrated that NM epithelial globlet cells expressed MUC5AC [16, 17] and MUC2 [12] mucins and that mucous cells in glands expressed MUC5B [18].

NP constitute an inflammatory disease, characterized by oedematous masses in the nasal cavities and paranasal sinuses affecting 2–4% of general population, whose aetiology is still unknown. Although commonly presented as a unique kind of disease, NP may occur in a variety of different diseases such as bilateral nasal polyposis, in patients with or without asthma and in which eosinophil infiltration constitutes a histological hallmark [19], NP in CF patients where neutrophils are supposed to be the main infiltrating inflammatory cell [20], and unilateral antrochoanal (AC) polyps where inflammatory cell infiltration seems to be less important [21].

As little is known about the heterogeneity of mucin gene expression in the healthy NM and in the different types of NP, the knowledge of these expression patterns may contribute to an improvement in the diagnosis and therapy for mucus hyper-secretion and/or mucosal inflammation. Thus, the aim of the present study was to investigate and compare mucin gene expression patterns, at both epithelial and glandular levels, in different types of nasal tissues including healthy NM, bilateral NP, NP from CF patients (CF polyps), and unilateral AC polypsin order to identify potential mucin expression patterns for specific upper airways pathologies.

Materials and methods

Subjects

Human NM was obtained from subjects ($n = 12$) undergoing nasal corrective surgery for septal deviation. Only one of the subjects was being treated with intranasal corticosteroids at the time of surgery. Bilateral NP were obtained from 38 patients undergoing nasal polypectomy. Among them, 22 patients presented a history of asthma, and 50% of the asthmatic patients ($N = 11$) presented a clear history of aspirin sensitivity. At the time of surgery, 19 patients (50%) were receiving both intranasal corticosteroids (budesonide, 400 μg twice daily) and oral prednisone (20 mg/day). Thirteen patients (34%) were atopic to common aeroallergens (Table 1). In the study, polyps from patients with CF ($N = 10$) and AC polyps ($N = 11$) were also analysed.

Antibodies

Monoclonal antibodies M8 and LDQ10, recognizing MUC1 and MUC2 respectively, were used as undiluted hybridoma supernatant for M8 [22] and as ascites fluid diluted 1 : 250 for LDQ10 [23]. B12 MoAb (Dr Castro, Barcelona, Spain), recognizing a synthetic dextran molecule, was used as negative control at 1/2 dilution. Polyclonal anti-MUC4 [24], anti-MUC6 [25], and anti-MUC8 [6] antibodies, recognizing MUC4, MUC6, and MUC8 respectively, were purified by affinity chromatography on the synthetic peptides coupled to AH-sepharose 4B (Pharmacia, Uppsala, Sweden). Rabbit polyclonal serum LUM5.1 [7], LUM5B.2 [26], and LUM7.1 [27] recognizing non-TR regions of MUC5AC, MUC5B, and MUC7 respectively, were also used. Pre-immune rabbit serum was used as negative control at 1 : 1000 dilutions. Except for MUC8, specificity of all the antibodies has been described previously. The specificity of anti-MUC8 affinity-purified antibodies was determined by ELISA and by peptide inhibition assays by immunohistochemistry on bronchial sections as described previously [28].

Table 1. Epidemiological characteristics of subjects and patients

Tissue	*N*	Age (years)*	Gender (M/F)	Steroid treatment (yes/no)	Atopy (yes/no)
NM	12	32.3 ± 6.5	9/3	1/11	1/10 (1)†
CF	10	14.5 ± 6.7	7/3	2/8	0/2 (8)
AC polyps	11	41.4 ± 20.2	8/3	2/9	4/4 (3)
NP	38	55.9 ± 13.6	24/14	19/19	13/25
No Asthma	16	50.6 ± 14.5	11/5	8/8	5/11
Asthma	22	58.8 ± 13.5	13/9	11/11	8/14
ASA tolerance	11	49.5 ± 16.3	5/6	5/6	4/7
ASA Intolerance	11	51.7 ± 13.2	8/3	6/5	4/7

*Mean ± standard deviation. †Values in brackets represent unknown data. AC, antrochoanal; CF, cystic fibrosis; M, male, F, female; NM, nasal mucosa; NP, nasal polyp.

Table 2. Mucin protein expression in nasal tissues detected by immunohistochemistry

	NM		NP		CF		AC polyps	
	Positivity*	Rate (%)	Positivity	Rate (%)	Positivity	Rate (%)	Positivity	Rate (%)
Epithelium								
MUC1	12/12	100	35/35	100	10/10	100	10/10	100
MUC2	10/10	100	31/35	88.6	8/8	100	4/9	44.4
MUC4	9/12	75.0	35/35	100	10/10	100	10/10	100
MUC5AC	11/11	100	35/36	97.2	10/10	100	10/10	100
MUC5B	5/12	41.7	28/36	77.8	8/8	100	8/10	80.0
MUC6	5/11	45.5	0/37	0.0	0/10	0.0	0/10	0.0
MUC7	0/12	0.0	0/37	0.0	0/10	0.0	0/10	0.0
MUC8	10/10	100	37/37	100	9/9	100	10/10	100
Glands								
MUC1	11/11	100	25/25	100	7/7	100	2/2	100
MUC2	1/9	11.1	0/25	0.0	1/5	20.0	0/2	0.0
MUC4	6/11	54.5	21/29	72.4	7/9	77.8	1/2	50.0
MUC5AC	3/9	33.3	15/22	68.2	5/7	71.4	1/3	33.3
MUC5B	10/10	100	27/27	100	8/8	100	3/3	100
MUC6	5/11	45.5	2/24	8.3	1/7	14.3	0/3	0.0
MUC7	6/11	54.5	0/26	0.0	1/10	10.0	1/3	33.3
MUC8	11/11	100	27/28	96.4	8/8	100	2/2	100

*Values are expressed as positive cases /total cases.

AC, antrochoanal; CF, cystic fibrosis; NM, nasal mucosa; NP, nasal polyp.

Immunohistochemistry

The indirect immunoperoxidase technique was performed on 3 μm sections of paraffin-embedded tissue sections. Samples were dewaxed, rehydrated, treated with 0.01 M sodium citrate buffer at 100 °C for 5 min, and fixed in cold acetone for 10 min. Endogenous peroxidase was blocked with 4% hydrogen peroxide in phosphate-buffered saline (PBS) containing 0.1% sodium azide for 10 min. Non-specific-binding sites were blocked with 5% skim milk in PBS. Primary antibodies were diluted in 1% PBS–bovine serum albumin and applied for 90 min. After washing with PBS, the slides were incubated for 60 min with peroxidase-labelled secondary antibodies (DAKO, Glostrup, Denmark). Peroxidase reaction was developed using the diaminobenzidine Substrate-cromogen System (DAKO) according to the manufacturer's recommendations. The slides were counterstained with haematoxylin, dehydrated, and mounted with DPX (BDH, Poole, UK).

Sections were examined by light microscopy and the antibody staining patterns were scored in a quantitative manner. The pattern of reaction was classified in the epithelium and glands, and the number of positive cells was expressed as a percentage of the total number of cells (400 cells counted). The percentage of positive among total cases was also quantified (Table 2). A positive case was considered when more than 5% of positive cells were detected. The scoring of reactivity was independently determined by two observers (A.M. and C.d.B) in a blind manner.

In Situ Hybridization

Sense and antisense synthetic oligonucleotides (48 bp) corresponding to the tandem repeat sequences of mucin genes were labelled with digoxigenin (DIG) following the manufacturer's instructions (Boehringer, Mannheim, Germany). After surgery, tissue samples were immediately fixed in 4% paraformaldehyde (PFA), embedded in paraffin, and stored at 4 °C until use. Sections were deparaffinized and rehydrated. Proteinase K digestion (1 μg/mL in 0.1 M Tris HCl, pH 7, 0.5 M EDTA) was performed for 15 min at 37 °C. Samples were fixed with 4% PFA for 15 min and treated with 0.1 M triethanolamine, pH 8, and 0.25% acetic anhydride for 10 min. After pre-hybridization in 4 × SSPE (300 mM NaCl, 20 mM NaH_2PO_4, 2 mM EDTA) with 1% Denhardt's at 42 °C for 60 min, sections were dehydrated and hybridized overnight at 42 °C with DIG-labelled oligonucleotides (400 μg/mL) diluted in 4 SSPE, 1% Denhardt's, 50% formamide, 20 mM DTT, 0.1 M phosphate buffer, pH 7.2%, 1% sarkosyl, and tRNA (250 μg/mL). Post-hybridization washes were performed stepwise from 4 × SSPE at room temperature (RT) to a final wash with 0.1 × SSPE at 42 °C. Sections were dehydrated, dried and incubated with 5% normal horse serum for 3 h at RT. Alkaline phosphatase-conjugated sheep anti-DIG antibodies (Boehringer) were incubated overnight at 4 °C. Alkaline phosphatase was developed overnight at RT using nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (Promega, Madison, WI, USA) in 100 m20 mM NaCl, 50 mM $MgCl_2$, 100 mM Tris HCl, pH 9.5. Slides were rinsed and mounted in Aquatex.

Table 3. Inflammatory component from healthy and pathologic tissues

Tissue	Lymphocyte (%)	Plasma cells (%)	Polymorphonuclear (%)	Eosinophils (%)
NM	40 (32.5–40)	50 (47.5–55)	5 (4–5)	5 (3.5–12.5)
NP	35 (15–45)	30 (25–40)*	5 (5–10)	25 (10–50)*
CFP	30 (20–36.3)	50 (48.8–61.3)†	5 (5–6.3)	10 (7.3–11.3)†
AC polyps	45 (21.3–65)	45 (22.5–50)	5 (3–8.8)	10 (2.5–18.8)†

*$P < 0.05$ compared with NM. †$P < 0.05$ compared with NP by the Mann–Whitney *U*-test.
Results are expressed as median and 25–75th percentile. NM, nasal mucosa; NP, nasal polyp; CFP, polyps from cystic fibrosis patients; AC, antrochoanal.

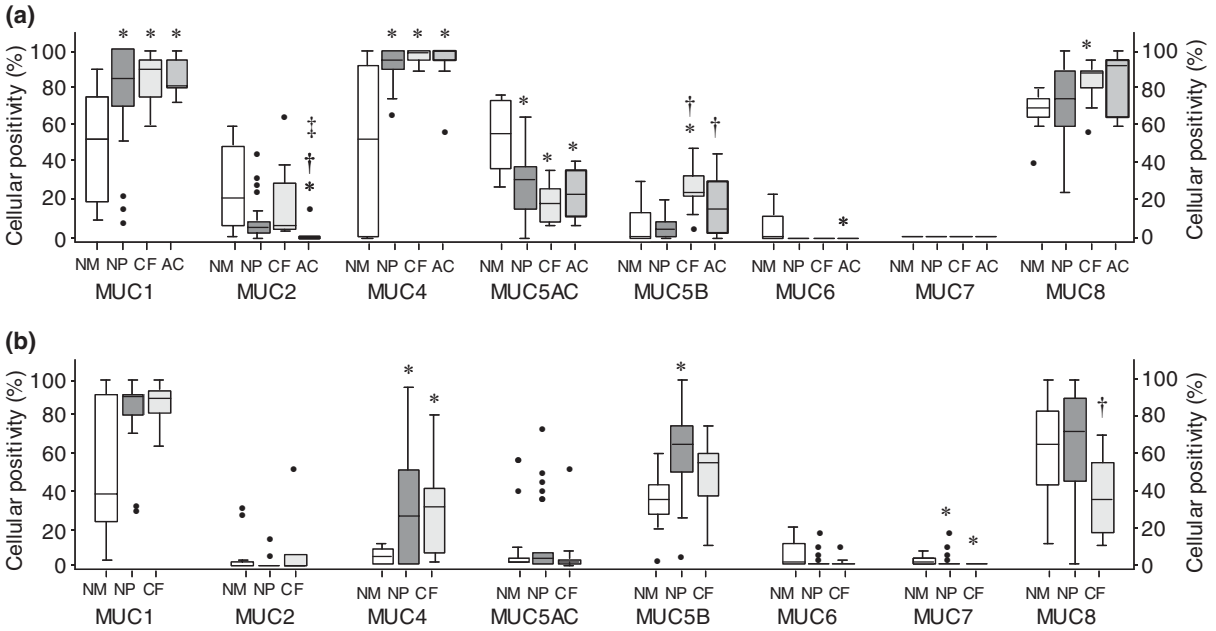

Fig. 1. Pattern of mucin gene expression in different types of upper airway tissues: nasal mucosa (NM), bilateral nasal polyps (NP), NP from patients having cystic fibrosis (CF), and antrochoanal (AC) polyps. The data are expressed as percentage of positive cells among total cells in the epithelium (a) and in the glands (b). Because of the absence of glands AC polyps are not represented in graph B. Box plots show the 25th, 50th (median), and 75th percentile values. Whiskers show the minimum and maximum values. Black points (•) represent outlayer values. Comparisons were made using the Mann–Whitney *U*-test (*$P < 0.05$ vs. NM; †$P < 0.05$ vs. NP; ‡$P < 0.05$ vs. CF).

The antisense oligonucleotide sequences used were as follows: MUC2 (5′-GGT CTG TGT GCC GGT GGG TGT TGG GGT TGG GGT CAC CGT GGT GGT GGT-3′), MUC4 (5′-GTC GGT GAC AGG AAG AGG GGT GGC GTG ACC TGT GGA TGC TGA GGA AGT-3′), MUC5AC (5′-AGG GGC AGA AGT TGT GCT CGT TGT GGG AGC AGA GGT TGT GCT GGT TGT-3′), and MUC6 (5′-CAT CTG TGC GTG GGT AGG GGT GAT GAC TGT GTG AGT ACT TGG AGT CAC-3′). The following tissues were used as controls: healthy colon for MUC2 and MUC4 and healthy stomach for MUC5AC and MUC6 [24, 28].

Inflammation

Inflammatory cells including eosinophils, lymphocytes, plasma cells, and polymorphonuclear cells were quantified, and the results were related to 100 inflammatory cells (Table 3).

Statistical Analysis

Mucin data were expressed as median and 25–75th percentile of positive cells among total cells. As the Kolmogorov–Smirnov test showed that most of the data did not reach a normal distribution, the non-parametric statistical Mann–Whitney's *U*-test was used for between-group comparisons. Rho Spearman's analysis was used to assess the correlation between mucin gene expression and inflammatory parameters in the different tissues. Statistical significance was set at $P < 0.05$.

Results

Mucin expression in human nasal mucosa

Epithelium. MUC1, MUC2, MUC5AC, and MUC8 mucins were detected in the epithelium of all cases (Table 2). The expression of MUC8 (median: 70%) was high, MUC1

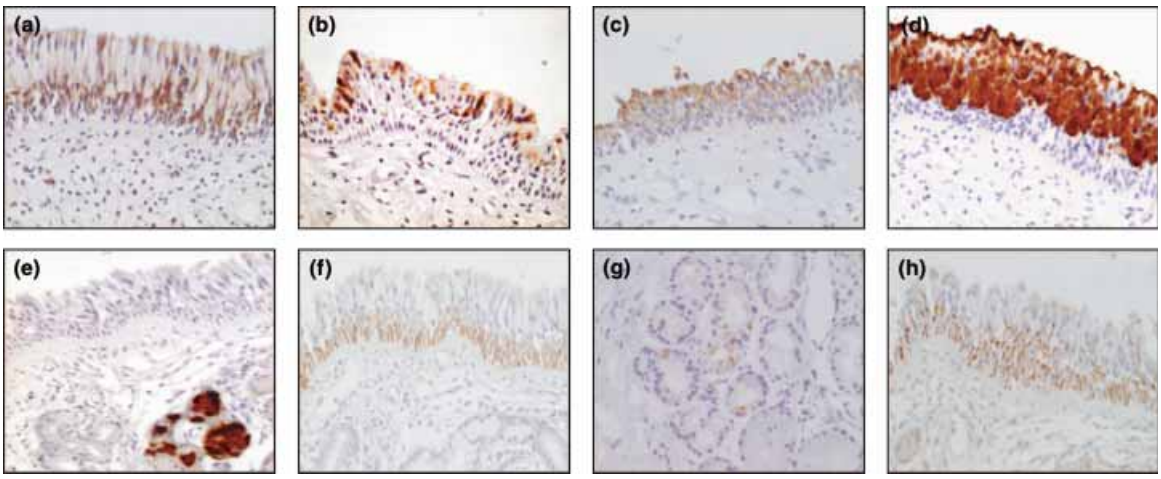

Fig. 2. (a)MUC1, (b) MUC2, (c) MUC4, (d) MUC5AC, (e) MUC5B, (f) MUC6, (g) MUC7, and (h) MUC8 mucins detected in human nasal mucosa by immunohistochemistry. MUC1, MUC4, and MUC8 were detected in nasal epithelial globlet and ciliated cells. MUC2 and MUC5AC were mainly detected in globlet cells. MUC5B was mainly detected in submucosal glands. MUC6 was detected at a low level while MUC7 was not expressed. (Original magnification: × 400).

(52.5%) and MUC5AC (55%) expression was moderate, and MUC2 (21.3%) showed a low expression. MUC5B and MUC6 were detected in approximately half of the cases and the cellular positivity was similar for both mucins (Fig. 1A). MUC4 (52.5%) was variably detected in 75% of the samples while MUC7 was undetectable in the epithelium.

Submucosal glands. MUC1, MUC5B, and MUC8 were detected in all analysed cases (Table 2). MUC8 mucin was highly expressed (65%), MUC1 (40%), and MUC5B (37.5%) were moderately detected, and the rest of the mucins were lowly expressed, MUC2 being the mucin detected at the lowest level (Fig. 1b). The number of positive cells and positive cases for MUC5B and MUC7 was increased compared with the epithelium (Table 2).

According to the stained cellular type, MUC1 and MUC4 immunoreactivity was found in both mucus-secreting and ciliated cells, whereas MUC2 and MUC5AC staining was restricted to the mucus-secreting cells. MUC6 and MUC8 staining was limited to the basal area of the superficial epithelium, while MUC5B was mainly detected in mucus-secreting cells of submucosal glands. MUC7 was undetectable in the epithelium although it was focally detected in glands (Fig. 2).

Mucin expression in nasal polyps

Epithelium. MUC1, MUC4, and MUC8 mucins were highly detected in all studied samples. MUC5AC was moderately expressed while MUC2 and MUC5B were poorly expressed, and MUC6 and MUC7 were not detected (Fig. 1a; Table 2).

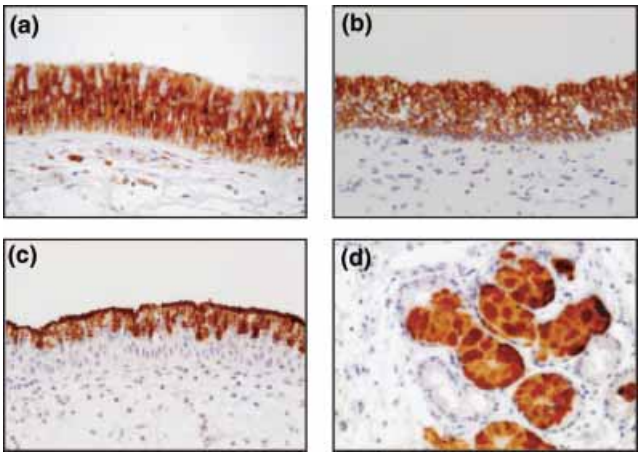

Fig. 3. Immunohistochemistry of mucin expression in bilateral nasal polyps: (a) MUC1, (b) MUC4, (c) MUC5AC, and (d) MUC5B. MUC1 and MUC4 were highly expressed throughout the epithelium. MUC5AC and MUC5B are mainly expressed in epithelial and glandular mucous cells, respectively. (Original magnification: × 400)

Submucosal glands. MUC1 and MUC8 were also highly expressed in all the cases. The expression of MUC5AC was decreased whereas MUC5B was increased compared with the epithelium (Fig. 1b). In contrast to a high positivity in the epithelium (90%), MUC2 was not detected in glands. While MUC6 was detected in few samples, MUC7 was totally absent (Table 2).

Expression pattern. Comparing the mucin expression pattern in NP with that found in healthy NM, an increased expression of MUC4 in both the polyp epithelium (95%; $P < 0.001$) and glands (25%; $P < 0.05$) as well as an increase of MUC1 (85%; $P < 0.001$) in the epithelium was observed. The expression of MUC5AC (30%; $P < 0.001$)

and MUC6 (0%; $P < 0.001$) was decreased in NP epithelium, whereas MUC7 (0%; $P < 0.01$) was decreased in NP glands. MUC5B (65%; $P < 0.001$) mucin was increased in NP glands with respect to NM (Fig. 3).

When comparing the mucin expression between asthma/no-asthma patients, a lower MUC2 mucin expression in asthmatics (3.8%; $P < 0.01$) than in non-asthmatic (10%) subjects was found in nasal epithelium. The expression of MUC5B was higher in NP glands from asthmatics patients with aspirin tolerance (67.5%; $P < 0.01$) than in polyps of patients with aspirin sensitivity (50%). Polyps of patients receiving corticosteroid treatment showed a higher expression of MUC1 (95%; $P < 0.05$) and MUC5B (7.5%; $P = 0.01$) but a lower expression of MUC8 (65%; $P = 0.01$) than the non-treated group (80%, 1.3%, and 87.5%, respectively) (Fig. 3). No differences in mucin expression between atopic and non-atopic subjects were found (data not shown).

Mucin expression in nasal polyps from cystic fibrosis patients

Epithelium. All the studied mucins, except MUC6 and MUC7, were detected in 100% of the cases (Table 3). The expression of mucins was high for MUC1, MUC4, and MUC8, moderate for MUC5B, and low for MUC2 and MUC5AC, while MUC6 and MUC7 were not expressed (Figs 1a and 4).

Submucosal glands. The number of positive cases was markedly decreased for MUC2 mucin compared with the epithelium (Table 3). The expression was high for MUC1, moderate for MUC4, MUC5B, and MUC8, and very low for MUC2, MUC5AC, and MUC7.

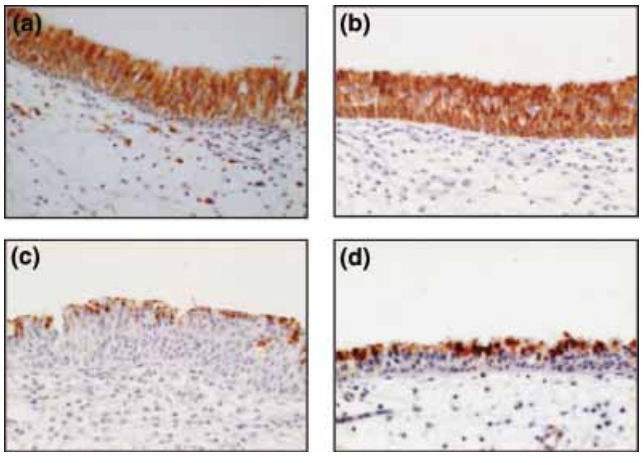

Fig. 4. Immunohistochemistry of mucin expression in nasal polyps from cystic fibrosis patients: (a) MUC1, (b) MUC4, (c) MUC5AC, and (d) MUC5B. MUC1 and MUC4 were highly expressed throughout the epithelium, whereas MUC5AC and MUC5B were mainly expressed in globlet cells. (Original magnification: × 400).

Expression pattern. When the mucin expression pattern detected in NP from CF patients, was compared with that found in healthy NM, an increased expression of MUC1 (90%; $P < 0.001$), MUC5B (25%; $P < 0.01$), and MUC8 (90%; $P < 0.001$) was observed in the epithelium. MUC4 expression was increased in both the epithelium (100%; $P < 0.001$) and the glands (30%; $P < 0.05$). MUC5AC expression was significantly lower (17.5%; $P < 0.001$) than in NM (Fig. 1). When comparing the mucin expression between CF polyps and bilateral NP, an increased expression of MUC5B mucin was observed in CF polyps epithelium ($P < 0.001$) and decreased MUC8 in glands ($P < 0.05$).

Mucin expression in antrochoanal polyps

Epithelium. MUC1, MUC4, and MUC8 mucins were detected in all the analysed cases in a high proportion of cells (Table 3). MUC5AC was also detected in all the samples but the expression intensity was low. The expression of MUC5B and MUC2 was low, while MUC6 and MUC7 were not detected (Fig. 1A).

Submucosal glands. Because of the low number of glands found in AC polyp samples, the cellular positivity could not be assessed at the glandular level.

Expression pattern. When the pattern of mucin expression in AC polyps was compared with that of healthy NM, an increased expression of MUC1 (80%; $P < 0.01$) and MUC4 (100%; $P = 0.001$) as well as a decreased expression of MUC2 (0%, 0–1.3; $P < 0.001$) and MUC5AC (22.5%; $P = 0.001$) was observed. MUC2 mucin expression was also decreased compared with CF polyps and bilateral NP (both

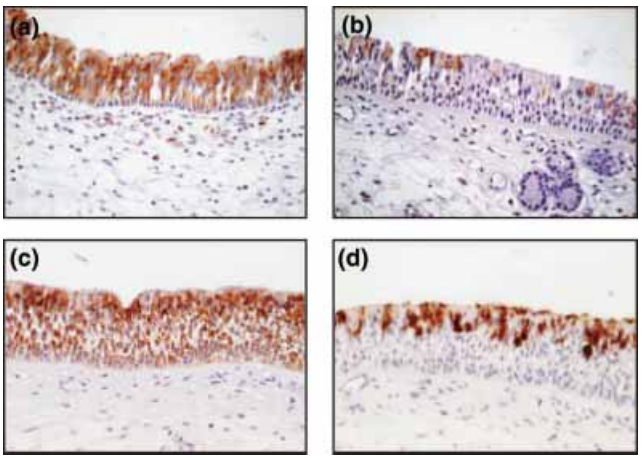

Fig. 5. Immunohistochemistry of mucin expression in AC polyps: (a) MUC1, (b) MUC2, (c) MUC4, and (d) MUC5AC. MUC1 and MUC4 were highly detected throughout the epithelium, whereas MUC2 and MUC5AC were mainly detected in globlet cells. (Original magnification: × 400).

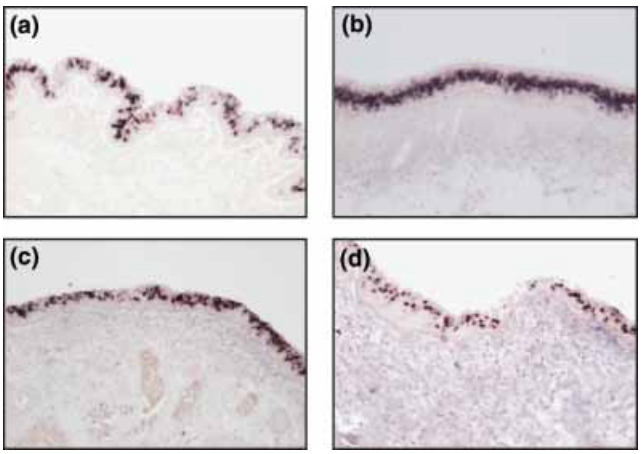

Fig. 6. MUC2, MUC4, and MUC5AC transcripts were detected by *in situ* hybridization. (a and c) MUC2 and MUC5AC transcripts expressed in nasal mucosa. (b and d) MUC4 and MUC5AC transcripts expressed in a bilateral nasal polyp. (Original magnification: × 150).

$P < 0.001$), while MUC5B was increased in AC polyps compared with bilateral NP ($P < 0.05$) (Figs 1a and 5).

Detection of mucin transcripts by in situ hybridization

To confirm the immunohistochemical findings at the RNA level, *in situ* hybridization for MUC2, MUC4, MUC5AC, and MUC6 was carried out on selected samples of healthy NM ($n = 4$) and bilateral NP ($n = 5$). MUC2 mRNA was expressed in the epithelium of NM in a low proportion of cells (Fig. 6A), but not in bilateral NP. MUC4 was expressed in all samples in a high proportion of epithelial cells (Fig. 6B). MUC5AC was mainly detected in globlet cells in both NM and polyps, although the expression was higher in NM than in NP (Figs 6C and D). MUC6 was not detected in either NM or polyps (data not shown).

Inflammation

The analysis of the haematoxylin–eosin staining showed a higher percentage of eosinophils in bilateral NP (25%; $P < 0.05$) than in the other tissues (NM = 5%; CF and AC polyps = 10%) (Table 3). Bilateral NP from patients receiving corticoids treatment (10%) showed a decreased eosinophil infiltration compared with NP from non-treated patients (50%; $P < 0.001$) (data not shown). There were no significant differences in the polymorphonuclear cells and lymphocyte content in the different tissues (Table 3). As a high variability in the correlations between mucin expression and inflammatory cells were found, no correlation data was reported.

Discussion

In the present study, the pattern of mucin gene expression in healthy NM has been analysed and compared with that of bilateral NP, NP from CF patients and AC polyps in order to assess the potential role of the mucins as pathologic markers for diagnosis and disease evolution in patients with chronic rhinosinusitis and NP. Our results indicate that an altered expression pattern is present in all NP samples.

Mucins in healthy nasal mucosa

In healthy NM, the major mucins expressed are MUC1, MUC4, MUC5AC, and MUC8 in the epithelium, and MUC1, MUC5B, and MUC8 in the glands. Previous data have reported that MUC4 and MUC5AC are the most highly expressed mucins in healthy inferior turbinates [5] and that MUC5AC and MUC5B are the two major mucin populations in respiratory secretions [7, 8]. MUC5AC has been exclusively detected in epithelial globlet cells of NM, while MUC5B has also been found in the mucous cells of submucosal glands [16]. In addition, MUC5AC has been also localized in goblet cells from other respiratory epithelia such as the bronchi, trachea, and lungs [6, 29, 30], as well as in non-respiratory tissues such as stomach [25] and gallbladder epithelia [31]. Little data regarding a mucin expression in the upper airways have been published. MUC2 and MUC7 were detected at a low level, while MUC6 was absent [5]. These observations are in accordance with our findings, in which MUC6 and MUC7 were exclusively detected in healthy NM at very low levels, and MUC2 was scarcely expressed in both healthy and pathogical nasal tissues.

Mucins in nasal polyps

Several authors have analysed the expression of mucin genes in different samples of upper airway mucosa. For instance, an increase of MUC8 mRNA and a decrease of MUC5AC mRNA expression in bilateral NP [13], as well as an up-regulation of MUC8 in chronic rhinosinusitis mucosa have been found, at both mRNA and protein levels [32]. By contrast, several studies have described an increased expression of MUC5AC in bilateral NP that can be associated to globlet cells hyperplasia [17, 33]. In this way, if hyperplasic epithelium appeared in pathologic tissues, the low percentage of MUC5AC-positive cells detected in our samples could be explained by either a higher proliferation of non-globlet cells (ciliated and basal) with respect to globlet cells or by the presence of less mature globlet cells with a differential phenotype. On the other hand, Kim et al. [18] have recently demonstrated an up-regulation of MUC5B mRNA expression in chronic rhinosinusitis mucosa compared with normal sinus mucosa. This result is comparable with our finding of increased MUC5B expression in bilateral NP glands.

Mucins in nasal polyp from various origins

In CF polyps, an up-regulation of MUC5AC mRNA in both human NP and bronchial tissues of CF subjects has been

reported [15], as well as an increase of MUC2 mRNA expression in CF NM [34]. However, we detected a decreased expression of MUC5AC and MUC2 in NP of CF patients similar to that present in bilateral NP. A potential explanation for the different results may be that all CF patients in the study of Li et al. were included in a gene therapy trial receiving either CFTR cDNA-liposome complexes or liposome alone by nasal topical application. The administration of liposomes may affect the surface of the epithelium and up-regulated MUC2 expression, as it has already been described for other inflammatory mediators [35]. On the other hand, our findings agree with those of Voynow and Henke, who detected a decreased expression of MUC5AC in nasal epithelial cells and sputum from CF subjects compared with NHNE cells and sputum of healthy subjects [36, 37].

In addition, our study reports, for the first time, the mucin gene expression in AC polyps. The results indicate that in this pathologic tissue, the mucin expression pattern is similar to that found in bilateral and CF NP, except for MUC2, which was found to be down-regulated in AC polyps.

Mucins in respiratory inflammation

Although several studies have tried to elucidate the role of inflammatory cells and their mediators in the regulation of mucin genes, the relationship between inflammation and mucin overproduction and/or hyper-secretion is still poorly understood. It has been reported that inflammatory cells present in NP could play an important role in the disease [38]. One of these implications could be the regulation of the mucin genes as it has been described in other respiratory cellular models. For example, Burgel et al. demonstrated that eosinophil products increased mucin production in airway epithelial cells [39] and pro-inflammatory cytokines (IL-1β, IL-9, and TNF-α) can regulate the expression of specific mucin genes (MUC2, MUC5AC, and MUC8) in bronchial epithelia cells, regulation that is mediated through a mechanism involving the ERK/MAPK/RSK1/CREB pathway [40–42]. In our tissue samples, bilateral and CF NP showed a higher inflammatory component compared with healthy NM and AC NP and eosinophils were the major inflammatory cells detected in bilateral NP. Altogether, these data could indicate that MUC1, MUC4, and MUC5B up-regulation, observed in bilateral NP and to a lower extent in CF and in AC NP, might be due to the increase of eosinophil infiltration, wherein activation and secretion of inflammatory factors would regulate the expression of specific mucin genes. However, factors secreted by other inflammatory cells present in NP, such as neutrophils, could also contribute to regulation of mucin gene expression [33].

The increased expression of secreted mucins may contribute to mucus hyper-secretion and to mucus viscoelasticity changes. On the other hand, the biologic significance of the membrane mucin (MUC1 and MUC4) activation in pathologic tissues may be related to the implication of these mucins for intracellular signalling pathways related to proliferative processes. MUC4 may carry out this action through the epidermal growth factor-like domains present in its sequence, which interact with ErbB2 [43]. Therefore, membrane mucins could be involved in epithelial cell hyperplasia reported to occur in several respiratory pathologies.

Recently, it has been shown that intranasal steroids decrease the number of eosinophils but not the level of mucins expression in bilateral NP [44]. Although the effect of corticosteroids on mucin gene expression was not the main aim of the present study, we found some remarkable differences between bilateral NP from steroid-treated and non-treated patients. In the treated group, a decrease in the number of eosinophils was detected in association with a decrease of MUC8 expression, suggesting that eosinophils could be involved in the regulation of MUC8.

Conclusions

These results suggest that bilateral NP have a different pattern of mucin expression, an increase of MUC1 and MUC4 and a decrease of MUC5AC, than healthy NM. Among NP, CF polyps, with an increase in MUC5B, and AC polyps, with a decrease in MUC2, also show a differential mucin expression pattern than bilateral nasal polyposis. These findings, together with further studies on the regulation of mucins by pro-inflammatory agents and anti-inflammatory drugs, might aid in the differential diagnosis and improved therapies of respiratory diseases with NP.

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Study 2. Corticosteroid therapy increases membrane-tethered while decreases secreted mucin expression in nasal polyps. *Allergy* 2008 *(in press) DOI: 10.1111/j.1398-9995.2008.01678.x.*

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Original article

Corticosteroid therapy increases membrane-tethered while decreases secreted mucin expression in nasal polyps

Background: Mucus hypersecretion is a hallmark of nasal polyposis (NP). Corticosteroids (CS) are first-line treatment for NP, decreasing their size and inflammatory component. However, their effect on mucin production is not well-understood. The aim of this (pilot) study was to investigate CS effect on mucin expression in NP.
Methods: Patients were randomized in *control* ($n = 9$) and *treatment* (oral prednisone for 2 weeks and intranasal budesonide for 12 weeks; $n = 23$) groups. Nasal polyposis from nonasthmatic (NP; $n = 13$), aspirin-tolerant (NP-ATA; $n = 11$) and aspirin-intolerant (NP-AIA; $n = 8$) asthmatics were studied. Nasal polyposis biopsies were obtained before (w0) and after 2 (w2) and 12 (w12) weeks of CS treatment. Secreted (MUC5AC, MUC5B and MUC8) and membrane-tethered (MUC1, MUC4) mucins (immunohistochemistry) and goblet cells (Alcian blue-periodic acid Schiff) were quantified in both epithelium and glands. Rhinorrea and nasal obstruction were also assessed.
Results: At w2, steroids increased MUC1 (from 70 to 97.5) and MUC4 (from 80 to 100) in NP-ATA patients' epithelium compared with baseline (w0). At w12, steroids decreased MUC5AC (from 40 to 5) and MUC5B (from 45 to 2.5) in NP-ATA patients' epithelium and glands, respectively, compared with baseline. No mucin presented significant changes in NP-AIA patients. MUC5AC and MUC5B expression correlated with goblet and mucous cell numbers, respectively, and MUC5AC also with rhinorrea score.
Conclusions: These results suggest: (i) CS up-regulate membrane (MUC1, MUC4) while down-regulate secreted (MUC5AC, MUC5B) mucins; (ii) there exists a link between secreted mucin expression and goblet cell hyperplasia; and (iii) NP from AIA may develop resistance to CS treatment.

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Key words: asthma; corticosteroids; goblet cells; nasal polyps; mucins.

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Airway mucus is composed from water, ions, lung secretions, serum protein transudates, antimicrobial proteins and mucus glycoproteins (mucins) (1). Mucins, the major component of mucus, are high-molecular weight proteins extensively glycosylated, synthesized and secreted by epithelial goblet cells and mucous cells of submucosal glands (SMG). To date, 20 human mucin genes (2) have been identified and subdivided into secreted and membrane-tethered mucins.

Mucus layer represents a protective barrier against pathogens and irritants and mucins, being responsible for the viscoelasticity of the mucus, are involved in this protective function. Notwithstanding that, different roles have been described for mucins depending on their secreted or membrane-tethered nature. In this manner, membrane-bound mucins such as MUC1 and MUC4 modulate cell–cell and cell–extra cellular matrix interactions on epithelial cell surfaces (3) and participate in cellular signaling (4). On the other hand, secreted mucins such as MUC2, MUC5AC, MUC5B and MUC6 have been directly involved in mucus formation (5–8). MUC8 is increased both *in vivo* and *in vitro* under inflammatory conditions (9–11), but its role in respiratory diseases has not been studied. Therefore, the implication of mucins in the development of respiratory diseases and its regulation might be completely different based on its nature.

The alteration of either the quantity or quality of mucus leads to a pathologic condition implying obstruction and infection of the airways. In fact, mucus

Abbreviations: AB-PAS, Alcian blue-periodic acid Schiff; AIA, aspirin-intolerant asthma; ATA, aspirin-tolerant asthma; CS, corticosteroids; GCH, goblet cell hyperplasia; NP, nasal polyps; SMG, submucosal glands.

hypersecretion usually occurs in respiratory diseases, such as asthma, cystic fibrosis and chronic rhinosinusitis with or without nasal polyps (NP) (2).

Nasal polyposis is an upper airways inflammatory disease affecting 2–4% of general population, 10–15% of asthmatic patients and over 90% of patients with aspirin-intolerant asthma (AIA) (12). Mucus hypersecretion, in the form of rhinorrea, is a common symptom of patients suffering from inflammatory sinonasal diseases, including NP. However, the mucin composition and its physiological role in the mucus overproduction of NP have not been deeply investigated. Although identical mucins and with a similar distribution have been found in healthy nasal mucosa and NP (9, 13, 14), these tissues differ in mucin amount. For instance, MUC1, MUC4, MUC5B and MUC8 mucins have been found increased while MUC2 and MUC5AC decreased in NP compared with healthy nasal mucosa (13). These differences could be partly due to goblet cell hyperplasia (GCH) usually present in airway diseases. In fact, changes in mucin production associated to GCH have been described in NP (15, 16).

Corticosteroids (CS) are the first line of therapy for the treatment of NP inducing a decrease in polyp size and inflammatory component (12, 17). Corticosteroids are known to decrease inflammation also in diseases such as asthma, cystic fibrosis and bronchitis (12, 18, 19), but their effects on mucus hypersecretion has been controversial. Although some works have studied CS effect on mucin expression in respiratory primary and culture cell lines, few studies have dealt with this topic in an *in vivo* situation and in a real disease.

In order to ascertain whether CS treatment represents a beneficial therapy for the mucus hypersecretion observed in NP, in the present (pilot) study we have investigated the *in vivo* effect of CS in both secreted (MUC5AC, MUC5B and MUC8) and membrane-tethered (MUC1, MUC4) mucins from NP. In addition, we have also assessed the effect of CS on the two main symptoms of NP (12), rhinorrea and nasal obstruction, and their correlation with mucin expression.

Methods

Study population

A total of 32 consecutive patients with severe NP, including patients without asthma, and with either aspirin-tolerant (ATA) or AIA, were included in this prospective and randomized study. All patients signed informed consent and the study was approved by the Ethics Committee of our Institution. In our study population, 20% of patients were atopic while 30% were female, this percentage increasing in the AIA group (50%), in agreement with previous studies (20) (Table 1).

Inclusion and exclusion criteria

All patients included in this study showed severe NP based on polyp size by nasal endoscopy (Lildholdt mean score: 2.7 over 3) (21) and bilateral sinus opacification by computed tomography (CT) scan.

Table 1. Epidemiological characteristics of study patients

Nasal polyp (NP) type	*n*	Age (years)*	Female, *n* (%)	Atopy, *n* (%)
All	32	54.2 ± 2.8	10 (31)	6 (19)
Control group	9	54.4 ± 5.5	2 (22)	1 (11)
No asthma	3	44.3 ± 6.4	0	1 (33)
Asthma	6	59.5 ± 7.0	2 (33)	0
Aspirin-tolerant	4	63.5 ± 8.7	1 (25)	0
Aspirin-intolerant	2	51.5 ± 13.5	1 (50)	0
Treatment group	23	54.1 ± 3.4	8 (35)	5 (22)
No asthma	10	56.0 ± 5.0	3 (30)	3 (30)
Asthma	13	52.6 ± 4.7	5 (38)	2 (15)
Aspirin-tolerant	7	51.3 ± 8.0	2 (29)	1 (14)
Aspirin-intolerant	6	54.1 ± 5.1	3 (50)	1 (17)

*Mean ± standard error of the mean (SEM).

The diagnosis of aspirin intolerance was made on the basis of a clear-cut history of asthma attacks precipitated by nonsteroidal anti-inflammatory drugs. In asthmatic patients with doubtful diagnostic, aspirin sensitivity was tested by nasal challenge with lysine acetylsalicylic acid and acoustic rhinometry outcomes (22). Asthmatic patients, depending on their severity, received inhaled steroids and/or beta-2 agonists but not leukotriene antagonists. This treatment of asthma was not modified during the study. None of the patients had cystic fibrosis and those with steroid contraindications were excluded from the study.

Study design

The study design used herein was as follows: after a washout period of 4 weeks for intranasal and 3 months for oral CS, patients were randomized (3 : 1) in: group A, the CS-treated group ($n = 23$) received oral prednisone (30 mg daily for 4 days followed by a 2-day tapered reduction of 5 mg) and intranasal budesonide (400 μg/twice a day) for 2 weeks (w2), followed by intranasal budesonide alone for 10 additional weeks (w12); and group B, the nontreated control group ($n = 9$) did not receive any steroid treatment over a 2-week period (w2). For ethical reasons, patients from the control group were not kept with ineffectual treatment for more than 2 weeks. Nasal polyp biopsies were obtained at w0, w2 and w12 in both A and B groups.

Immunohistochemistry

The indirect immunoperoxidase technique was performed on 3-μm sections of paraffin-embedded tissue sections for the detection of membrane-tethered (MUC1 and MUC4) and secreted (MUC5AC, MUC5B, MUC8) mucins, as previously described (13). The monoclonal antibody M8 recognizing MUC1 was used as undiluted hybridoma supernatant (23). Polyclonal anti-MUC4 (24) and anti-MUC8 (25) antibodies, and rabbit polyclonal serum LUM5.1 (26) and LUM5B.2 (27) recognizing non-TR regions of MUC5AC and MUC5B, respectively, were also used. B12 MoAb (Dr Castro, Barcelona, Spain) recognizing a synthetic dextran molecule and preimmune rabbit serum were used as negative controls.

Quantification analysis

Sections were examined by light microscopy (×400) and the patterns of antibody staining were scored in a quantitative manner. The pattern of reaction was analysed in both, the epithelium and SMGs, the number of immunoreactive positive cells (brown staining) being expressed as a percentage of total cell number (500 counted cells).

The immunoreactivity score was assessed by two independent observers in a blind manner, and the results averaged. The level of mucin expression was classified in *high* (>70% to 100%), *moderate* (>30% to 70%) and *poor* (0% to 30%) depending on their percentage of cell positivity.

Goblet cell staining

In order to assess changes in the number of goblet cells before and after CS treatment, Alcian blue-periodic acid Schiff (AB-PAS) staining was performed. Positive cells with purple/blue color were counted by light microscopy (×400) and expressed as a percentage of total epithelial cells (500 cells counted).

Nasal symptoms

Rhinorrea and nasal obstruction were assessed at w0, w2 and w12. The severity of nasal symptoms was scored as follows: 0, no symptom; 1, mild but not troublesome; 2, moderate symptom somewhat troublesome; and 3, severe and troublesome that interferes with the daily activity or sleep.

Statistical analysis

Mucin data was expressed as median and 25–75th percentile of positive cells among total cells. The nonparametric statistical Mann–Whitney *U*-test was used for between-group comparisons and the Wilcoxon test was used for paired comparisons of the expression of mucins before and after CS treatment. *Rho* Spearman's analysis was used to assess the correlation between mucin gene expression and goblet cell number in the different tissues, as well as to correlate mucin expression and nasal symptoms. Statistical significance was set at $P < 0.05$.

Results

Expression of MUC genes at baseline

At w0, there were no significant differences in mucin expression between CS-treated and control groups neither at epithelial nor at glandular level (Table 2).

Membrane-tethered mucins. At w0, MUC1 was highly detected in NP' epithelium and glands whereas MUC4 was highly detected in the epithelium but poorly detected in SMG (Table 2). Regarding the epithelial mucin expression in the different groups of NP, a nonsignificant increase was found in the AIA group compared with nonasthmatic and ATA patients (Fig. 1A,B). Membrane-tethered mucins showed no variations between groups in glands.

Secreted mucins. Mucin expression levels were high for MUC8, moderate for MUC5AC and poor for MUC5B in the epithelium. In glands, MUC5B and MUC8 were moderately detected while MU5AC was poorly expressed (Table 2). In the epithelium, MUC5AC in ATA (median, 25–75th percentile: 40, 35–60) and MUC8 in AIA (100, 100–100) groups showed an increased expression compared with nonasthmatic patients (MUC5AC: 20, 10–30; MUC8: 75, 55–92.5; $P < 0.05$) (Figs 1C and 2A,C). In glands, MUC5B was found decreased in AIA patients (5, 1.3–23.4) compared with ATA (45, 12.5–56.3; $P < 0.05$) and nonasthmatic (35, ns) patients (Fig. 1D).

Mucin regulation by 2 weeks of both oral and intranasal corticosteroids

No significant differences in mucin expression were found in controls between w0 and w2 in the epithelium. Nonsignificant increases were observed in glands (Table 2).

Membrane-tethered mucins. At w2, membrane-tethered mucins increased in the epithelium of the treated group compared with w0 while no variations were found in glands (Table 2).

Regarding the different subgroups, MUC1 increased at w2 compared with w0 in both nonasthmatic (w0: 80, w2: 87.5; ns) and ATA (w0: 70, w2: 97.5; $P < 0.05$) patients,

Table 2. Mucin protein expression in nasal polyps (NP) detected by immunohistochemistry

		Control group (C)		Treatment group (T)		
Mucin		C-w0	C-w2	T-w0	T-w2	T-w12
MUC1	Epithelium	80 (70–88)	85 (71–94)	80 (70–90)	90 (80–100)**	88 (50–100)
	Glands	35 (8–63)	73 (54–88)	73 (34–80)	73 (28–90)	73 (35–80)
MUC4	Epithelium	95 (90–100)	100 (100–100)	90 (70–100)	100 (90–100)	90 (55–100)***
	Glands	5 (0–23)	10 (3–18)	0 (0–3)	5 (0–8)	0 (0–3)
MUC5AC	Epithelium	25 (9–38)	23 (11–80)	30 (14–51)	28 (8–53)	13 (3–28)**,***
	Glands	0 (0–3)	8 (3–18)	0 (0–3)	3 (0–3)	0 (0–3)
MUC5B	Epithelium	1 (0–3)	3 (0–3)	0 (0–3)	3 (0–3)	3 (0–3)
	Glands	10 (5–80)	60 (13–85)	30 (5–45)	30 (8–45)	5 (3–20)*,****
MUC8	Epithelium	98 (73–100)	100 (100–100)	90 (68–100)	100 (93–100)	100 (100–100)**
	Glands	8 (1–10)	28 (2–70)	8 (2–58)	10 (3–40)	30 (5–70)*

Results are expressed as median and 25–75th percentile. C-w0, control group at baseline (week 0); C-w2, control group after 2 weeks without treatment; T-w0, treated group at baseline (week 0); T-w2, treated group after 2 weeks of oral and intranasal corticosteroids (CS); T-w12, treated group after 12 weeks of intranasal CSs.
Wilcoxon test: *$P < 0.05$, and **$P < 0.01$ compared with T-w0; ***$P < 0.05$, and ****$P < 0.01$ compared with T-w2.

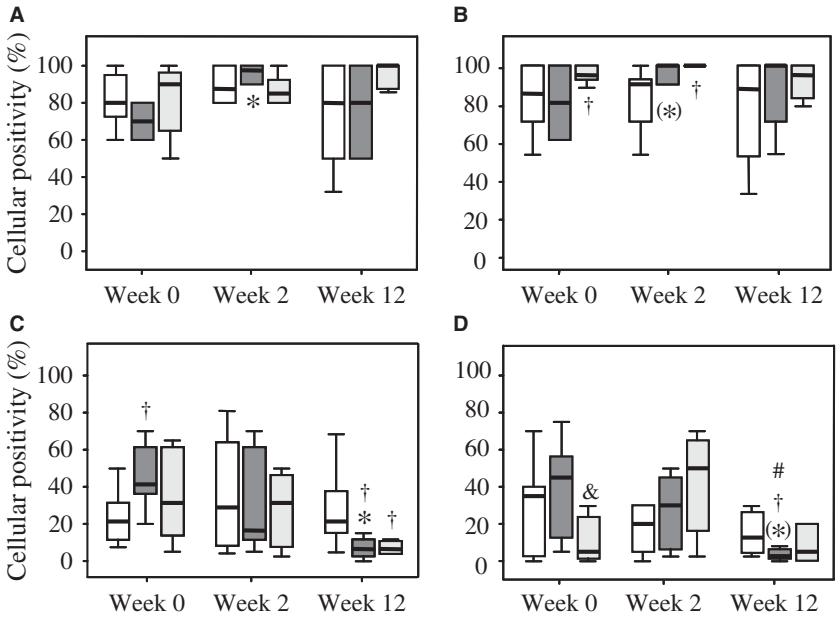

Figure 1. MUC1 (A), MUC4 (B), MUC5AC (C) and MUC5B (D) regulation by corticosteroids (CS) in nasal polyps (NP) from nonasthmatic (open boxes) aspirin-tolerant (ATA) (dark gray), and aspirin-intolerant asthmatic (AIA) (light gray) patients. A, B and C epithelial and D glandular expression. *, $P < 0.05$ compared with week 0; #, compared with week 2; †, compared with NP-NA and &, compared with NP-ATA. Symbols in brackets represent $P < 0.06$.

and MUC4 in ATA patients (w0: 80, w2: 100; $P = 0.06$) (Fig. 1A,B). Interestingly, polyps from AIA patients showed no variations after 2 weeks of steroid treatment. In glands, no variations were found in any of the studied groups.

Secreted mucins. At w2, secreted mucins showed no significant variations compared with w0, neither in the epithelium nor in glands (Table 2).

Mucin regulation by 12 weeks of intranasal corticosteroids

Membrane-tethered mucins. In the epithelium, both membrane-tethered mucins (MUC1, MUC4) showed a similar pattern of regulation by CSs, increasing at w2 and returning to basal levels at w12 while no variations were detected in glands (Table 2). In the epithelium, MUC1 expression decreased in w12 respect to w2 in both nonasthmatics and ATA patients but not in AIA reaching w0 levels, suggesting that the increase detected after oral steroids therapy was not maintained by long-term intranasal steroids in none of these groups (Fig. 1A). At w12, MUC4 showed a trend to decrease in ATA patients and to return to basal levels (w0) (Fig. 1B).

Secreted mucins. After 12 weeks of intranasal steroid treatment, secreted mucins, MUC5AC and MUC5B, showed a significant decrease compared with w0 and w2 in the epithelium and glands, respectively (Table 2).

Regarding the different subgroups, MUC5AC showed a significant decrease in the epithelium at w12 compared with w0 and w2 in the asthmatic group, mainly in ATA patients (w0: 40, 35–60; w2: 15, 10–60; w12: 5, 1.3–10; $P < 0.05$) (Figs 1C and 3A,C). A similar but not significant decrease pattern was found for MUC5B in glands in both nonasthmatic (w0: 35, w2: 20, w12: 12.5; ns) (Fig. 1D) and ATA (w0: 45, w2: 30, w12: 2.5; $P = 0.06$) (Figs 1D and 4A,C) patients. This decrease was not observed in the AIA group (Fig. 1D).

In the epithelium, the soluble mucin MUC8 was found markedly increased in nonasthmatics at w12 (100; $P = 0.06$) compared with w0 (75) and slightly increased compared with w2. Moreover, although the expression of MUC8 in NP glands showed a high variability, especially in asthmatic patients, a significant increase in MUC8 was found in ATA patients at w12 (45; 8.7–85, $P < 0.05$) compared with w0 (2.5; 0–6.2) (Fig. 2B,D) and w2 (2.5; 1.3–75).

Mucins and goblet cells

Alcian blue-periodic acid Schiff staining was observed in epithelial goblet cells and mucous cells of SMG from NP biopsies. While goblet cells stained exclusively blue, mucous cells in glands stained mainly blue, but also pink or purple, when acidic and neutral mucins were jointly expressed. A decrease in goblet cell number was observed after both oral (w2) and intranasal (w12) CS treatment, specifically in ATA patients (Fig. 3B,D). The decrease on

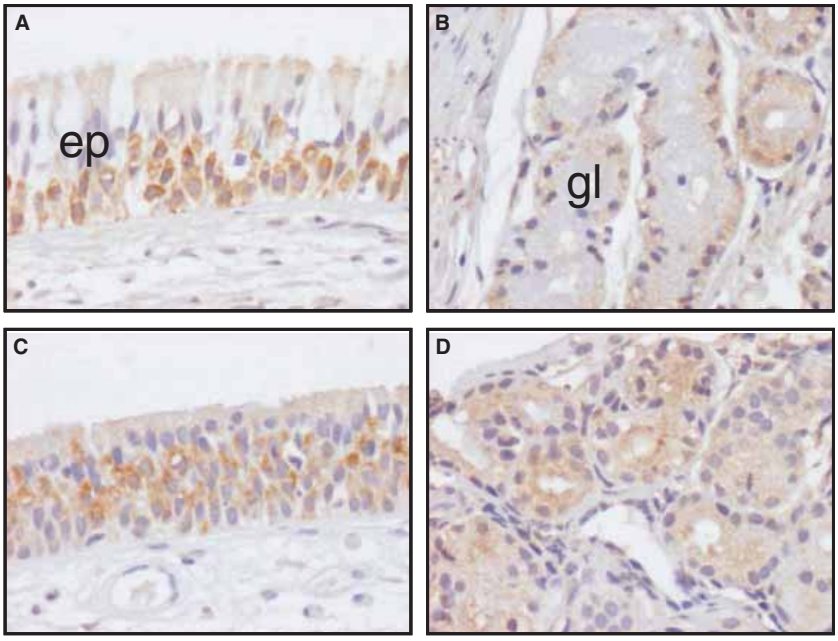

Figure 2. Photomicrographs of MUC8 expression in the epithelium of NP from nonasthmatic (A) and AIA (C) patients at baseline (w0) and in the glands of NP-ATA before (B) and after (D) 12 weeks of CS treatment. ep, Epithelium; gl, glands (original magnification: ×400).

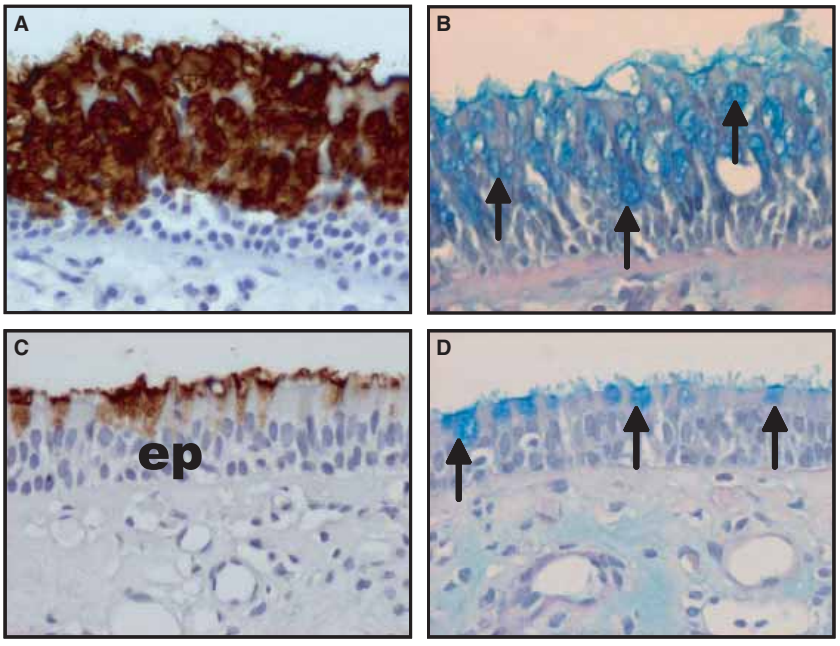

Figure 3. Photomicrographs of MUC5AC (A and C) and Alcian blue-periodic acid Schiff (AB-PAS) staining for goblet cell detection (B and D) in the epithelium (ep) of NP-ATA patients. Changes in MUC5AC mucin due to corticosteroid (CS) therapy correlate with changes in goblet cell (arrows) amount in the epithelium (original magnification: ×400).

goblet cell content in the epithelium correlated with MUC5AC (r: 0.725; $P < 0.01$) (Fig. 3). In addition, a correlation with the AB-PAS staining pattern in glands mucous cells was also found for MUC5B (r: 0.782, $P < 0.01$) (Fig. 4).

Nasal symptoms

Control group showed no variations over the time on nasal symptoms. At w0, there were no significant differences in rhinorrea and nasal obstruction scores

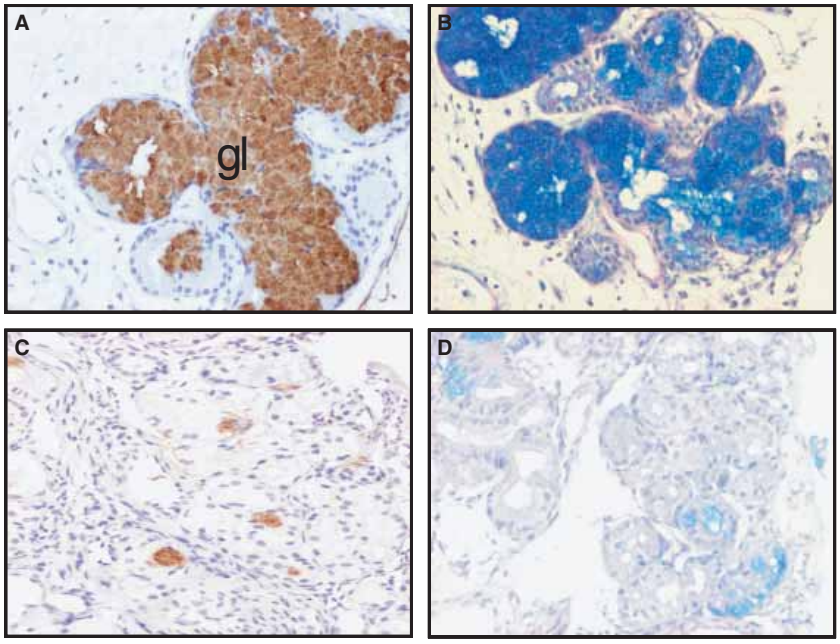

Figure 4. Photomicrographs of MUC5B (A and C) and Alcian blue-periodic acid Schiff (AB-PAS) staining for mucous cell detection (B and D) in submucosal glands (SMGs) (gl) of NP-ATA patients. Changes in MUC5B mucin due to corticosteroid (CS) therapy correlate with changes in the number of mucous cells of SMGs glands (original magnification: ×200).

between treated and control groups. At w2, treated patients showed a significant improvement in nasal obstruction (w0: 3, 2–3; w2: 0, 0–1) and rhinorrea (w0: 3, 2–3; w2: 0, 0–2) compared with w0. At w12, intranasal budesonide maintained the improvement in both nasal obstruction (0, 0–2) and rhinorrea (1, 0–2), similar to w2 (Table 3). No significant differences at baseline (w0) were found between asthmatic and no-asthmatic or ATA and AIA patients. At w2, the improvement on nasal obstruction was higher in asthmatics (3, 1.5–3; $P < 0.01$) than in nonasthmatic (1, 0–1.3) patients.

A significant correlation was observed between the improvement in rhinorrea and the reduction of MUC5AC after treatment ($r = 0.403$, $P < 0.05$). MUC5B showed a similar tendency but with no significance. No correlation was found between secreted mucin expression and nasal obstruction.

Discussion

In the present study, different regulation patterns by CSs were observed depending on the type of mucins, secreted *vs* membrane-tethered, on the duration of steroid treatment, short courses *vs* long-term therapy and on the phenotypic characteristics of NP. While a short-term treatment with oral prednisone combined with intranasal budesonide seemed to up-regulate membrane-tethered mucins (MUC1 and MUC4) in almost all NP epithelia and the long-term therapy failed to maintain this effect, secreted mucins MUC5AC and MUC5B appeared to strongly respond to the long-term treatment by decreasing their expression in the epithelium and glands, respectively. This is at variance with two previous studies in which no variations were found in MUC5AC expression after either 8 weeks of intranasal fluticasone in NP (28) or 1 month of intranasal budesonide in lung

Table 3. Effect of oral and intranasal corticosteroids (CS) on nasal symptoms in nasal polyp (NP) patients

	Control group (C)		Treatment group (T)			Improvement after treatment	
Nasal symptoms	C-w0	C-w2	T-w0	T-w2	T-w12	T-w2 (from T-w0)	T-w12 (from T-w0)
Rhinorrea	3.0 (2.0–3.0)	2.5 (1.0–3.0)	3.0 (2.0–3.0)	0.0 (0.0–1.0)*,**	0.0 (0.0–2.0)*	2.0 (1.0–3.0)	1.0 (0.0–3.0)
Obstruction	3.0 (2.8–3.0)	3.0 (2.0–3.0)	3.0 (2.0–3.0)	0.0 (0.0–2.0)*,**	1.0 (0.0–2.0)*	2.0 (1.0–3.0)	1.0 (0.0–3.0)

Results are expressed as median and 25–75th percentile. C-w0, control group at baseline (week 0); C-w2, control group after 2 weeks without treatment; T-w0, treated group at baseline (week 0); T-w2, treated group after 2 weeks of oral and intranasal CSs; T-w12, treated group after 12 weeks of intranasal CSs.
Wilcoxon test: *$P < 0.01$ compared with T-w0; **$P \leq 0.01$ compared with C-w2.

tissue biopsies (29). These differences could be explained by the small number of patients analysed in both studies as well as to the short duration of treatment. In agreement with our findings, several *in vitro* studies have reported that dexamethasone decreases MUC5AC mRNA in airway epithelial cell lines (30–32), primary normal human bronchial epithelial (NHBE) cells (32) and rat primary airway epithelial cells (31) while dexamethasone increased MUC1 in cancer cell lines (33, 34).

These different regulation patterns might reflect a variety of pathophysiological roles of mucins in mucus production and secretion. Given that MUC5AC and MUC5B are the major mucins found in respiratory tract secretions (26, 35) they might play an important role in mucus formation. Therefore, the down-regulation caused by CSs in MUC5AC and MUC5B levels could result in a decrease of mucus hypersecretion from NP. In this direction, down-regulation of MUC5AC after CS treatment clearly correlated with the improvement of rhinorrea in all groups of NP patients. Completely different functions have been described for the two membrane-tethered mucins here studied. MUC1 has been reported to be involved in metastasis, angiogenesis and immune regulation (33, 36, 37) while MUC4 has been identified as a ligand of ErbB2 (38), a receptor that modulates epithelial cell proliferation following damage in airways of asthmatics (39). The increase of MUC1 and MUC4 levels after CS treatment may be related to the epithelial repairing and remodeling processes in which they seem to be involved.

Although MUC8 has been reported to be increased in chronic rhinosinusitis and NP compared with healthy nasal mucosa (13, 40), its potential role as one of the major compounds of mucus has not been well-established. In the present study, CS treatment increased MUC8, like membrane-tethered mucins, with a maximum response after long-term CSs. Since MUC8 does not seem to be a major secreted mucin, the different CS regulation pattern of MUC8 compared with other secreted mucins could account for a different role of this mucin in NP.

Since NP is an inflammatory disease affecting 10–15% of asthmatic patients and over 90% of patients with AIA, a special attention was paid to these groups of patients.

The groups of NP patients showed a differential response to CS therapy. Nasal polyps from ATA showed the most significant changes for all analysed mucins, while those from nonasthmatics showed variations in MUC1, MUC5B and MUC8, and those from AIA patients showed changes almost exclusively in MUC5AC, suggesting a trend of resistance to CS treatment. In accordance to these findings, aspirin sensitivity has been reported to be a risk factor for steroid resistance in patients with NP (41) as well as in steroid nonresponder severe asthmatic (42). A greater inflammatory component and/or a reduced number of CS receptors in AIA patients could account for its CS resistance. Another potential explanation for this lack of response could be the high basal levels of membrane-tethered mucins and the low levels of secreted mucins in AIA patients, almost comparable with the levels found in ATA patients after CS treatment.

Goblet cell hyperplasia has been reported in airways diseases such as NP (15) and asthma (43, 44). In a GCH rat model, CSs inhibited the hyperplasia induced by tobacco smoke (45) and neutrophils products (46). In this sense, since CSs could decrease GCH, the changes in mucin content found in our NP biopsies after CS treatment might be due to changes in the number of goblet cells. In fact, a correlation was found between MUC5AC expression and goblet cell numbers, as well as between mucous cells in SMG and MUC5B. Since MUC1, MUC4 and MUC8 are not goblet cell-specific mucins we could speculate that their increase after CS treatment might be explained by an increased number of nongoblet cells (basal, ciliated) that might take place in NP epithelium to counteract the decrease of goblet cell number after CS treatment. Although there are no studies dealing with CS effects on GCH in NP, Laitinen et al. have demonstrated that long-term treatment of asthmatic subjects with inhaled CS significantly increased the ratio of ciliated cells to goblet cells in the airways (47). However, other steroid effects should be taken into account: CSs could exert their action directly regulating MUC gene expression (32) or indirectly through their inhibitory effects on pro-inflammatory cytokines (48).

In conclusion, our study demonstrates that a short course of oral steroids increases membrane-tethered (MUC1 and MUC4) mucins and that long-term intranasal steroid treatment is able to decrease major secreted mucins (MUC5AC and MUC5B). The down-regulation of secreted mucins could result from the ability of CSs to reduce GCH, and could account for the reduction of mucus production and rhinorrea. Since CSs are capable to reduce the number of the main mucin-producing cells and they also decrease rhinorrea, our results suggest that CS may be considered a beneficial therapy for mucus hypersecretion in NP. Notwithstanding that, regarding mucin expression, patients with NP and aspirin-sensitive asthma seems to show a trend of resistance to CS treatment.

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Study 3. Dexamethasone decreases basal and IL-1β-induced MUC5AC expression in A549 cells (in preparation).

DEXAMETHASONE DECREASES BASAL AND IL-1β-INDUCED MUC5AC EXPRESSION IN A549 CELLS

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Abstract

Background: In airway inflammatory diseases such as asthma and nasal polyposis, several proinflammatory cytokines have been found increased and involved in the up-regulation of mucins, the major component of mucus. Glucocorticoids (GC) seem to be the most effective drug controlling the mucus hypersecretion associated to airway inflammation. Although some studies have reported glucocorticoid effect on mucin steady-state levels, few have dealt with this matter after proinflammatory agent induction.

Objectives: To analyze the effect of dexamethasone (DEX) on both basal and IL-1β-induced MUC5AC and MUC5B mRNA expression (RT-PCR) and protein secretion (ELISA) in A549 human lung adenocarcinoma cells.

Methods: A549 cells were incubated with or without IL-1β (from 0.1 to 20 ng/ml), DEX (from 10^{-9} to 10^{-6} M), or IL-1β + DEX during 1, 6, 12, and 24h. Both, cells and culture media were collected and MUC5AC and MUC5B mRNA and protein secretion were analyzed by RT-PCR and ELISA, respectively.

Results: Our results showed that: a) IL-1β induced MUC5AC mRNA and protein secretion in a dose dependent manner; b) DEX caused a dose-dependent decrease of MUC5AC mRNA expression at both basal and after IL-1β induction, and a decrease of MUC5AC protein secretion after IL-1β induction; and c) DEX slightly reduced MUC5B mRNA abundance at baseline.

Conclusion: Glucocorticoids decrease both MUC5AC gene and protein expression at basal and under inflammatory conditions in human respiratory cells.

Introduction

Mucins, which are high-molecular-weight glycoproteins produced by epithelial goblet cells and mucous cells from submucosal glands, are essential for the viscoelastic properties of airways mucus. To date, twenty human mucin genes have been identified and subdivided into secreted and membrane-tethered mucins (1). Among them, MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC7, MUC8, and MUC13 [2-4] are normally expressed in the human respiratory tract, although only MUC5AC and MUC5B have been convincingly demonstrated to be major components of human airway secretion [5, 6].

Mucus hypersecretion is commonly observed in respiratory diseases such as chronic rhinosinusitis, asthma, chronic bronchitis, and cystic fibrosis (1). Moreover, in these diseases an abnormal mucin composition of the mucus gel has been reported regarding the amount, type, and size of mucins [4, 7-9], these changes contributing to the rheological properties of airways mucus and leading to an impaired mucociliary clearance.

Several cytokines and inflammatory mediators that are found elevated in chronic airway diseases (10-15) have been found to stimulate mucus hypersecretion (16). Among them, interleukin (IL)-1β is one of the most important multifunctional proinflammatory cytokines playing a role in mucin overproduction (17-21).

Glucocorticoids remain the most effective anti-inflammatory drug in the treatment of inflammatory airway disorders such as chronic rhinosinusitis with/out nasal polyps and asthma (22, 23). The inhibitory effects of glucocorticoids on the synthesis of inflammatory mediators is considered the central mechanism of their efficacy. Although glucocorticoid effects on mucus hypersecretion has always been controversial, paying attention to mucin overproduction, a recent *in vivo* study has reported a reduction in MUC5AC and MUC5B mucins linked to the improvement of rhinorrhea after GC therapy in patients suffering from nasal polyps (24).

Moreover, some *in vitro* studies have confirmed a decrease in MUC gene mRNA expression in human airway cell lines (25-27) and primary rat tracheal epithelial cells (27) after glucocorticoid treatment. Although these studies dealt with glucocorticoid effect on basal MUC gene expression and/or mucin secretion, only one study have reported the GC effect on cytokine-induced mucin over-expression/secretion (28).

Since mucin overproduction in inflammatory airway diseases might be partly due to the mucin upregulation prompted by proinflammatory cytokines, the aim of this study was: first, to determine which inflammatory mediators were able to increase MUC5AC and MUC5B gene expression in a human respiratory cell line (A549); second, to determine whether the proinflammatory cytokine IL-1β regulated MUC5AC and MUC5B mucin gene expression and protein secretion in A549 cell cultures; and third, to determine whether the glucocorticoid dexamethasone (DEX) was able to inhibit both basal and IL-1β-induced MUC5AC and MUC5B gene expression and protein secretion from A549 cells.

Material and methods.

Cell culture and treatment.

A549 human adenocarcinoma cells (American Type Culture Collection Rockville, MD) were seeded at a density of 1 X 10^6 cells per well into 6-well plates (Costar, Corning, NY). Cultures were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), penicillin (100 U/ml), and streptomycin (100μg/ml) at 37ºC in a humidified 5% CO_2 atmosphere. Cells were grown until confluence and maintained in serum-free media for 24h before stimulation. Plates were rinsed twice with phosphate buffered saline (PBS) and incubated with the following agents: human recombinant IL-1β (from 0.1 to 20 ng/ml), human recombinant TNF-α (20ng/ml), bacterial lipopolysaccharide (LPS; 10μg/ml), a cytokine mixture (CytMix) composed of IL-1β, TNF-α and interferon-γ (all at 10ng/ml), metacholine (10^{-6}M), and 10% FCS. To study the effect of DEX

on IL-1β-induced MUC gene expression cell cultures were pretreated with increasing concentrations of DEX (from 10^{-9} to 10^{-6} M) for 1h before incubation with IL-1β (20ng/ml) while control cultures were incubated with culture media alone. IL-1β was dissolved in distilled water, and DEX was dissolved in RPMI 1640 media. All experiments were performed in triplicate on three separated occassions.

After 1, 6, 12, and 24h of treatment cells and culture media were collected to be analyzed by real time RT-PCR (MUC gene expression) and enzyme-linked immunosorbent assay (ELISA) (mucin protein secretion), respectively.

Enzyme-linked immunosorbent assay of cell culture media.

MUC5AC and MUC5B mucins were measured in cell culture media by means of direct ELISA using the mouse monoclonal antibody 45M1 (Neomarkers, Fremont, CA) and the polyclonal antibody LUM5B.2 (29), respectively. To assay MUC5AC mucin, cell culture media were diluted 1:100 with PBS1X and 100 μl/well were added in duplicate in MaxiSorp microtiter 96-well plates (Nunc, Rochester, NY) and let dry at 37ºC for 90 minuts. Wells were washed three times with PBS-0.05% Tween-20 (PBS-T) and blocked with 1% bovine serum albumin (BSA) in PBS for 1h at 37ºC. After 5 washes with PBS-T between each step, wells were sequentially incubated with the 45M1 antibody (100μl/well; 1:200 dilution) for 1h at 37ºC, peroxidase-labeled horse anti-mouse antibody (Vector Laboratories, Burlingame, CA) (100 μl/well; 1:5000; 1h at 37ºC), and 3,3',5,5'-tetramethylbenzidine (Pierce, Rockford, IL) substrate [100μl/well; 1:5000; 30 min at room temperature (RT)]. The reaction was terminated by adding 1N HCl stop solution (50μl/well; 15 min RT) and absorbance was read at 450nm. To assay MUC5B mucin, a similar protocol was followed but using the LUM5.B2 polyclonal antibody as primary antibody and the peroxidase-labeled goat anti-rabbit antibody (Vector Laboratories, Burlingame, CA) as secondary antibody. The relative amount of MUC5AC and MUC5B mucins in each sample was determined from their A_{450} values using standard curves

constructed with serial dilutions of the commercial *Mucins from Porcine Stomach type II* (Sigma-Aldrich, Steinheim, Germany) for MUC5AC and human saliva for MUC5B. Results were expressed as percentage of control. Sample and standard duplicates showed a variation rate of < 5%.

Real-time RT-PCR analysis of gene expression

Total RNA from A549 cells was isolated using Qiagen (Valencia, CA) 96 RNeasy kit according manufacturers' instructions. cDNA was obtained from total RNA (2μg) using Retroscript kit (Ambion) following manufacturers' recommendations. Real-time PCR was performed on the generated cDNA products in the Lihgtcycler System (Roche diagnostics) using a reaction containing: 3mM MgCl2, 0.5 μM of primers, 2μl of Lightcycler Fast Start DNA Master SYBR Green I (Roche Diagnostics), 1U of Uracil-DNA glycosilase, and 10-40 ng of cDNA up to a 20μl volum. The following primer sequences were used: MUC5AC *sense* 5'-GTACCAGAACAGTCGACCT-3', *antisense* 5'-CTCTTCCACCTCGGTGTAGC-3'; MUC5B *sense* 5'-CACATCCACCCTTCCAAC-3', *antisense* 5'-GGCTCATTGTCGTCTCTG-3'; β-actin *sense*, 5'-GAAACTACCTTCAACTCCATC-3', *antisense* 5'-CTAGAAGCATTTGCGGTGGAC-3'. β-Actin was unchanged by the incubation with DEX and was used as an internal control for normalizing MUC5AC and MUC5B mRNA levels in control and experimental samples. Quantification was done by means of relative expression ratios (30) normalized with β-actin gene expression.

Statistical Analysis

All experiments were performed on multiple occasions using triplicate samples. All data was represented as mean ± standard error of the mean (SEM), except for the results on the effect of proinflammatory stimuli (IL-1β, TNF-α, CytMix, LPS, and FCS) on MUC5AC and MUC5B mRNA expression that was represented as median and 25-75th percentils. Differences between conditions were assessed by the one way ANOVA and Dunnet's posthoc test. The non-parametric Mann-Whitney U test was used for comparisons between pro-inflammatory mediators (IL-1β, TNF-α, CytMix,

LPS, and FCS) and control groups. A *P* value of less than 0.5 was considered statistically significant.

Results.

IL-1β, CytMix, and FBS increased MUC5AC mRNA expression in A549 cells. From all analyzed pro-inflammatory stimuli, only IL-1β, CytMix, and FBS were able to significantly increase, after 6h of treatment, MUC5AC mRNA expression compared to control (Fig. 1A). After 24h of treatment, IL-1β, CytMix, and FBS, failed to maintain this induction (data not shown). None of the stimuli induced MUC5B mRNA expression in A549 cell cultures (Fig. 1B). Since IL-1β was the proinflammatory agent that most homogeneously increased MUC5AC mRNA expression, this cytokine was selected to study the effect of glucocorticoids (dexamethasone) on MUC5AC-inflammatory induced expression.

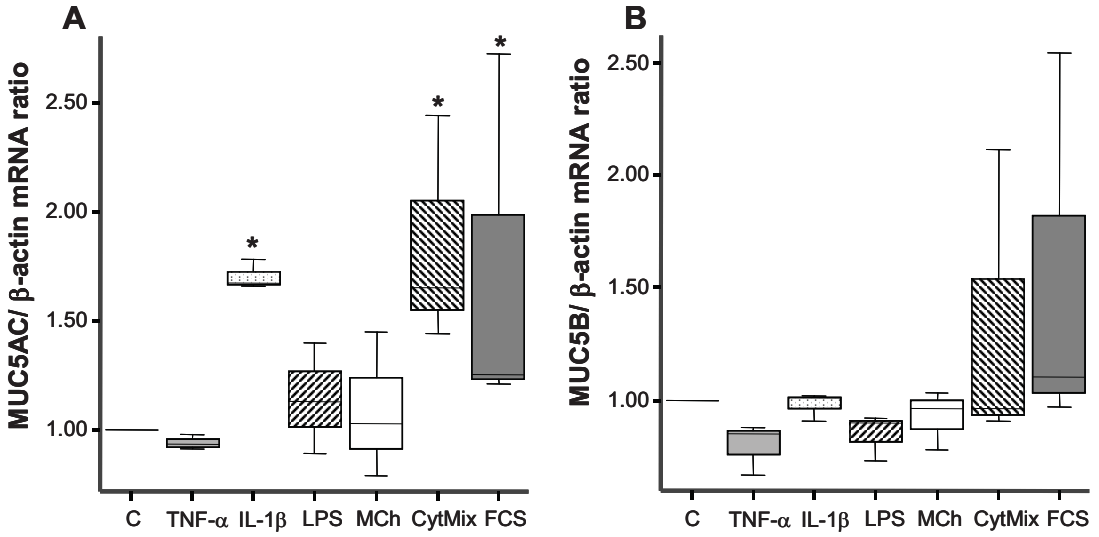

Figure 1. Effect of inflammatory mediators on MUC5AC (A) and MUC5B (B) mRNA expression. A549 cells were exposed to tumor necrosis factor-α (TNF-α, 20 ng/ml), interleukin-1β (IL-1β, 20 ng/ml), bacterial lipopolisaccharide (LPS, 10 μg/ml), metacholine (MCh, 10^{-6} M), a mix of cytokines (CytMix, all at 10 ng/ml), fetal calf serum (FCS, 10%), or culture media alone (C) for 6h. (A) IL-1β, CytMix, and FCS induced MUC5AC expression compared to control. (B) None of the stimuli caused a significant induction of MUC5B mRNA expression. Box plots show the 25th, 50th (median) and the 75th percentile values. Each experiment was performed in triplicate on three separated occasions. Comparisons were made using the Mann-Whitney *U* test. **P < 0.05* compared to control.

IL-1β increased MUC5AC mRNA expression and protein secretion in A549 cells. In order to analyze IL-1β effect on MUC5AC and MUC5B mRNA expression and protein secretion dose-response experiments were performed in A549 cell cultures. IL-1β was found to increases both, MUC5AC mRNA and protein levels in a dose-dependent manner in A549 cells (Fig. 2). In addition, IL-1β failed to stimulate MUC5B gene and protein expression (data not shown).

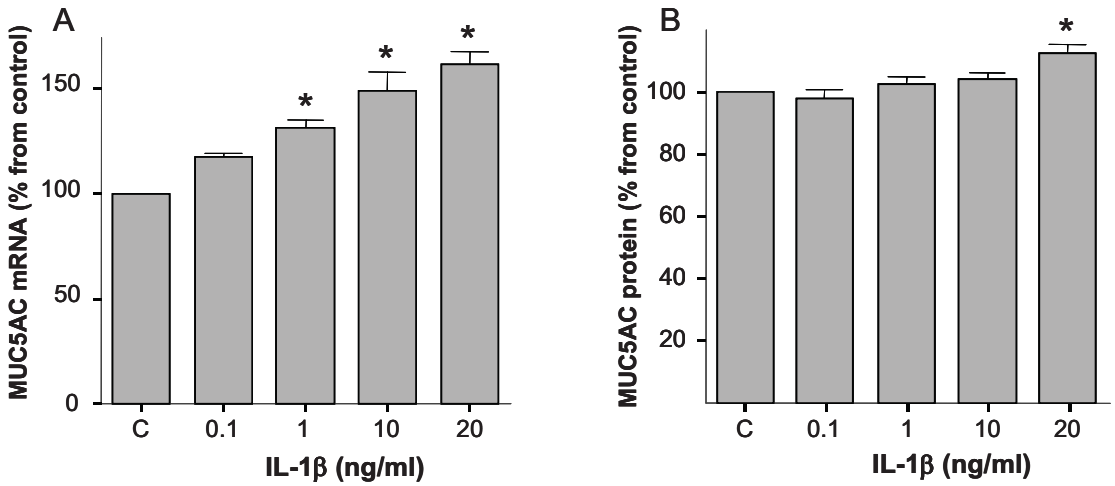

Figure 2. Interleukin (IL)-1β increases MUC5AC mRNA expression (A) and protein secretion (B) in A549 cells. A549 cells were exposed to increasing concentrations of IL-1β (0.1-20 ng/ml) or culture media alone (C) for 6h. Results are expressed as mean ± SE. Each experiment was performed in triplicate on three separated occasions. Comparisons were made using the Dunnett's test. *$P < 0.05$ compared to control (C).

Dexamethasone decreased basal and IL-1β-induced MUC5AC mRNA expression in A549 cells. In order to asses DEX effect on basal and IL-1β-induced mucin expression, MUC5AC and MUC5B mRNA levels were assessed in a dose-response (10^{-6} to 10^{-9}M) and time-response (1, 6, 12, 24h) manner in A549 cell cultures. In addition, the effect of dexamethasone on basal and IL-1β-induced MUC5AC and MUC5B protein secretion was analyzed in over-the-time experiments.

DEX decreased both basal (Fig. 3A) and IL-1β-induced (Fig. 4A) MUC5AC mRNA expression in a dose-dependent manner (10^{-9}-10^{-6} M), showing a peak effect at the concentration of 10^{-6} M. DEX decreased both basal and induced expressions in approximately 50% with respect to control. Moreover, DEX caused a significant reduction of IL-1β-induced MUC5AC protein secretion at the concentration of 10^{-6} M (Fig. 4B). On the

other hand, DEX decreased MUC5B basal expression dose-dependently but without statistical significance (Fig. 3B).

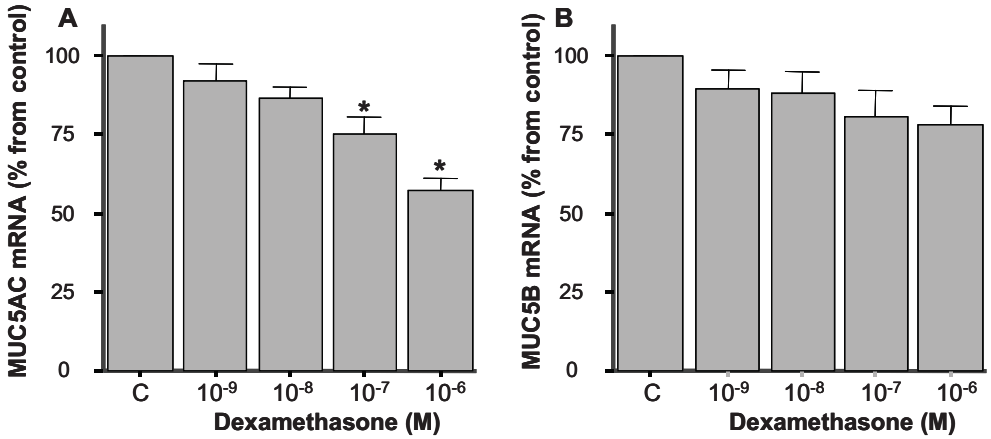

Figure 3. Dexamethasone decreases basal MUC5AC (A) but not MUC5B (B) mRNA abundance in A549 cells. A549 cells were exposed to DEX (10^{-9}-10^{-6} M) or culture media alone (C) for 24h. Results are expressed as mean ± SE. Each experiment was performed in triplicate on three separated occasions. Comparisons were made using the Dunnett's test. *$P < 0.05$ compared to control (C).

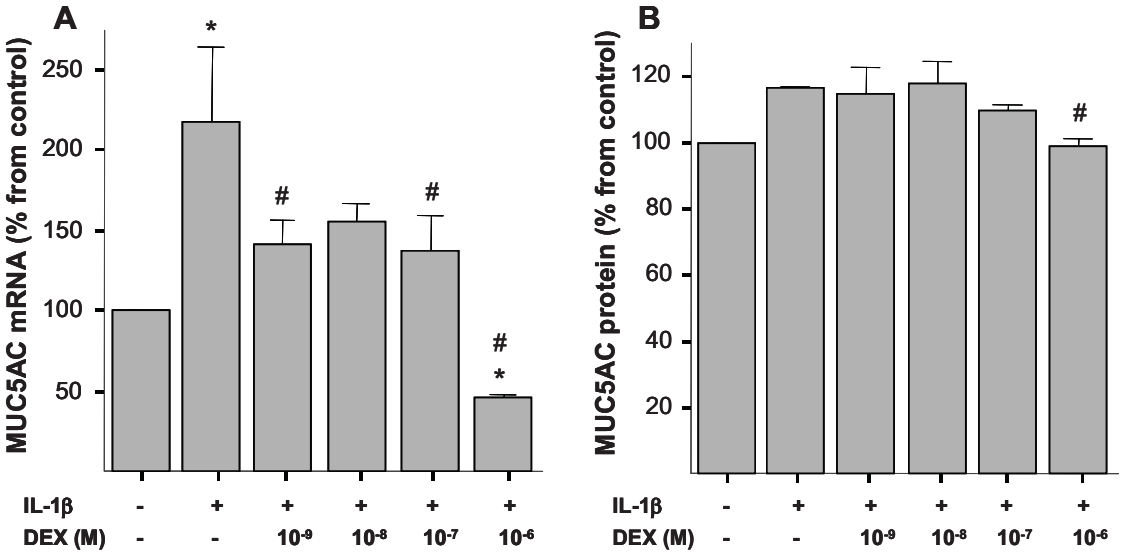

Figure 4. Effect of dexamethasone (DEX) on interleukin (IL)-1β induced MUC5AC gene (A) and secreted protein (B) levels. A549 cells were cotreated with IL-1β (20 ng/ml) and increasing concentrations of DEX for 12h. DEX decreases IL-1β–induced MUC5AC mRNA and protein expression, especially at 10^{-6} M. Results are expressed as mean ± SE and represent three independent experiments. Comparisons were made using the Dunnett's test. *$P < 0.05$ compared to control (C); #$P < 0.05$ compared to IL-1β. Significances between brackets represent $P < 0.06$.

Over-the-time experiments (1h to 24h) showed spontaneous increases in MUC5AC and MUC5B expression in the control untreated cells. This reflects an increase in A549 cell number over the time; therefore, mRNA and protein levels in cells exposed to DEX and/or IL-1β were compared at each time point with control cells.

Over-the-time experiments showed DEX to decrease MUC5AC mRNA in A549 cells after 12 and 24h of exposure, both at baseline and after IL-1β induction (Fig. 5A). Although IL-1β failed to stimulate MUC5B mRNA expression in A549 cells, DEX significantly decreased MUC5B expression at basal levels after 12 and 24h of incubation (Fig. 6).

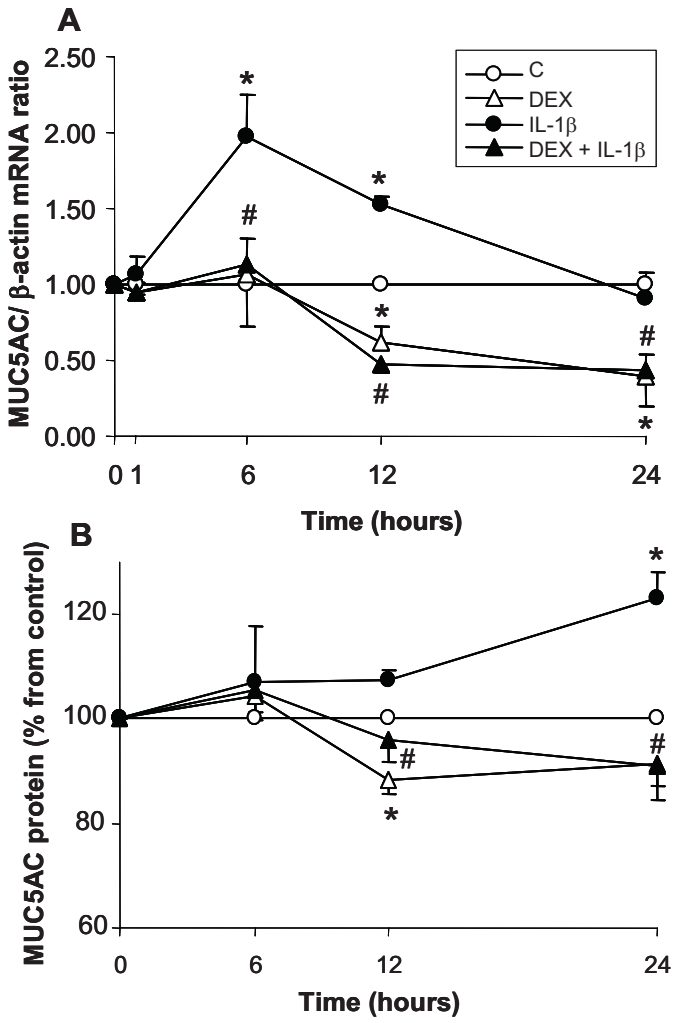

Figure 5. Kinetics of dexamethasone (DEX) effect on MUC5AC mRNA and secreted protein levels at basal and after interleukin (IL)-1β stimulation. A549 cells were incubated with IL-1β (20ng/ml), DEX (10^{-6} M), DEX + IL-1β or culture media alone (C) for 1, 6, 12, and 24 hours. (A) IL-1β significantly stimulated MUC5AC mRNA expression after 6 and 12h of incubation while DEX decreased MUC5AC mRNA abundance at basal and after IL-1β induction after 12 and 24h. (B) IL-1β significantly stimulated MUC5AC secretion after 24h of incubation whereas DEX decreases MUC5AC secretion at basal and after IL-1β induction after 12h and 24h of incubation. Results are expressed as mean ± SE. Each experiment was performed in triplicate on three separated occasions. Comparisons were made using the Dunnett's test. *$P < 0.05$ compared to control (C); #$P < 0.05$ compared to IL-1β.

In addition, dexamethasone decreases both basal and IL-1β-induced MUC5AC protein secretion after 12h and 24h of incubation (Fig. 5B). IL-1β failed to stimulate MUC5B secretion and dexamethasone did not show a clear down-regulatory effect on this mucin secretion (data not shown).

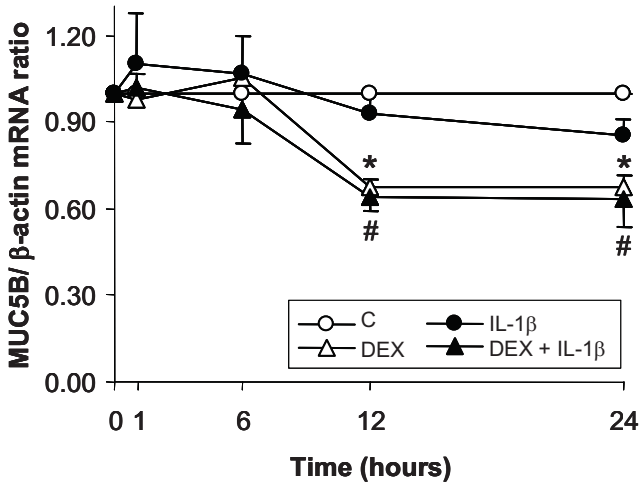

Figure 6. Kinetics of dexamethasone (DEX) effect on MUC5B mRNA levels at basal and after interleukin (IL)-1β stimulation. A549 cells were incubated with IL-1β (20ng/ml), DEX (10^{-6} M), DEX + IL-1β or culture media alone (C) for 1, 6, 12, and 24 hours. IL-1β failed to stimulate MUC5B mRNA expression while DEX decreases MUC5B mRNA levels after 12 and 24 h of incubation. Results are expressed as mean ± SE. Each experiment was performed in triplicate on three separated occasions. Comparisons were made using the Dunnett's test. *$P < 0.05$ compared to control (C); #$P < 0.05$ compared to IL-1β.

Discussion.

In this study, we demonstrate that dexamethasone decreased MUC5AC mRNA dose-dependently and after different time exposures in A549 cells, both at basal and after induction with IL-1β. In addition, dexamethasone decreased MUC5AC protein secretion under inflammatory conditions. By contrast, IL-1β failed to induce MUC5B mRNA and protein expression, while dexamethasone slightly decreased this mRNA expression.

Glucocorticoids are the only class of drugs available and found to be effective to clinically control mucus hypersecretion associated with airway inflammation. Because cytokines are well known to stimulate mucin gene expression and mucus production and secretion (1, 31, 32), it is reasonable to speculate that the mechanism of the inhibitory effects of glucocorticoids on mucus hypersecretion in airway epithelial cells involves an indirect effect of their anti-inflammatory properties (22). In fact, previous studies have demonstrated glucocorticoids to reduce basal and both IL-1β and TNF-α induced glandular secretion in bronchial and nasal mucosa (32, 33).

Among all the analyzed proinflammatory stimuli only IL-1β, a mixture of cytokines consisting of IL-1β, TNF-α, and IFN-γ, and the unspecific stimulus FCS were able to upregulate MUC5AC mRNA expression. In accordance with our findings, IL-1β has been found to upregulate MUC5AC mRNA expression in NCI-H292 cells and human nasal epithelial cells (17, 21). However, Gray et al. reported no changes in MUC5AC mRNA expression although MUC5AC secretion was down-regulated in normal human tracheobronchial epithelial cells after dexamethasone exposure (19). In our study, although TNF-α alone failed to induce MUC5AC mRNA expression in A549 cells, some studies have reported TNF-α to stimulate MUC5AC expression and/or secretion in human nasal epithelial cells from healthy and inflamed (nasal polyps) upper airway mucosa (21, 34), as well as in human nasal mucosa explants (35). The different cell type analyzed could be a potential explanation for the discrepancies here found.

The dexamethasone-induced decrease in MUC5AC mRNA abundance at basal levels has been previously reported in both NCI-H292 and A549 cell lines (25, 26). Moreover, Chen et al. reported that dexamethasone partly exerted its action directly on MUC5AC gene promoter region, reducing this way MUC5AC mRNA expression (25). In our study, the dexamethasone effect of reducing the basal MUC5AC mRNA expression was corroborated by performing dose-response studies, demonstrating that dexamethasone reduces MUC5AC mRNA levels in a dose dependent manner.

Besides the effect on basal MUC5AC mRNA expression, our dose-response experiments also demonstrated dexamethasone being able to decrease IL-1β-induced MUC5AC mRNA expression. Thus, our findings were in accordance to previous findings where dexamethasone suppressed steady-state MUC5AC gene expression in both NCI-H292 (26) and A549 cells (25, 27), as well as to the inhibition caused by dexamethasone on IL-1β-induced MUC2 mRNA expression in NCI-H292 cells (28).

A unique previous study on the effect of dexamethasone on steady-state MUC5B mRNA expression, reported no changes in MUC5B mRNA

abundance after dexamethasone incubation in nasal polyp epithelial cells (36). By contrast, in our study dexamethasone produced a slight dose-dependent decrease in MUC5B mRNA expression in A549 cells. Hence, primary cells and cell lines seem to differently behave in front of similar stimulus.

In conclusion, we here report that IL-1β increases MUC5AC gene expression and protein secretion and that dexamethasone is able to dose-dependently decrease both basal and IL-1β-induced MUC5AC mRNA expression, as well as IL-1β-induced MUC5AC protein secretion. In addition, dexamethasone slightly down-regulates MUC5B gene expression.

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Study 4. Mucin gene expression in rhinitis syndromes. *Curr Allergy Asthma Rep* 2006; 6(3):189-97 *[Review].*

Mucin Gene Expression in Rhinitis Syndromes

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Rhinitis and rhinosinusitis are often associated with airway diseases such as asthma, cystic fibrosis, and nasal polyposis. In these diseases, the alteration of both the quantity and quality of mucus results in an impaired mucociliary clearance, and this produces, in extreme cases, the airway obstruction. Mucins are the major component in mucus and are responsible for its viscoelastic properties. Mucin expression patterns have been shown to be altered in rhinitis-associated diseases. It has been proposed that this is one of the causes of hyperviscid mucus plugs in these pathologies. For this reason, the study of mucin expression and regulation in upper- and lower-airway diseases, such as asthma, cystic fibrosis, and nasal polyposis, may be crucial for the development of new therapies against mucus hypersecretion. In this review, we report major findings regarding mucin expression and regulation in rhinitis syndromes.

Introduction

Rhinitis and rhinosinusitis are common respiratory diseases associated with lower-airway diseases, mainly asthma [1••]. They are characterized by the presence of one or more of the following symptoms: sneezing, itching, rhinorrhea, nasal congestion, and loss of the sense of smell.

Airway mucus is composed of water, ions, lung secretions, serum protein transudates, antimicrobial proteins, and mucus glycoproteins (mucins) [2]. The main role of this mucus is to cover and protect the respiratory tract by trapping pathogens and irritants and allowing their removal by mucociliary clearance. This function is carried out by the action of epithelial-cell cilia that are embedded in the mucus gel phase and sweep it along with a coordinated stroke. The diffusion of microorganisms through the mucus barrier may be limited by binding to antibodies and by physiochemical interactions with glycoconjugates and antimicrobial proteins [3,4].

Mucus secretions mainly contain plasma, glandular, and goblet cell products. Plasma exudation is the source of albumin, immunoglobulin (Ig)G, IgM, fibrinogen, complement, and other plasma proteins [4]. Glandular secretions mainly contain products from serous and mucous cells of the submucosal glands. Secretory IgA, lactoferrin, lysozyme, and peroxidase are serous products that play a defensive role against infectious agents [5]. Mucous cells of submucosal glands and goblet cells synthesize and secrete mucins, the major macromolecular component of mucus.

In physiologic conditions, the airway glandular secretion is under the control of a variety of mechanisms, but the nervous system seems to have a prominent role. Glandular structures are innervated by parasympathetic (cholinergic), sensory (nonadrenergic noncholinergic), and sympathetic (adrenergic) nerves. However, the physiologic regulation of airway glandular secretion is mainly due to parasympathetic and sensory nervous systems [4]. In pathologic conditions, inflammatory cells participate in the regulation of glandular secretion secreting inflammatory mediators such as prostaglandins, leukotrienes, histamine, cytokines, and eosinophil-derived proteins [3] (Fig. 1).

In several airway diseases, such as chronic rhinosinusitis, asthma, and cystic fibrosis (CF), airway inflammation and mucus hypersecretion are usually accompanied by histologic and physiologic changes, such as goblet cell hyperplasia, submucosal gland hypertrophy, and an increase of inflammatory mediators [6,7].

Because mucin production is part of the innate immunity of the airway, the study of the mucin expression patterns in health and disease will help to improve the current therapies against mucus hypersecretion, taking into account that an effective treatment entails a return to a normal phenotype and not the mere inhibition of secretion. In this article, we review the expression of mucins in various rhinitis syndromes, paying attention to their localization and their regulation as well as to the histologic changes of the respiratory tract in the different pathologies.

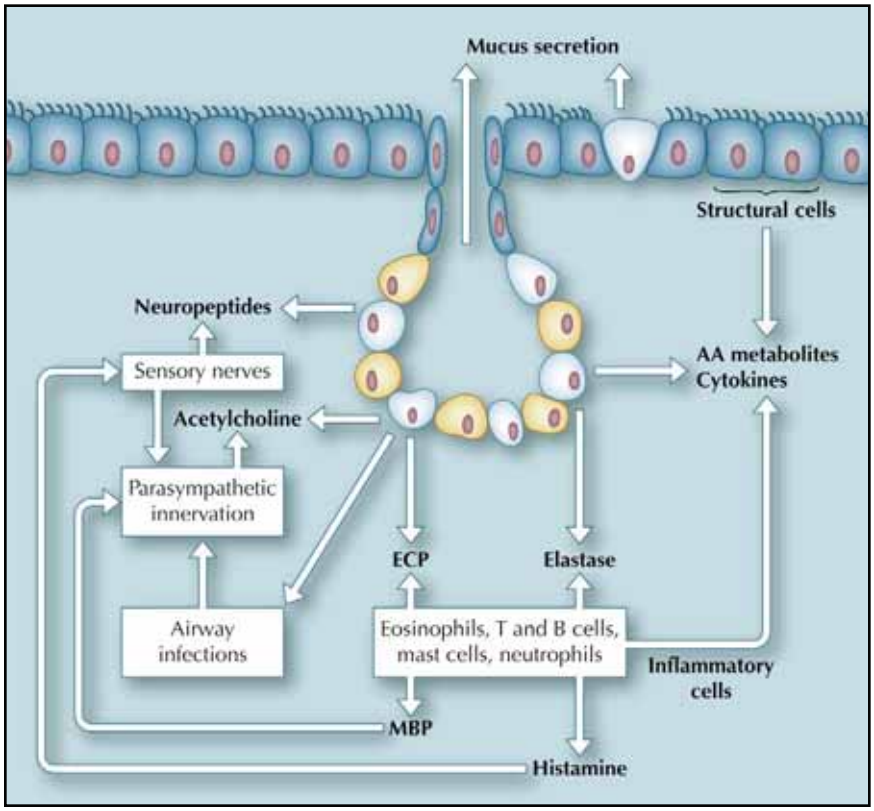

Figure 1. Regulation of airway glandular secretion. Mucus secretion during both physiologic and inflammatory situations is regulated by sensory and parasympathetic nerves and by activated structural and inflammatory cells. Neuropeptides and neurotransmitters released by parasympathetic and sensory nerves directly stimulate mucus secretion. Parasympathetic activity is stimulated by sensory nerves, histamine, and major basic protein (MBP). Viral infections induce mucus secretion by a cholinergic mechanism. Arachidonic acid (AA) metabolites and cytokines released by inflammatory and structural cells and other inflammatory cell products, such as elastase and eosinophil cationic protein (ECP), stimulate mucus secretion.

Airway Mucins

Mucins are high–molecular-weight proteins with a large proportion of O-linked oligosaccharides (up to 80% of the total mass) and represent the major macromolecular components of mucus [8••]. A typical feature of mucins is their main core, composed of variable numbers of tandemly repeated amino acid sequences that are rich in serine, threonine, and praline. It is in these tandem repeats that the O-glycosylation occurs. Goblet cells of the surface epithelium and mucous cells of the submucosal glands are the main cells involved in the synthesis and secretion of mucins.

To date, 19 human mucin genes [9–16] have been identified and subdivided into two groups: secreted mucins and membrane-bound mucins. Specifically, MUC2, MUC5AC, MUC5B, MUC6-MUC9, and MUC19 are secreted mucins, while MUC1, MUC3, MUC4, MUC11–MUC13, MUC17, MUC18, and MUC20 are membrane-bound mucins characterized by containing a transmembrane domain and a short cytoplasmatic tail. The other mucin genes (MUC15, MUC16) have not been fully characterized. Although eight of them, MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC7, MUC8, and MUC13 [17•,18,19] are normally expressed in the human respiratory tract, only MUC5AC and MUC5B have been convincingly demonstrated to be major components of human airway secretion [20,21]. For this reason, most of the studies dealing with mucin expression in the respiratory tract have been focused on the expression of the two secreted mucins.

In mucus from healthy airways, MUC2, MUC5AC, and MUC5B, known to be the gel-forming mucins, probably dimerize, and interact with other secreted mucus components (proteins, lipids), and are finally responsible for the viscoelastic properties of mucus. In several airway diseases, such as chronic rhinosinusitis, asthma, and cystic fibrosis, an abnormal mucin composition of the mucus gel, with regard to the amount, type, and size of mucins, has been reported [22–24]. These changes may contribute to the rheologic properties of airway mucus, producing a hyperviscid mucus in the case of CF and asthma and watery mucus in the case of allergic rhinitis and nasal polyps (NP). However, the functional consequences of mucus with different mucin compositions are still poorly understood.

Chronic Rhinosinusitis and Nasal Polyposis

Chronic rhinosinusitis (CRS) is one of the most commonly reported diseases in the United States, affecting approximately 14% of the population. It is characterized by at least two of the following symptoms: nasal obstruction, smelling decrease, rhinorrea, and facial pain. Most patients with CRS who require endoscopic sinus surgery (4% of the general population) have nasal polyps [25••]. Mucus hypersecretion is a common feature of patients with CRS with and without polyps, but the composition of this excessive mucus is not well known.

Regarding the production of the three gel-forming mucins expressed in the airways, several studies have

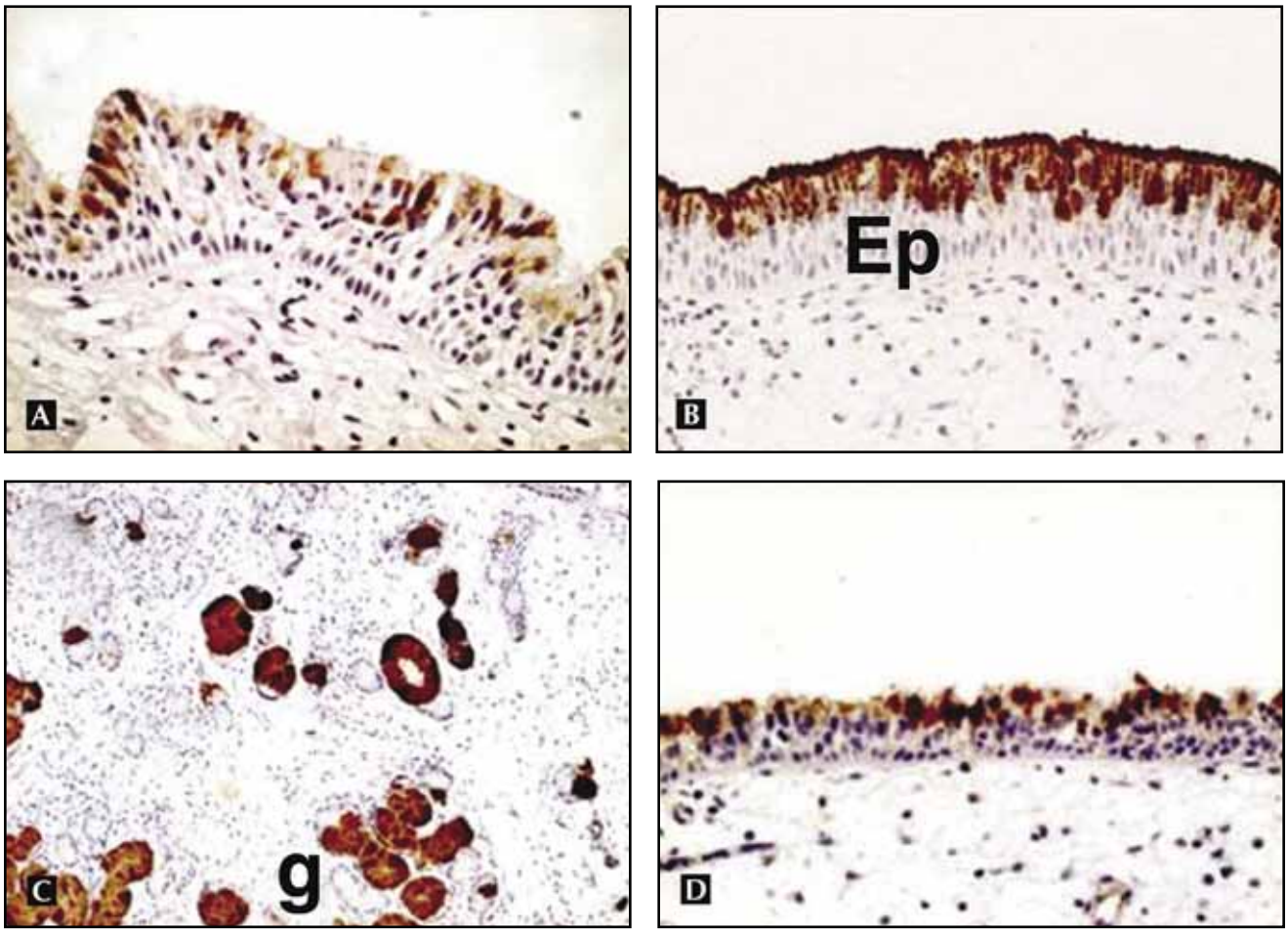

Figure 2. Mucin expression detected by immunohistochemistry. MUC2 mucin expression in healthy nasal mucosa (**A**), MUC5AC and MUC5B mucins in bilateral nasal polyp (**B** and **C**, respectively), and MUC5B in nasal polyp from a CF patient (**D**). MUC2 and MUC5AC are mainly expressed in goblet cells. MUC5B is mainly detected in submucosal glands, although it is highly expressed in CF polyp epithelium. CF—cystic fibrosis; Ep—epithelium; g—glands. Original magnification: ×150 (**C**) and ×400 (**A**, **B**, and **D**). (*Adapted from* Martínez-Antón [19].)

demonstrated that nasal mucosa epithelial goblet cells express MUC2 and MUC5AC [19,26], and that mucous cells in submucosal glands express MUC5B (Fig. 2) [19,27]. A similar distribution seems to be found in nasal polyps [19,28,29], although the healthy and the pathologic tissues differ in mucin amounts. The remaining respiratory mucins, both secreted and membrane-tethered, do not seem to have such a well-defined distribution pattern, being found at both epithelial and glandular levels [19], with the exception of MUC7, which, together with MUC5B, has been shown to be exclusively expressed in submucosal glands [19,27].

Comparing mucin expression in nasal polyp and healthy nasal mucosa, several differences were found. For instance, MUC8 mRNA expression was found to be increased and MUC5AC mRNA decreased in bilateral nasal polyps compared to normal inferior turbinates [30]. MUC8 was also found increased, at both mRNA and protein levels, in chronic rhinosinusitis mucosa [31]. By contrast, several studies have described an increased expression of MUC5AC in bilateral nasal polyps [29,32]. These discrepancies may be due to the different samples used in the mentioned studies, because Seong et al. [30] used epithelial cells isolated from nasal polyps and Kim et al. [29] used tissues from chronic maxillary rhinosinusitis mucosa tissues. Therefore, and taking into account that goblet cell hyperplasia has been described to occur in nasal polyps and other respiratory pathologies [6,33], the increased MUC5AC expression found by Kim et al. could be explained by the increase in the number of goblet cells, a theory that is not dealt with in the studies carried out using isolated epithelial cells. On the other hand, MUC2 and MUC5B mRNA expression have been found increased in mucosa from nasal polyps and CRS, respectively, compared with healthy mucosa [28,29].

All changes regarding mucin expression reported here could be explained by different regulation pathways. Several inflammatory proteins, such as granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL)-1β, IL-3, IL-5, IL-6, IL-8, IL-12, and tumor necrosis factor (TNF)-α have been shown to

Table 1. MUC gene regulation by inflammatory mediators in both nasal mucosa and polyp epithelial cells

Study	Stimuli	Effect and via	Cell or tissue
Seong et al. [30]	Mix: TNF-α, IL-1β, LPS, IL-4, PAF	Induce MUC8 mRNA	NHNE and NP epithelials cells
Shirasaki et al. [37]	TNF-α	Induces MUC1 mRNA	NHNE cells
Kim et al. [38]	TNF-α	Induces MUC5AC mRNA via ERK	NP epithelial cells
Song et al. [39•]	IL-1β, TNF-α	Induce MUC5AC mRNA via ERK/p38-MSK1-CREB	NHNE cells
Song et al. [40]	IL-1β	Induces MUC8 mRNA via ERK-RSK1-CREB	NHNE cells
Cho et al. [41]	PGE_2	Induces MUC8 mRNA via ERK-RSK1-CREB	NHNE cells

CREB—cAMP-response element binding protein; ERK—extracellular signal-regulated kinase; IL—interleukin; LPS—lipopolysaccharide; MSK—mitogen- and-stress-activated protein kinase; NHNE—normal human nasal epithelial; NP—nasal polyps; PAF—platelet-activating factor; PGE_2—prostaglandin E_2; RSK1—p90 ribosomal S6 protein kinase; TNF-α—tumor necrosis factor-α.

be increased in nasal polyps [34–36], and, therefore, they may play an important role in mucin upregulation (Table 1) [30,37,38,39•,40,41].

Cystic Fibrosis

Cystic fibrosis (CF) is the most common severe genetic disease among whites, with an incidence rate varying from 1 per 2000 to 1 per 6500 living newborn babies. Defective expression of the CF transmembrane conductance regulator (CFTR) in CF airway epithelial cells is associated with mucus hypersecretion, inflammation, and infection that begin in early life and lead to a marked cyclical airway obstruction and infection responsible for the morbidity and mortality in patients with CF. Mucus hypersecretion, together with defective chloride secretion and elevated sodium absorption [42], typical of CF respiratory cells, lead at least in part to an alteration of the airway mucociliary clearance. This would favor lung infection that is characterized by the predominance of *Staphylococcus aureus* in early life and of *Pseudomona aeruginosa* later on. Some 92% to 100% of CF patients present with radiologic signs of sinonasal affectation [43]. Hence, the sinonasal disease may be responsible for a significant morbidity in these patients.

Thornton et al. [24] have reported that although mucins are heterogeneous in size and buoyant density, they share the same architecture and macromolecular properties and are similar to mucins from normal respiratory secretions. However, other authors have reported that, at least regarding mucin glycosylation, several differences could be found between healthy and pathologic mucins. Thus, a higher sulphation level was reported to occur in CF compared to non-CF airway tissues [44,45], and the sialic acid content of mucins secreted by CF patients was increased in the most severely infected patients [45]. Taking into account these glycosylation changes, it is important to observe that some authors have described an increased affinity of *P. aeruginosa* for different respiratory mucins in CF patients [46]. Carnoy et al. [47] have made similar conclusions for CF salivary mucins. In the same way, several studies deal with the regulation of mucins by bacterial extracts and exoproducts (Table 2) [48–54].

Most of the studies regarding mucin expression in CF and healthy airways have been focused on the gel-forming mucins MUC2, MUC5AC, and MUC5B. Several authors reported a decrease in MUC5AC mRNA and mucin expressions in nasal epithelial cells and sputum of CF patients compared to normal nasal epithelial cells and sputum of healthy subjects, respectively [55,56]. A decrease of MUC5B has also been reported in CF sputum [56]. However, immunohistochemical staining showed a similar histologic pattern in CF compared to normal tissues, with an increase of MUC5AC-positive cells due to goblet cell hyperplasia and metaplasia [57]. MUC2 mRNA also showed a similar expression level in CF and normal nasal epithelial cells [55].

Because a high percentage of CF patients develop sinonasal problems, including nasal polyposis, some CF studies have been carried out on nasal polyps of this group of patients. Dohrman et al. [49] demonstrated that MUC5AC mRNA and protein were increased in nasal polyps from CF patients compared to non-CF nasal polyps. By contrast, a recent study has shown that MUC5AC mucin was downregulated in CF nasal polyps compared to healthy nasal mucosa, but no differences were found compared to nasal polyps of non-CF patients. The same group has also demonstrated that MUC5B mucin was increased in nasal polyps from CF-patient epithelium compared to healthy nasal mucosa and nasal polyps of non-CF patients (Fig. 2) [19]. The different methodology performed in these studies could account for the discrepancies.

Given that mucin expression did not show many differences between CF patients and healthy subjects, some authors have focused their studies on the high DNA content found in CF purulent mucus [58] or on the low fluid volume found in CF airway mucus [59,60] as the potential main causes for the hyperviscid mucus and the reduction of mucociliary clearance found in CF.

Table 2. MUC gene regulation by bacterial extracts, exoproducts, and neutrophil elastase in bronchial epithelial cell lines

Study	Stimuli	Effect and via	Cell or tissue
Li et al. [48]	PA, LPS	MUC2 mRNA via Src-dependent Ras-MEK1/2-ERK1/2-pp90rsk-NFκB	NCI-H292 cells
Dohrman et al. [49]	PA exoproducts	MUC5AC mRNA and protein	NCI-H292 cells
Shao et al. [50]	PMA, PA, LPS	Induces MUC5AC mRNA via TACE/TGF-α/ EGFR	NCI-H292 cells
Belcher et al. [51]	Bordatella pertussis	MUC2 and MUC5AC mRNA	BEAS-2B cells
Wang et al. [52]	*Haemophilus influenzae*	Induces MUC5AC mRNA	A549 cells
Fischer et al. [53]	Neutrophil elastase	Induces MUC4 mRNA	NHBE cells
Fischer et al. [54]	Neutrophil elastase	Induces MUC5AC mRNA via ROS	NHBE and A549 cells

A549—human lung carcinoma cell line; BEAS-2B—human bronchial epithelial cell line; EGFR—epidermal growth factor receptor; ERK—extracellular signal-regulated kinase; LPS—lipopolysaccharide; MEK—MAPK/ERK kinase; NCI-H292—human lung mucoepidermoid carcinoma cell line; NFκB—nuclear factor κB; NHBE—normal human bronchial epithelia; PA—*Pseudomonas aeruginosa*; PMA—phorbol 12-myristate 13-acetate; pp90rsk—90-KDa ribosomal S6 kinase; ROS—reactive oxygen species; TACE—TNF-α converting enzyme; TGF-α—transforming growth factor-α.

Asthma

Asthma is a chronic inflammatory condition of the lower airways, although asthmatic patients frequently present with rhinitic symptoms in a concomitant way (>80% of allergic asthmatic patients). In fact, it has been proposed that prevention and early treatment of allergic rhinitis could help prevent asthma or decrease the severity of bronchial symptoms [61]. Asthma is clinically characterized by variable airflow limitation that is at least partially reversible, both spontaneously and after treatment. Sputum production is a common symptom in asthma, especially during asthma exacerbations [62], and a history of sputum production is independently associated with an accelerated rate of decline in forced expiratory volume in 1 second (FEV_1) [63]. Furthermore, hypersecretion of mucus plays a central role in the pathogenesis of severe airway obstruction, particularly in patients who die in status asthmaticus, the airways of whom are occluded by gelatinous plugs [64]. Whether goblet cells or submucosal gland mucus cells are the principal sources of mucins in airway secretions in asthma is not well known. In fatal asthma, at least, it is clear that goblet cells strongly contribute to mucin secretion [6].

Goblet cell metaplasia/hyperplasia in the airway epithelium is a common finding in pathologic studies of asthma, and it is associated with mucus hypersecretion [65•]. In spite of the importance of goblet cell hyperplasia in airways, the analysis of mechanisms of goblet cell production has been difficult because of the heterogeneity of the hypersecretory diseases.

Thornton et al. [66,67] have demonstrated that the total gel-forming mucin pool in an asthmatic exudate is composed of three mucin species, namely the MUC5AC mucin and two glycoforms of MUC5B mucin. The low-charge population of the MUC5B mucin described in these studies was by far the predominant species (approximately 79% of the total gel-forming mucins), and results obtained on the mucin preparation from normal individuals indicate that the MUC5AC mucin was predominant [66]. The reason for the high presence of MUC5B low-charge population in asthmatic samples remains unclear, but it may account for abnormal and rheologically compromised mucus [68]. On the other hand, Ordóñez et al. [65•] demonstrated that the most frequently expressed MUC gene in the bronchial biopsy specimens from both healthy and asthmatic subjects was MUC5AC, this expression being 60% higher in asthmatic than in normal subjects. The same group found that the expression levels of MUC2 and MUC4 were significantly increased in asthmatic subjects, whereas the expression of MUC5B was significantly decreased. By contrast, Groneberg et al. [69] found no differences in the MUC5AC and MUC5B mucin content in lung tissues from asthmatic patients and healthy subjects. The discrepancies they reported may be due to the different origins of the samples used for the different studies: airway sputum, bronchial biopsies, and lung tissue, respectively.

While most of the mucin-regulation studies regarding CF were focused on the regulation that bacterial products exert on mucin expression, in the case of asthma, the attention has been focused on inflammatory mediators such as interleukins and growth factors. Moreover, because eosinophils are the inflammatory cells characteristic of asthmatic airway inflammation, and they also produce and release TGF-α, activated eosinophils have been proposed to stimulate mucin synthesis in human airway epithelial cells (Table 3) [70–76].

Allergic Rhinitis

Allergic rhinitis (AR) is a heterogeneous disorder characterized by the presence of one or more of the

Table 3. MUC gene regulation by inflammatory mediators in bronchial epithelial cell lines

Study	Stimuli	Effect and via	Cell or tissue
Kim et al. [70]	IL-1β	Induces MUC2 and MUC5AC at both mRNA and protein levels via ERK or p38/Cox-2	NCI-H292 cells
Gray et al. [71]	IL-1β	Induces MUC5AC mRNA and protein	NHTBE cells
Chen et al. [72]	IL-17	Induces MUC5AC and MUC5B mRNA and protein via IL-6	NHTBE cells
Mata et al. [73]	EGF	Induces MUC5AC mRNA and protein	A549 cells
Hewson et al. [74]	PMA	Induces MUC5AC mRNA and protein via PKC-EGF/TGF-α- Ras/Raf-MEK-ERK-Sp1	NCI-H292
He et al. [75]	Rhinovirus, LPS	Induce MUC5AC protein secretion	NHBE cells and HB tissue
Burgel et al. [76]	Eosinophils	Induce MUC5AC mRNA and protein via EGFR	NCI-H292 cells

A549—human lung carcinoma cell line; Cox-2—cyclooxygenase-2; EGF—epidermal growth factor; EGFR—epidermal growth factor receptor; ERK—extracellular signal-regulated kinase; HB—human bronchial; IL—interleukin; LPS—lipopolysaccharide; MEK—MAPK/ERK kinase; NCI-H292—human lung mucoepidermoid carcinoma cell line; NHTBE—normal human tracheobronchial epithelial; PKC—protein kinase C; PMA—phorbol 12-myristate 13-acetate; Sp1—specificity protein 1; TGF-α—transforming growth factor-α.

following nasal symptoms: sneezing, itching, rhinorrhea, and obstruction. The incidence of this pathology is approximately 10% to 25% of the world population. AR symptoms are secondary to the appearance of a Th2 immunologic response mediated by IgE antibodies [1••].

The increase in nasal mucin secretion, as in other diseases associated with mucus hypersecretion, is related to an increased amount of mucin-secreting tissue, either submucosal glands or goblet cells. However, changes in the number of goblet cells are equivocal [77]. Some authors have detected a transitory increase in the number of goblet cells in patients with AR after nasal allergen challenge [78,79]. In contrast, nasal goblet cell hyperplasia was not detected in other studies of patients with AR, including the influence of natural allergen exposure [80,81]. On the other hand, nasal cell samples of AR patients were found to contain few secretory cells and more columnar/indeterminant cells than nasal cell samples from CF and normal subject groups [55]. Because goblet cell hyperplasia is often accompanied by goblet cell metaplasia, these discrepancies might be due to difficulties in the quantification of goblet cell number due to the variable distribution of these cells along the nasal epithelium.

Regarding mucin expression in patients with AR, few reports are published. Voynow et al. [55] described that MUC5AC was the main mucin detected in AR patients, and that MUC5AC mRNA expression was at fivefold to tenfold higher levels than MUC2 or MUC1. Other studies have reported that mucin content was increased in AR nasal lavage fluids compared to normal subjects' secretions [82]. However, MUC5AC mucin expression was found to be increased in lung tissue from a model of AR rats compared to normal lung tissue [83].

Conclusions

Rhinitis and rhinosinusitis are often associated with several airway diseases, such as asthma, cystic fibrosis, and nasal polyposis. In these diseases, mucus hypersecretion forebodes nasal obstruction and infection of the airways. Regarding mucin expression, the pathology of these diseases has involved MUC5AC and MUC5B mucins in most studies. For instance, MUC5B and MUC5AC seem to be the predominant mucins in secretions of asthmatic and allergic rhinitis patients, respectively. However, these two mucins have been reported to be increased in nasal polyps and decreased in nasal epithelial cells and sputum of CF patients compared to normal subjects. Although it is quite clear that MUC5AC and MUC5B are the main mucins detected in human airway secretions, other respiratory mucins, both secreted and membrane-tethered, should be further analyzed to provide a complete view of the mucin expression patterns found in the various airway diseases.

Several advances have been achieved regarding the pathophysiology of respiratory diseases, including mucus hypersecretion. However, to clarify the role of mucus in pathologic conditions, further elucidation is needed regarding the following aspects: 1) cell and structural biology of mucins and mucus; 2) regulation of specific airway mucin genes and identification of the key mediators involved in this regulation; and 3) the mechanism that results in goblet cell hyperplasia. All these studies will contribute to finding possible targets for future therapeutic strategies for the treatment of mucus hypersecretion.

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Articles' summary

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Study 1. Mucin genes have different expression patterns in healthy and diseased upper airway mucosa. *Clin Exp Allergy. 2006; 36(4):448-57.*

In order to determine if there exist specific mucin expression patterns for nasal polyp associated diseases, mucin expression was analyzed in healthy nasal mucosa (NM) and in nasal polyps from different origins: bilateral nasal polyps (NP), NP from cystic fibrosis (CFP) patients, and antrochoanal polyps (ACP). Immunohistochemistry for MUC1, MUC2, MUC4, MUC5AC, MUC5B, MUC6, MUC7, and MUC8 showed that: a) MUC1, MUC4, and MUC5AC mucins were highly expressed in the epithelium of both healthy nasal mucosa and nasal polyps, and their expression pattern was similar in all nasal polyp types, but different from the one found in healthy nasal mucosa. In this manner, MUC1 (NP:85%, CFP:90%, ACP:80%) and MUC4 (NP:95%, CFP:100%, ACP:100%) were found increased while MUCAC (NP:30%, CFP:17.5%, ACP:22.5%) decreased compared to healthy nasal mucosa (MUC1: 52.5%, MUC4:52.5%, MUC5AC:55%); b) MUC8 was highly detected at both epithelial and glandular levels with marked variability between groups; c) MUC5B was mainly detected in glands and its expression in all nasal polyp types was higher than in nasal mucosa (NM:37.5%, NP: 65%, CFP: 67%). In addition, MUC5B expression was increased in NP epithelia from CF patients (25%) compared to bilateral nasal polyps (5%) and nasal mucosa (<5%); d) MUC2 showed a low expression especially in ACP, and MUC6 and MUC7 were scarcely detected in all tissues.

In situ hybridization for MUC2, MUC4, MUC5AC, and MUC6 showed similar results to the ones found for protein expression, regarding cellular and histological distribution and MUC genes amount.

Regarding inflammatory cell infiltration of the analyzed tissues, bilateral nasal polyps (25%) showed the highest eosinophilic content compared to both healthy nasal mucosa (5%) and the other inflammatory tissues (CFP:10%, ACP:10%). The other evaluated inflammatory cells (lymphocytes, plasma cells, and polymorphonuclear cells) showed no significant differences between tissues. In addition, no correlation was found between inflammatory cell content and mucin expression.

These results suggest that nasal polyps have a different pattern of mucin expression than healthy nasal mucosa and that cystic fibrosis polyps (increased MUC5B) and antrochoanal polyps (decreased MUC2) have also a different mucin expression pattern than bilateral NP.

Study 2. Corticosteroid therapy increases membrane-tethered while decreases secreted mucin expression in nasal polyps. *Allergy 2008 (in press) DOI: 10.1111/j.1398-9995.2008.01678.x.*

In order to investigate the *in vivo* effect of oral and intranasal corticosteroids on the expression of mucins and their producing cells in nasal polyps, a prospective study with patients suffering from nasal polyps, randomized in control and treatment (oral prednisone + intranasal budesonide for 2 weeks, and intranasal budesonide alone for 10 weeks) groups, was performed. Nasal polyps biopsies were obtained before and after 2 and 12 weeks of corticosteroid treatment. Immunohistochemistry for membrane-tethered (MUC1, MUC4) and secreted (MUC5AC, MUC5B, MUC8) mucins showed that: a) a short-term treatment with oral prednisone combined with intranasal budesonide upregulated membrane-tethered mucin expression (MUC1: from 70 to 98%; MUC4: from 80 to 100%) compared to baseline, specially in nasal polyp epithelium from aspirin-tolerant asthmatic patients; b) a long-term treatment with intranasal budesonide downregulated secreted mucin expression compared to baseline, especially MUC5AC in the epithelium of asthmatic patients (from 40 to 5%), and MUC5B in the glands of aspirin-tolerant asthmatic patients

(from 45 to 2.5%); c) the soluble mucin MUC8 increased after 12 weeks of intranasal corticosteroid treatment in nasal polyp epithelium of non-asthmatic (from 10 to 75%) and in glands of aspirin-tolerant asthmatic (from 2.5 to 45%) patients compared to baseline. In summary, nasal polyps from aspirin-tolerant asthmatics showed the most significant changes for all analyzed mucins, while those from non asthmatics showed variations in MUC1, MUC5B, and MUC8. Nasal polyps from aspirin-intolerant patients showed changes almost exclusively in MUC5AC, suggesting a trend of resistance to corticosteroid treatment.

Corticosteroid treatment decreased goblet cells in the epithelium (from 20 to 10%) and mucous cells in submucosal glands (from 40 to 10%), as well as rhinorrhea (from 3 to 0) and nasal obstruction (from 3 to 1) symptoms in nasal polyp patients. The decrease in secreted mucin expression, both MUC5AC and MUC5B, after corticosteroid treatment correlated with the reduction of goblet cells ($r:0.725$, $p<0.01$) and mucous cells ($r:0.782$, $p<0.01$), respectively, as well as with the decrease of the rhinorrhea ($r:0.403$, $p<0.05$) in nasal polyp patients.

These results suggest that corticosteroids, reducing mucin-producing cells and consequently mucin production and rhinorrhea, could be considered a beneficial therapy for mucus hypersecretion in nasal polyps, except for the NP from aspirin-tolerant asthmatic patients which showed a tendency of resistance to corticosteroid treatment.

Study 3. Dexamethasone decreases basal and IL-1β-induced MUC5AC expression in A549 cells. *(in preparation)*

In order to investigate glucocorticoid effect on MUC gene and protein expression at baseline and under inflammatory conditions, A549 cell cultures were treated with different proinflammatory stimuli and/or dexamethasone. Cells and culture media were collected after 1, 6, 12 and 24 hours of treatment to be analyzed by real time RT-PCR (mRNA) and

ELISA (protein), respectively. Among the tested inflammatory stimuli (IL-1β, TNF-α, LPS, cytokine mixture (CytMix), methacoline, and foetal bovine serum (FBS)), only IL-1β, CytMix and FBS were able to induce MUC5AC mRNA expression. None of the stimuli increased MUC5B mRNA expression. Since it caused the most powerful and homogeneous effect, IL-1β was finally chosen as the proinflammatory stimulus to be used in the experiments.

IL-1β stimulated both MUC5AC gene and protein expression in a dose-dependent manner (0.1-20 ng/ml), showing the peak effect at 6h for mRNA and at 24h for protein expression. IL-1β failed to induce MUC5B gene and protein expression. Dexamethasone decreased both basal and IL-1β-induced MUC5AC gene and protein expressions in a dose-dependent manner (from 10^{-9} to 10^{-6} M), showing a peak effect after 24h of incubation. Although IL-1β failed to induce an increase in MUC5B gene and protein expression, DEX slightly decreased MUC5B basal levels after 12 and 24h of treatment.

These results suggest that dexamethasone is able to induce a decrease in MUC5AC mucin expression at both baseline and under inflammatory conditions, while MUC5B only at basal level.

"Review" study. Mucin gene expression in rhinitis syndromes. *Curr Allergy Asthma Rep. 2006;6(3):189-97.*

Rhinitis and rhinosinusitis are commonly associated to several airway diseases such as asthma, cystic fibrosis, and nasal polyposis. In these diseases mucus hypersecretion causes nasal obstruction and infection of the airways. Mucins are the main component of mucus and they are responsible for its rheological properties. Since mucin expression patterns have been found to be altered in rhinitis-associated diseases, mucins have been proposed as one of the most important agents involved in the formation of abnormal mucus, whether it is a hyperviscid or watery mucus. MUC5AC and

MUC5B mucins have been found to be the predominant secreted mucins in airways secretions, and changes in their quantity and/or quality have been reported in several airway diseases. In general, both mucins show an increased expression in respiratory diseases such as asthma, allergic rhinitis, and nasal polyposis, but some studies have reported a diminution of these mucins in cystic fibrosis secretions compared to normal subjects.

The mechanisms involved in the regulation of respiratory mucins differ depending on the disease. In this way, cytokines, growth factors, and inflammatory cells seem to be the main agents involved in mucin regulation in asthma and nasal polyposis while bacteria and their exoproducts are responsible for mucin regulation in cystic fibrosis patients.

Another characteristic that could be essential for the development of a hypersecretory phenotype is the goblet cell hyperplasia and the submucosal gland enlargement reported to occur in the above mentioned diseases.

Although several advances have been achieved regarding the pathophysiology of respiratory diseases, including mucus hypersecretion, further elucidation is needed regarding: a) cell and structural biology of mucins and mucus in health and disease, b) regulation of specific airway mucin genes and identification of the key mediators involved in this regulation, and c) the mechanism that result in goblet cell hyperplasia.

Resum dels articles

Resum dels Articles

Estudi 1. Mucin genes have different expression patterns in healthy and diseased upper airway mucosa. *Clin Exp Allergy. 2006; 36(4):448-57.*

Amb el propòsit de determinar si hi ha patrons d'expressió específics per a la poliposi nasal i les seves malalties associades, es va analitzar l'expressió de mucines en mucosa nasal sana (MN) i en pòlips nasals de diferents orígens: pòlips nasals bilaterals (PN), pòlips nasals de pacients amb fibrosi quística (PFQ) i pòlips antrocoanals (PAC). Es van emprar tècniques immunohistoquímiques per a la detecció de les mucines MUC1, MUC2, MUC4, MU5AC, MUC5B, MUC6, MUC7 i MUC8, obtenint els següents resultats: a) MUC1, MUC4 i MUC5AC es van trobar altament expressades tant a l'epiteli de la mucosa nasal sana com al dels pòlips nasals, mostrant un patró d'expressió similar en tots els tipus de pòlips nasals i diferent al trobat a la mucosa nasal sana. Així, MUC1 (PN:85%, PFQ:90%, PAC:80%) i MUC4 (PN:95%, PFQ:100%, PAC:100%) es trobaven incrementades i MUC5AC (PN:30%, PFQ:17,5%, PAC:22,5%) disminuïda en teixits patològics comparat amb el teixit sà (MUC1: 52,5%, MUC4:52,5%, MUC5AC:55%); b) MUC8 es va detectar en grans quantitats tant a nivell epitelial com glandular, mostrant una gran variabilitat entre grups; c) MUC5B es va detectar principalment en glàndules i la seva expressió fou superior en els teixits patològics que en la mucosa nasal sana (MN:37,5%, PN: 65%, PFQ: 67%). A més a més, l'expressió de MUC5B es va trobar incrementada a l'epiteli dels pòlips nasals de pacients amb fibrosi quística (25%) respecte als pòlips nasals bilaterals (5%) i la mucosa nasal sana (<5%); d) MUC2 presentava una expressió molt baixa, especialment als pòlips antrocoanals, mentre MUC6 i MUC7 van ser escassament detectats a tots els teixits.

Tant quant a la distribució cel·lular i tissular, com a la quantitat de mucines als diferents teixits, l'anàlisi de l'ARNm de MUC2, MUC4, MUC5AC i

MUC6 per hibridació *in situ* va mostrar resultats similars als trobats a nivell de proteïna.

L'estudi de l'infiltrat de cèl·lules inflamatòries, ens va mostrar un increment en el contingut eosinofílic dels pòlips nasals bilaterals (25%) en comparació tant amb el teixit sà (5%) com amb els altres teixits inflamats (PFQ:10%, PAC:10%). La resta de cèl·lules inflamatòries analitzades (limfòcits, cèl·lules plasmàtiques i polimorfonuclears) no van presentar diferències significatives entre teixits.

Aquests resultats suggereixen que el patró d'expressió de mucines als pòlips nasals difereix del trobat a la mucosa nasal sana i, alhora, que els pòlips nasals de pacients amb fibrosi quística (amb MUC5B incrementada) i els pòlips antrocoanals (amb MUC2 disminuïda) també difereixen quant a l'expressió de mucines amb els pòlips nasals bilaterals.

Estudi 2. Corticosteroid therapy increases membrane-tethered while decreases secreted mucin expression in nasal polyps. *Allergy 2008 (en premsa) DOI: 10.1111/j.1398-9995.2008.01678.x.*

Amb el propòsit d'investigar la regulació *in vivo* per corticoides orals i intranasals sobre l'expressió de mucines i les seves cèl·lules productores en pòlips nasals, es va realitzar un estudi prospectiu en pacients amb poliposi nasal, els quals es van distribuir aleatòriament en un grup control i un de tractatment (prednisona oral + budesonida intranasal durant 2 setmanes, i budesonida sola durant 10 setmanes). Les biòpsies de pòlip nasal es van obtenir abans i després de 2 i 12 setmanes de tractament amb corticoides. Les mucines de membrana (MUC1 i MUC4) així com les secretades (MUC5AC, MUC5B i MUC8) es van analitzar mitjançant immunohistoquímica obtenint els següents resultats: a) el tractament amb tandes curtes de prednisona oral combinada amb budesonida intranasal va produir un increment en l'expressió de mucines de membrana (MUC1: de 70 a 98%; MUC4: de 80 a 100%) comparat amb els nivells basals, especialment en els

pòlips de pacients amb asma tolerant; b) el tractament de llarga durada amb budesonida intranasal va produir una regulació a la baixa de les mucines secretades comparat amb els nivells basals, especialment MUC5AC a l'epiteli de pacients asmàtics (de 40 a 5%), i MUC5B a les glàndules de pòlips nasals de pacients amb asma tolerant (de 45 a 2,5%); c) després del tractament de 12 setmanes amb budesonida intranasal la mucina soluble MUC8 va incrementar a l'epiteli de pòlips nasals de pacients no asmàtics (de 10 a 75%) i a les glàndules dels asmàtics tolerants (de 2,5 a 45%), comparats amb el basal. En resum, els pòlips de pacients amb asma-tolerant van mostrar els canvis més significatius en totes les mucines analitzades i els de pacients no asmàtics presentaven variacions en MUC1, MUC5B i MUC8. Els pòlips de pacients amb asma intolerant només van presentar canvis en l'expressió de la mucina MUC5AC, mostrant d'aquesta manera una certa resistència al tractament corticoide.

Els corticoides van ser capaços de disminuir el nombre de cèl·lules caliciformes a l'epiteli i de cèl·lules mucoses a les glàndules submucoses, així com també la rinorrea i l'obstrucció nasal en pacients amb pòlips nasals. La davallada en l'expressió de mucines secretades, MUC5AC i MUC5B, després del tractament amb corticoids es correlacionava amb la reducció en el nombre de cèl·lules caliciformes i mucoses, respectivament, així com també amb la disminució de la rinorrea en pacients amb pòlips nasals després del tractament corticoide.

Aquests resultats suggereixen que els corticoides, mitjançant la reducció en el nombre de cèl·lules productores de mucines i conseqüentment en la producció de mucines i la rinorrea, poden ser considerats una teràpia beneficiosa en el tractament de la hipersecreció mucosa present en els pòlips nasals, a excepció dels pacients amb triada de Widal els quals mostren una tendència a la resistència enfront el tractament corticoide.

Estudi 3. Dexamethasone decreases basal and IL-1β-induced MUC5AC expression in A549 cells *(en preparació).*

Amb el propòsit d'investigar l'efecte dels glucocorticoides sobre l'expressió dels gens de les mucines tant a nivell basal com en condicions d'inflamació, es van tractar cultius de cèl·lules A549 amb diferents estímuls proinflamatoris i/o amb dexametasona. Les cèl·lules i els medis de cultiu es van recollir a les 1, 6, 12 i 24h d'incubació per tal de ser analitzats mitjançant RT-PCR a temps real (ARNm dels gens MUC5AC i MUC5B) i ELISA (proteïnes MUC5AC i MUC5B), respectivament. Dels estímuls proinflamatoris que es van testar (IL-1β, TNF-α, LPS, mescla de citocines, metacolina i sèrum fetal boví) només la IL-1β, la mescla de citocines i el sèrum fetal boví van ser capaços d'induir l'expressió de l'ARNm de MUC5AC. Cap dels estímuls testats va regular l'expressió de l'ARNm de MUC5B. La IL-1β es va triar com a estímul proinflamatori en la resta d'experiments, ja que va ser l'agent que va causar l'efecte proinflamatori més potent i homogeni.

La IL-1β va regular a l'alça l'expressió de MUC5AC de manera depenent de dosi (0,1-20 ng/ml), tant a nivell d'ARNm com de proteïna, mostrant màxim efecte a les 6 i a les 24h, respectivament. D'altra banda, la IL-1β no va causar cap efecte sobre l'expressió de l'ARNm i la proteïna de MUC5B. La dexametasona va regular a la baixa de manera depenent de dosi (10^{-9}-10^{-6} M) l'expressió de l'ARNm i la proteïna de MUC5AC, a nivell basal i induït per IL-1β, amb efecte màxim després de 24h d'incubació. Tot i què MUC5B no va ser induïda per la IL-1β, la dexametasona va ser capaç de disminuir lleugerament l'expressió d'ARNm de MUC5B a nivell basal, tant a les 12 com a les 24h d'incubació.

Aquests resultats suggereixen que la dexametasona és capaç de disminuir l'expressió del gen de MUC5AC i la secreció d'aquesta mucina a nivell basal i en situació d'inflamació. D'altra banda, la dexametasona regula a la baixa l'expressió basal de MUC5B.

Estudi de revisió. Mucin gene expression in rhinitis syndromes. *Curr Allergy Asthma Rep. 2006;6(3):189-97. Review*.

La rinitis i la rinosinusitis es troben freqüentment associades a d'altres malalties respiratòries com ara l'asma, la fibrosi quística i la poliposi nasal. En aquestes malalties la hipersecreció de moc causa obstrucció nasal i infecció de les vies respiratòries. Les mucines són el component majoritari del moc i són responsables de les seves propietats reològiques. Degut a que s'han trobat alteracions en els patrons d'expressió de mucines a les malalties associades amb la rinitis, les mucines han estat directament implicades en la formació de moc amb característiques anormals, ja sigui de tipus hiperviscós o aquós. Les mucines predominants a les secrecions respiratòries són MUC5AC i MUC5B, i en diverses malalties respiratòries s'han descrit canvis tant en la seva quantitat com en la seva qualitat. En general, l'expressió d'ambdues mucines s'ha trobat incrementada en patologies respiratòries com ara l'asma, la rinitis al·lèrgica i la poliposi nasal però, en canvi, alguns estudis han demostrat una disminució d'aquestes a les secrecions de pacients amb fibrosi quística comparat amb les d'individus sans.

Els mecanismes involucrats en la regulació de les mucines de vies respiratòries són diversos i depenents de la malaltia. En aquest sentit, les citoquines, els factors de creixement i les cèl·lules inflamatòries serien els principals agents implicats en la regulació de les mucines a l'asma i la poliposi nasal, enfront de la regulació exercida pricipalment per bacteris i els seus exoproductes en pacients amb fibrosi quística.

Un altre factor que podria ser essencial en el desenvolupament d'un fenotip hipersecretor seria l'aparició d'hiperplàsia de cèl·lules caliciformes i l'engrandiment de les glàndules submucoses, característiques que d'altra banda han estat descrites en les malalties anteriorment esmentades.

Tot i què s'han assolit grans avenços en relació a la fisiopatologia de les malalties respiratòries, incloent-hi la hipersecreció mucosa, més

informació es fa encara necessària en relació a: a) la biologia cel·lular i estructural de les mucines i del moc, tant en situacions fisiològiques com patològiques; b) la regulació dels gens MUC i la identificació dels mediadors claus implicats en aquesta regulació; i c) el mecanisme que condueix a la hiperplàsia de les cèl·lules caliciformes.

Resumen de los artículos

Resumen de los artículos

Estudio 1. Mucin genes have different expression patterns in healthy and diseased upper airway mucosa. *Clin Exp Allergy. 2006; 36(4):448-57.*

Con el propósito de investigar si existen patrones de expresión específicos para la poliposis nasal y sus enfermedades asociadas, se analizó la expresión de mucines en mucosa nasal sana (MN) y en pólipos nasales de diferentes orígenes: pólipos nasales bilaterales (PN), pólipos nasales de pacientes con fibrosis quística (PFQ) y pólipos antrocoanales (PAC). Se utilizaron técnicas inmunohistoquímicas para la detección de las mucinas MUC1, MUC2, MUC4, MU5AC, MUC5B, MUC6, MUC7 y MUC8, obteniéndose los siguientes resultados: a) MUC1, MUC4 y MUC5AC se hallaron altamente expresadas tanto en el epitelio de mucosa nasal sana como en el de pólipo nasal, mostrando un patrón de expresión similar en todos los tipos de pólipos nasales estudiados y diferente al encontrado en mucosa nasal sana. De este modo, MUC1 (PN:85%, PFQ:90%, PAC:80%) y MUC4 (PN:95%, PFQ:100%, PAC:100%) se hallaron incrementadas mientras que MUC5AC (PN:30%, PFQ:17,5%, PAC:22,5%) se halló disminuida en tejidos patológicos comparándose con el tejido sano (MUC1: 52,5%, MUC4:52,5%, MUC5AC:55%); b) MUC8 se detectó en grandes cantidades tanto a nivel epitelial como glandular mostrando una gran varibilidad entre grupos; c) MUC5B se detectó principalmente en las glándulas, siendo su expresión superior en tejidos patológicos que en la mucosa nasal sana (MN:37,5%, PN: 65%, PFQ: 67%). Además, la expresión de MUC5B se halló incrementada en el epitelio de pólipos nasales de los pacientes con fibrosis quística (25%) respecto a los pólipos nasales bilaterales (5%) y la mucosa nasal sana (<5%); d) MUC2 presentó una expresión muy baja, especialmente en los pólipos antrocoanales, mientras que MUC6 y MUC7 fueron escasamente detectados en todos los tejidos estudiados.

En cuanto a la distribución tisular y celular, así como a la cantidad de mucinas en los diferentes tejidos, el análisis por hibridación *in situ* del ARNm de MUC2, MUC4, MUC5AC y MUC6 mostró resultados similares a los encontrados a nivel de proteína.

El estudio del infiltrado celular inflamatorio nos muestra un incremento en el contenido eosinofílico de los pólipos nasales bilaterales (25%) en comparación tanto con el tejido sano (5%) como con otros tejidos inflamados (PFQ:10%, PAC:10%). El resto de las células inflamatorias analizadas (linfocitos, células plasmáticas y células polimorfonucleares) no presentaron diferencias significativas entre los diferentestejidos.

Estos resultados sugieren que el patrón de expresión de mucinas en los pólipos nasales difiere del hallado en la mucosa nasal sana y, a la vez, que los pólipos de pacientes con fibrosis quística (con MUC5B incrementada) y los pólipos antrocoanales (con MUC2 disminuida) también difieren, en cuanto a la expresión de mucinas, de los pólipos nasales bilaterales.

Estudio 2. Corticosteroid therapy increases membrane-tethered while decreases secreted mucin expression in nasal polyps. *Allergy 2008 (en prensa) DOI: 10.1111/j.1398-9995.2008.01678.x.*

Con el propósito de investigar la regulación *in vivo* por corticosteroides orales e intranasales sobre la expresión de mucinas y sus células productoras en pólipos nasales, se realizó un estudio prospectivo en pacientes con poliposis nasal los cuales fueron aleatoriamente distribuidos en un grupo control y en uno de tratamiento (prednisona oral + budesonida intranasal durante 2 semanas, y budesonida sola durante 10 semanas). Las biopsias de pólipos nasales se obtuvieron antes y después de las 2 y las 12 semanas de tratamiento con corticoides. Las mucinas de membrana (MUC1 y MUC4) así como las secretadas (MUC5AC, MUC5B y MUC8) se analizaron mediante inmunohistoquímica obteniéndose lo siguientes resultados: a) el tratamiento con tandas cortas de prednisona oral combinada con

budesonida intranasal produjo un incremento en la expresión de mucinas de membrana (MUC1: de 70 a 98%; MUC4: de 80 a 100%) comparado con su expresión basal, especialmente en pólipos de pacientes con asma tolerante; b) el tratamiento de larga duración con budesonida intranasal produjo una regulación a la baja de las mucinas secretadas, comparado con sus niveles basales, especialmente MUC5AC en el epitelio de pacientes asmáticos (de 40 a 5%), y MUC5B en las glándulas de pólipos nasales de pacientes con asma tolerante (de 45 a 2,5%); c) después del tratamiento durante 12 semanas con budesonida intranasal la mucina soluble MUC8 incrementó en el epitelio de pólipos nasales de pacientes no asmáticos (de 10 a 75%) y en las glándulas de los asmáticos tolerantes (de 2,5 a 45%), comparado con el basal. En resumen, los pólipos de pacientes con asma tolerante mostraron los cambios más significativos en todas las mucinas analizadas mientras que los de pacientes no asmáticos mostraron variaciones de expresión en MUC1, MUC5B y MUC8. Los pólipos de pacientes con asma intolerante sólo presentaron cambios de expresión en la mucina MUC5AC, mostrando de este modo una cierta resistencia al tratamiento corticoideo.

Los corticoides fueron capaces de disminuir el número de células caliciformes del epitelio y el de células mucosas de las glándulas submucosas, así como también la rinorrea y la obstrucción nasal de los pacientes con pólipos nasales. La disminución en la expresión de mucinas secretadas MUC5AC y MUC5B después del tratamiento con corticoides correlacionaba con la reducción en el número de células caliciformes y mucosas, respectivamente, así como también con la disminución de la rinorrea en pacientes con pólipos nasales después del tratamiento corticoideo.

Estos resultados sugieren que los corticoides, al disminuir el número de células productoras de mucinas y, consecuentemente, la producción de mucinas y la rinorrea, pueden ser considerados una terapia beneficiosa para el tratamiento de la hipersecreción mucosa presente en los pólipos nasales,

a excepción de los pacientes con triada de Widal ya que éstos muestran una tendencia a la resistencia frente al tratamiento corticoideo.

Estudio 3. Dexamethasone decreases basal and IL-1β-induced MUC5AC expression in A549 cells *(en preparación).*

Con el propósito de investigar el efecto de los glucocorticoides sobre la expresión de los genes MUC y sus proteínas a nivel basal y en condiciones de inflamación, los cultivos de células A549 fueron tratados con diferentes estímulos proinflamatorios y/o dexametasona. Las células y los medios de cultivo se recogieron a las 1, 6, 12 y 24 horas de incubación para ser analizadas mediante RT-PCR a tiempo real (ARNm de los genes MUC5AC y MUC5B) y ELISA (proteinas de MUC5AC y MUC5B), respectivamente. De los estímulos proinflamatorios que se probaron (IL-1β, TNF-α, LPS, mezcla de citocines, metacolina y suero fetal bovino) sólo la IL-1β, la mezcla de citocinas y el suero fetal bovino fueron capaces de inducir la expresión de ARNm de MUC5AC. Ninguno de estos estímulos reguló la expresión del ARNm de MUC5B. La IL-1β fue elegida como estímulo proinflamatorio en el resto de experimentos ya que fue el agente que causó el efecto proinflamatorio más ptente y más homogéneo.

La IL-1β incrementó la expresión de MUC5AC de manera dosis-dependiente (0,1-20 ng/ml), tanto a nivel de mensajero como de proteína, mostrando un efecto máximo a las 6 y a las 24h, respectivamente. Por otro lado, la IL-1β no causó ningún efecto sobre la expresión de ARNm ni de proteína de MUC5B. La dexametasona reguló a la baja de manera dosis-dependiente (10^{-9}-10^{-6} M) la expresión de ARNm y la proteína de MUC5AC tanto a nivel basal como el inducido por IL-1β, con efecto máximo después de 24h de incubación. A pesar de que MUC5B no fue inducida por la IL-1β, la dexametasona fue capaz de disminuir ligeramente la expresión de su ARNm a nivel basal, tanto a las 12 como a las 24h de incubación.

Estos resultados sugieren que la dexametasona es capaz de disminuir la expresión del gen de MUC5AC y la secreción de esta mucina, tanto a nivel basal como en situación de inflamación. Además, la dexametasona regula a la baja la expresión basal de MUC5B.

Estudio de Revisión. Mucin gene expression in rhinitis syndromes. *Curr Allergy Asthma Rep. 2006;6(3):189-97.[Revisió].*

La rinitis y la rinosinusitis se encuentran frecuentemente asociadas a otras enfermedades respiratorias tales como el asma, la fibrosis quística y la poliposis nasal. En estas enfermedades la hipersecreción de moco causa obstrucción nasal e infección de las vías respiratorias. Las mucinas son el componente mayoritario del moco y son responsables de sus propiedades reológicas. Debido a que se han hallado alteraciones en el patrón de expresión de las mucinas en enfermedades asociadas a la rinitis, las mucinas han sido directamente implicadas en la formación de moco con características anormales, ya sea de tipo hiperviscoso o acuoso. Las mucinas predominantes en las secreciones respiratorias son MUC5AC y MUC5B y se han descrito cambios tanto en su cantidad como en su calidad en diversas enfermedades respiratorias. En general, la expresión de estas dos mucinas se ha hallado incrementada en patologías respiratorias tales como el asma, la rinitis alérgica y la poliposis nasal pero, en cambio, algunos estudios han demostrado una disminución en las secreciones de pacientes con fibrosis quística comparado con las de individuos sanos.

Los mecanismos involucrados en la regulación de las mucinas de vías respiratorias son diversos y dependientes de la enfermedad. En este sentido, las citocinas, los factores de crecimiento y las células inflamatorias serían los principales agentes implicados en la regulación de mucinas en el asma y la poliposis nasal, mientras que una regulación ejercida principalmente por bacterias y sus exoproductos sería típica de pacientes con fibrosis quística.

Otro factor que podría ser esencial en el desarrollo de un fenotipo hipersecretor sería la aparición de hiperplasia de células caliciformes y el agrandamiento de las glándulas submucosas, características que por otro lado han sido ya descritas en las enfermedades mencionadas anteriormente.

A pesar de que se han realizado grandes avances en relación al conocimiento de la fisiopatología de las enfermedades respiratorias, incluyendo la hipersecreción mucosa, todavía es necesaria mayor información con respecto a: a) la biología celular y estructural de las mucinas y del moco, tanto en situaciones fisiológicas como patológicas; b) la regulación de los genes MUC y la identificación de los mediadores clave implicados en esta regulación; y c) el mecanismo que conduce a la hiperplasia de las células caliciformes.

4. DISCUSSION

Discussion

Mucins, which are major component of mucus, have been found to be altered in several respiratory diseases such as asthma, cystic fibrosis and chronic rhinosinusitis with/out nasal polyps, and have been involved in the mucus hypersecretion present in these pathologies (46, 158, 279). Although many articles have reported mucin expression and secretion patterns in healthy and diseased cells and tissues in the lower airways (46), few have dealt with this topic in the upper respiratory tract.

For a better understanding of the pathophysiology of nasal polyposis and its associated diseases, specially regarding mucus hypersecretion, in the present thesis:

a) Mucin expression patterns in healthy and inflamed nasal mucosa have been studied and compared, taking as a model of inflammation nasal polyp tissue from different origins: NP from patients suffering of nasal polyposis alone or associated to cystic fibrosis, asthma, and/or aspirin-sensitivity, and antrochoanal polyps.

b) Additionally, since glucocorticoids are the recommended therapy for the treatment of nasal polyposis and their efficacy on mucus hypersecretion has been poorly studied and still remains controversial, in order to clarify the efficacy of GC therapy on mucus hypersecretion present in nasal polyposis, we aimed to investigate the *in vivo* effect of both oral and intranasal glucocorticoids on mucin expression and their producing cells in nasal polyps.

c) Moreover, the effect of glucocorticoids on mucin expression and secretion in normal and under inflammatory conditions was also assessed in a respiratory cell line (A549).

These studies will help characterizing both the abnormal mucus composition of secretions and the membrane-tethered mucin expression

profiles present in nasal polyposis and their associated diseases. The knowledge of these expression patterns might contribute to an improvement in the diagnosis and therapy of mucus hypersecretion in upper airway mucosal inflammation.

1. Mucin expression in healthy nasal mucosa.

In the study 1 (186), the expression of both membrane-tethered and secreted mucins was analyzed in healthy nasal mucosa at baseline. MUC1, MUC4, MUC5AC, and MUC8 were the mucins most highly expressed in the epithelium, while MUC1, MUC5B and MUC8 were the highest in glands. MUC2 and MUC7 were poorly expressed in the epithelium and glands, respectively, and MUC6 was scarcely detected in both locations. The expression of MUC2, MUC4, MUC5AC, and MUC6 mucins in healthy nasal mucosa was supported by the analysis of their MUC genes by *in situ* hybridation (ISH), showing similar amounts and distribution at both gene and protein levels. Partly in accordance with our results, a previous study found MUC4 and MUC5AC being the highest expressed MUC genes in healthy inferior turbinates (280). Apart from MUC8, that was not analyzed in the study by Aust and co-workers, the rest of analyzed mucins showed the same cellular distribution than in our study, founding the membrane-tethered MUC1 and MUC4 mucins detected throughout the epithelium while the secreted mucins showed more specific-cell expressions. In this manner, MUC2 and MUC5AC expression was restricted to epithelial goblet cells, MUC8 to basal epithelial cells, and MUC5B mainly to mucous cells in the submucosal glands.

Since MUC5AC and MUC5B have been reported to be the main mucins found in respiratory secretions (187, 188), the few articles dealing on mucin expression in the upper airways have focused on the expression of these two secreted mucins, leaving aside the rest of mucins that surely also have important roles in the pathophysiology of respiratory diseases, including nasal polyposis.

From the *gel*-forming mucins (MUC2, MUC5AC, MUC5B, and MUC6) analyzed in our study, only MUC5AC and MUC5B have been consistently detected in healthy secretions (281-283) hence at least these two mucins must be involved in healthy mucus formation. The role of the rest of mucins is not as clear. For instance, the MUC8 mucin is expressed in large amounts in our healthy nasal mucosa tissues and has been found increased in upper respiratory diseases (186, 284), but no function has been described yet for this soluble mucin. MUC2 is a *gel*-forming mucin found to be expressed in the upper airways (186, 280) and, although Ali and co-workers reported MUC5B and MUC2 being predominant mucins in healthy nasal mucus (285), it has not been consistently detected in airways secretions.

Airway mucus consists of two layers, the inner layer called *sol* phase and the outer called *gel* phase. While membrane-tethered mucins are closely associated with the periciliary liquid layer surrounding the microvilli and cilia, secreted mucins (mainly MUC5AC and MUC5B) are excluded from this area and form the *gel* phase that is moved continuously from the lung (189), conferring to this *gel* layer the viscoelasticity required for efficient mucus-cilia interaction (158). Mucins, within the airways mucus, are responsible for entrapping external agents and pathogens and eliminating them together with beating cilia by mucocilliary clearance. In healthy nasal mucosa, the *gel*-forming mucins MUC5AC and MUC5B found highly expressed in our Study 1 might be probably involved in this defensive role.

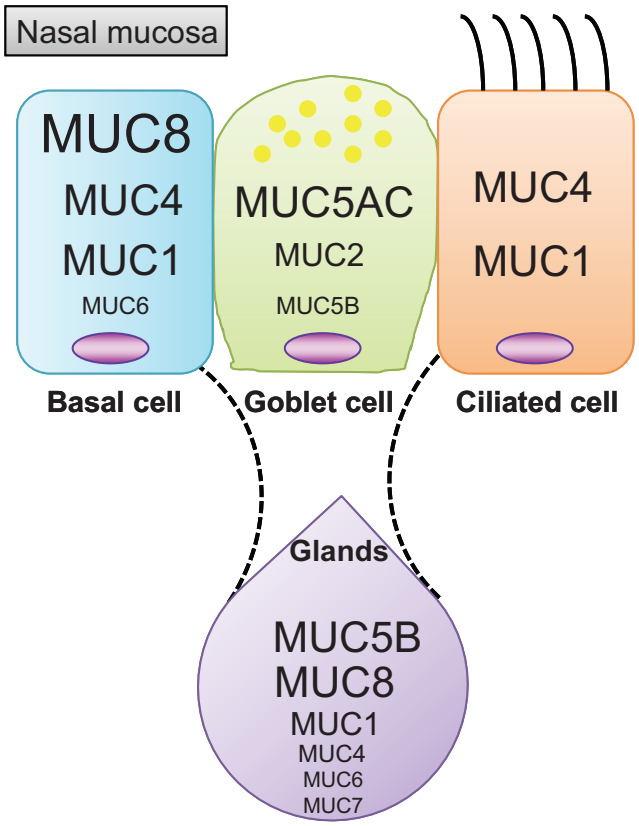
As for secreted mucins, the potential role for membrane-tethered MUC1 and MUC4 mucins highly detected in healthy nasal mucosa (186) might be related to the epithelia hydration, lubrication, protection from proteases, and defense against pathogens functions reported for these mucins in several tissues (122, 123, 160). Notwithstanding that, other actions have been described for these membrane-tethered mucins related to anti- and proadhesive capacities (166, 286-289) and intracellular cell signaling (290, 291), although it is not clear if these actions are carried out only in pathologic conditions or instead they also occur in healthy situation.

At least one example of defense against pathogens combined with intracellular signaling function has been reported for Muc1 in CHO cells. In this way, the bacterial protein flagellin of *Pseudomonas aeruginosa* interacts with the Muc1 extracellular domain (164) and stimulates cellular signaling pathways that may represent the initial stages of host response to infection (165).

2. Mucin expression in nasal polyps. Comparison with healthy nasal mucosa.

In several airway diseases such as chronic rhinosinusitis, asthma, and cystic fibrosis, an abnormal mucus composition of the mucus *gel*, with regards to the amount, type, and size of mucins has been reported (292-294). These changes may contribute to rheological properties of airways mucus, producing watery mucus in the case of allergic rhinitis and nasal polyposis and a hyperviscid mucus in the case of cystic fibrosis and asthma. However, the mucin expression patterns and their implication in this differential mucus presentation are still poorly understood, especially in relation to upper airways diseases.

In order to clarify whether disease-specific mucin expression patterns exist in nasal polyps in relation to their associated diseases, and if these expression profile differ from the one found in healthy nasal mucosa, the expression of membrane-tethered and secreted mucins in nasal polyps, nasal polyps from CF patients, and antrochoanal polyps, were analyzed and compared with this expression in healthy nasal mucosa (186). In Study 2 the mucin expression profile was analyzed and compared between nasal polyps from non-asthmatic and tolerant/intolerant asthmatic patients.



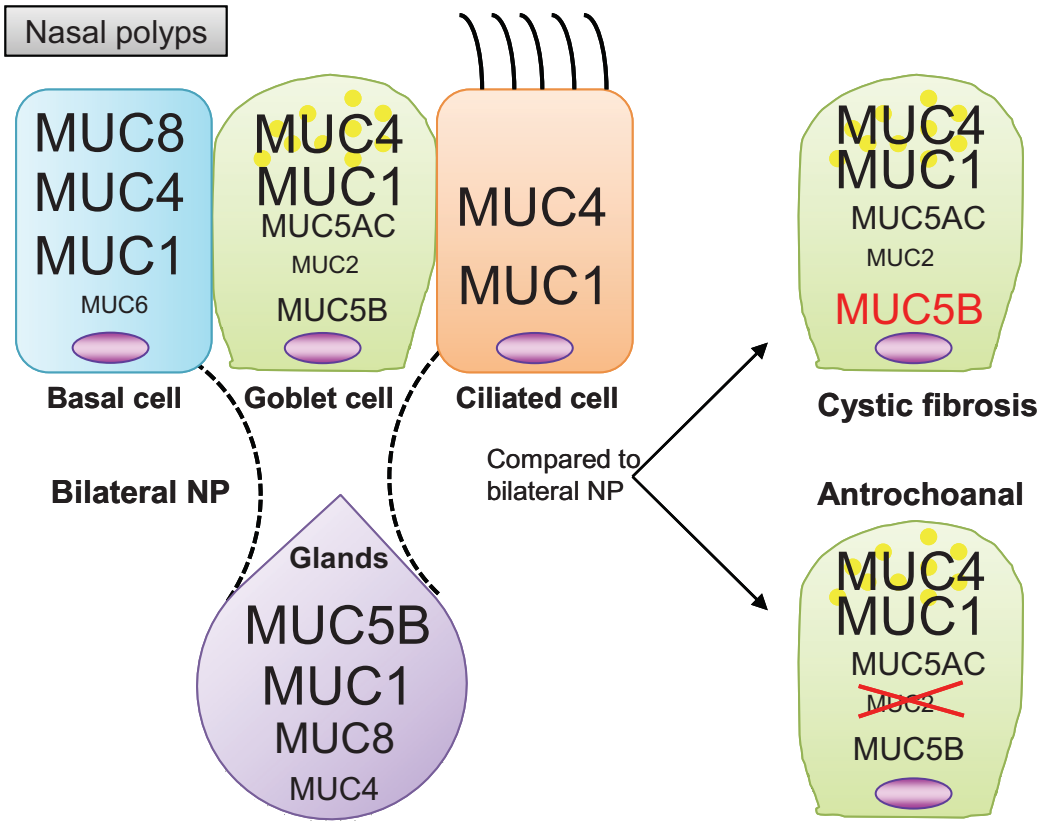


Figure 14. Mucin expression in healthy nasal mucosa and nasal polyp subtypes.
MUC1, MUC4, and MUC8 in the epithelium and MUC5B and MUC8 in glands were the highest expressed mucins in both healthy nasal mucosa and nasal polyps. MUC5AC in the epithelium and MUC1 in glands were also predominant mucins in healthy and nasal polyp mucosa, respectively.
The main differences were: a) increased MUC1 and MUC4, and decreased MUC5AC expression in nasal polyp epithelium compared to healthy nasal mucosa, b) MUC5B increase in nasal polyp glands compared to the healthy tissue, and c) increased MUC5B and decreased MUC2 in the epithelium of cystic fibrosis nasal polyps and antrochoanal polyps, respectively, compared to bilateral nasal polyps.

In Studies 1 and 2 we showed that, in general, in all types of nasal polyps MUC1, MUC4, and MUC8 and MUC1, MUC5B and MUC8 were the most expressed mucins in the epithelium and glands, respectively (Fig. 14). This is partly at variance with a previous study in which MUC5AC and MUC4 mRNA were reported to be the most expressed while MUC1 was one of the less expressed MUC genes in nasal polyp tissue (285). Since we analyzed the product of MUC genes (mucins) and Ali and co-workers studied MUC genes at mRNA levels by *in situ* hybridization, the differences here found can be directly explained by mismatches between protein and gene expressions.

Although all nasal polyps showed similar mucin expression patterns, in Study 1 MUC5B was found increased while MUC2 was found decreased in CF and antrochoanal nasal polyps' epithelium, respectively, compared to bilateral nasal polyps. Interestingly, current thinking states that expression of MUC5B in goblet cells from human lower respiratory tract epithelium is atypical and may be a marker of airway disease (192), being this a possible explanation for the MUC5B increase present in the epithelium of NP from CF patients. The expression of MUC5AC in nasal polyps from CF patients was previously studied by Dorhman et al (295), reporting an increase in MUC5AC mRNA levels compared to NP from non-CF patients. The fact that we did not found this MUC5AC mucin increase might be due to the different methodology performed by Dorhman et al. (ISH) and our group (immunohistochemistry). No previous studies exist regarding mucin expression in antrochoanal polyps.

In Study 2 (296), MUC5AC and MUC8 mucins were more detected in NP from asthmatic patients than in non-asthmatic patients. In this regard, a marked increase of MUC5AC mRNA levels in goblet cells of bronchial brushings from asthmatics was found compared to non-asthmatic patients (297). By contrast, Groneberg et al. found no differences in the MUC5AC and MUC5B mucin content beetween bronchial biopsies from asthmatic patients and healthy subjects (298).

Although in Studies 1 and 2, a similar tissue and cellular mucin distribution patterns have been found in healthy and inflamed (nasal polyps) upper airway mucosa, mucin amount differed in healthy and pathologic tissues. In this way, in Study 1, MUC1 and MUC8 in the epithelium, MUC5B in glands, and MUC4 in both epithelium and glands have been found increased in all types of nasal polyps compared to healthy nasal mucosa. On the other hand, MUC2 and MUC5AC mucins were decreased in pathologic compared to healthy tissue. In agreement with our results, an increased MUC8 and a decreased MUC5AC mRNA expression in bilateral nasal polyps (299), as well as an increased MUC5B and MUC8, at both mRNA and protein levels, in chronic rhinusinusitis compared with healthy tissues have been reported (284, 300). In addition, MUC5AC has been found decreased in nasal epithelial cells and sputum from CF patients compared to healthy subjects (301, 302). By contrast, several studies have reported an increase in MUC5AC mRNA and protein levels in bilateral nasal polyps associated to goblet cell hyperplasia (300, 303). In this respect, half of the patients suffering from bilateral nasal polyps in Study 1 were receiving glucocorticoids at the time of surgery, and as we have observed in Study 2, glucocorticoids are able to decrease goblet cell in the NP epithelium, fact that could explain the decreased MUC5AC mucin expression in bilateral nasal polyps.

3. Inflammatory mechanisms involved in mucus hypersecretion with/without altered rheological properties.

It has been reported that inflammatory cells present in NP could play an important role in the pathophysiology of the disease (304), the regulation of MUC genes being one of these implications. In this sense, eosinophil and mast cell products induce mucin production and/or secretion in airway epithelial cells (224, 305), and proinflammatory cytokines (IL-1β, IL-9, and TNF-α), mainly produced by inflammatory cells, can regulate the expression of specific mucins in bronchial and nasal [205, 223, 225, 229,

306 (personal observation)] epithelial cells. Since inflammatory cells could account for mucin production/secretion induction, in the Study 1, the inflammatory cell infiltrate of healthy and diseased nasal mucosa was evaluated, finding that both bilateral and CF nasal polyps showed a higher inflammatory cell content than healthy NM and ACP, being eosinophils the major inflammatory cells observed in bilateral nasal polyps. However, clear correlations were not found between mucin expression and inflammatory cell infiltrates in nasal polyps.

Goblet cell hyperplasia has been reported to occur in airways diseases such as nasal polyposis and asthma (245, 307, 308), while submucosal gland hypertrophy has been detected in asthma and cystic fibrosis airways (194, 309). These cellular changes often result in mucus hypersecretion usually accompanied by altered physiological properties. In addition, different studies have demonstrated that mucus-secreting cells in NP epithelium contribute to increased mucus secretion (299, 303). In this sense the increased expression patterns found in NP compared to healthy nasal mucosa in Study 1, could be related to these cellular changes reported in nasal polyposis.

4. Biologic significance of altered mucin composition of mucus.

The altered amounts of secreted mucins in pathologic conditions may contribute to mucus hypersecretion and to mucus viscoelasticity changes. Both increased or decreased amounts of secreted mucins might lead to the formation of an hyperviscid mucus, in the first case due to the formation of abnormal bonds between mucin subunits, changes in acidity, size, and glycoforms of the excessive mucus (292), and in the second case due to the reduced hydration of the mucus, since mucins, because of the abundant negative charges present in their carbohydrate terminals, are responsible for the large hydration of mucus and consequently of the epithelium (158).

The biologic significance of the membrane-tethered mucin (MUC1 and MUC4) activation in pathologic tissues may be related to the implication of these molecules in intracellular signaling pathways related to proliferative processes. In this way, MUC1 has been reported to be involved in metastasis, angiogenesis, and immune regulation (310-312) while MUC4 has been identified as a ligand of ErbB2 (313), a receptor that modulates epithelial cell proliferation following damage in asthmatic airways (175). Hence, the increase of MUC1 and MUC4 in nasal polyps could contribute to epithelial remodeling processes occurring during polyp formation.

In Studies 1 and 2, the quantity but not the quality of mucins in healthy and inflamed nasal mucosa has been analyzed. However, several reports deal with the presence of abnormal mucin forms and/or with altered glycosylation patterns in pathologic airway mucus. In this direction, a low charge glycoform of MUC5B have been found increased in airway secretions from asthmatic patients compared to these secretions of control subjects (188, 314, 315). Additionally, some studies have found clear differences in the glycosylation patterns of the major *gel*-forming mucins from CF patients compared with non-CF healthy controls (316, 317). This is in contrast with other structural studies where no changes between CF and non-CF secretions were found (194, 318, 319), although they have reported that presence of fragmented mucins is a common feature of CF mucin preparations, likely as a consequence of increased levels of bacterial- and neutrophil-derived proteases in the CF lungs (194, 318-320). Future glycosylation studies would elucidate if mucins from NP secretions present altered glycosylation profiles compared to healthy subjects secretions.

5. Corticosteroid effects on mucin expression in nasal polyps.

Corticosteroids (CS) are first-choice therapy in the management of inflammatory respiratory diseases including nasal polyposis (15). They are effective in reducing the polyp size and their inflammatory component, mainly represented by eosinophilic infiltration (15, 79), but their efficacy on

regulating mucin production and consequently mucus hypersecretion is not well established. Although some studies have assessed CS effect on mucin expression in respiratory primary and cell lines cultures, few studies have dealt with this topic in an *in vivo* situation. In the Studies 2 and 3, the *in vivo* and *in vitro* effect of CS on mucin expression/secretion in nasal polyps and in a respiratory cell line (A549) has been investigated.

In the Study 2 (296), CS seemed to differentially regulate mucin expression depending on mucin nature, secreted vs membrane-tethered, on the duration of the treatment, short-courses vs long-term therapy, and on the origin of NP, from non-asthmatic or aspirin-tolerant/-intolerant asthmatic patients. In this manner, while a short-term treatment with oral prednisone combined with intranasal budesonide seemed to up-regulate membrane-tethered mucins (MUC1 and MUC4) in almost all NP epithelia, secreted mucins (MUC5AC and MUC5B) appeared to strongly respond to the long-term treatment by decreasing their expression in the epithelium and glands, respectively (Fig. 15). Previous studies reported no variations in MUC5AC expression in NP (321) and lung biopsies (298) after 8 weeks of intranasal fluticasone or one month of intranasal budesonide, respectively. These discrepancies could be explained by the small number of patients analyzed, specifically in the latter study (n=5), as well as to the different and short duration of the treatment in those studies. In agreement with our findings, several *in vitro* studies reported that dexamethasone (DEX) decreases MUC5AC mRNA in airway epithelial cell lines [306 (in preparation), 322-324] and in primary normal human bronchial epithelial cells (322), while DEX increased MUC1 in cancer cell lines (312, 325).

Although MUC8 has been found increased in chronic rhinosinusitis and NP compared to healthy nasal mucosa (186, 284) its potential role as one of the major compounds of mucus has not been well-established. In the Study 2, MUC8, being a secreted mucin, increased after long-term intranasal budesonide treatment (Fig. 15). The differential regulation that CS exerted

on this secreted mucin compared to the others secreted mucins could be related to a different role of this mucin in NP.

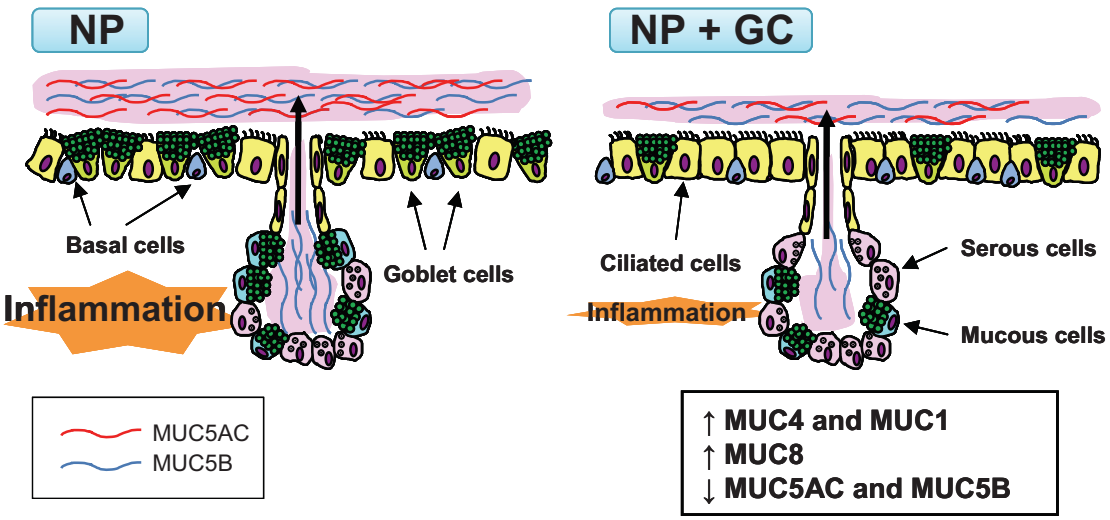


Figure 15. Glucocorticoid effect on mucin production and on mucus-producing cells in nasal polyps. Glucocorticoids (GC) decrease inflammation of nasal polyps (NP). Additionally, they are able to reduce the number of goblet cells in the epithelium and mucous cells in submucosal glands, consequently decreasing the expression of secreted mucins (MUC5AC and MUC5B) which are mainly produced by these cell types. The reduction of secreted mucins could be translated in a diminution of mucus secretion (pink) in the form of rhinorrhea. The increase of membrane mucins (MUC1 and MUC4) could be a reflex of a relative increase of their main producing cells (non-goblet cells: ciliated, basal) after glucocorticoid treatment.

Since nasal polyps are often associated to aspirin-tolerant (ATA) and –intolerant (AIA) asthma, in the study 2 a special attention was paid to these groups of patients regarding CS effect on mucin expression. In this way, nasal polyps from ATA patients showed the most significant changes for all studied mucins, while those from non-asthmatic patients showed variations mainly in MUC1, MUC5B, and MUC8, and those from AIA patients showed changes almost exclusively in MUC5AC, suggesting a trend of resistance to CS treatment. In accordance to these findings, aspirin sensitivity has been reported to be a risk factor for steroid resistance in patients with NP (326) as well as in steroid non-responder severe

asthmatics (327). The reason for this resistance is still poorly understood. Another potential explanation for this lack of response could be the high levels of membrane-tethered and low levels of secreted mucins that AIA patients showed at baseline, almost comparable with the profile found in ATA patients after CS treatment.

6. Corticosteroid effects on mucin-producing cells and on nasal symptoms related to mucus hypersecretion in nasal polyps.

As previously mentioned in the *discussion-section 3*, goblet cell hyperplasia is a feature of the remodeling process reported to occur in respiratory diseases, including nasal polyposis and asthma (245, 307, 308), and it has also been related to the mucus overproduction/secretion present in nasal polyposis and asthmatic patients (297, 307, 328). Although several studies have investigated the effect of CS on goblet cell hyperplasia (GCH) in murine models of asthma (329-331) finding both positive and negative effects, no studies have dealt with this matter in human nasal polyps. For this reason, in the Study 2 (296) the number of goblet cells in the epithelium and of mucous cells in submucosal glands before and after CS treatment was analyzed, finding a decrease of both mucin-producing cells after CS short- and long-term treatments. Interestingly, the reduction caused by CS in the number of goblet and mucous cells in nasal polyps, correlated with the decrease of secreted mucins MUC5AC and MUC5B after CS treatment. In this sense, CS would produce a beneficial indirect effect on mucus hypersecretion reducing the number of mucin-producing cells and consequently decreasing secreted mucins (Fig. 15). Since MUC1, MUC4, and MUC8 are not goblet cell specific-mucins we could speculate that their increase after CS treatment could be due to an increase in the number of non-goblet epithelial cells (basal, ciliated), as a consequence of a reconstruction process that may occur in NP epithelium after CS treatment leading eventually to a more "healthy-like" tissue. In this direction, although there are no studies based on CS effect on GCH in NP, Laitinen et al. have

demonstrated that long-term treatment of asthmatic subjects with inhaled CS significantly increased the ratio of ciliated to goblet cells in the airways (332).

To further analyze the efficcacy of CS therapy on some clinical symptoms commonly present in patients suffering from nasal polyposis and to investigate whether the improvement in any of these clinical symptoms correlated with the effect of CS on mucin expression, nasal obstruction and rhinorrhea were also assessed in patients included in the Study 2. CS short- and long-term treatments were able to reduce nasal obstruction and rhinorrhea in all groups of NP, although the improvement in nasal obstruction after 2 weeks of treatment was higher in the asthmatic group. The improvement in rhinorrhea correlated with the reduction of MUC5AC and slightly of MUC5B expression after treatment. Thus, the effect of CS reducing mucin-producing cells and consequently mucin amount could be translated in a decrease in the rhinorrhea present in patients with nasal polyposis.

7. Glucocorticoid effects on in vitro mucin expression and secretion. Potential regulatory mechanism.

As mentioned before, glucocorticoids remain the most effective anti-inflammatory drug available in the treatment of inflammatory airway disorders such as chronic rhinosinusitis with/without nasal polyps and asthma (15, 333), their capacity of inhibiting the synthesis of inflammatory mediators being considered the basis of their efficacy. Several cytokines and inflammatory agents found elevated in chronic airway diseases (197, 199-201, 334, 335) have been found to stimulate mucus hypersecretion (46). Among them, interleukin (IL)-1β is one of the most important multifunctional proinflammatory cytokines playing a role in mucin overproduction (205, 222, 225, 336-338).

Although glucocorticoid effect on mucus hypersecretion has always been controversial, especially paying attention to mucin overproduction, in the Study 2 we found a reduction in MUC5AC and MUC5B mucins linked to rhinorrhea fall after GC therapy in patients suffering from nasal polyps. On this basis, in the Study 3, the effect of dexamethasone on MUC5AC and MUC5B mRNA expression and protein secretion, both at baseline and after induction by IL-1β, was investigated in the respiratory cell line A549. IL-1β was found to upregulate MUC5AC, but not MUC5B, mRNA and secreted protein. Dexamethasone was able to decrease the basal expression of both MUC5AC and MUC5B mRNA and protein levels as well as IL-1β-induced MUC5AC expression. In agreement with our findings, previous studies have reported IL-1β to stimulate MUC5AC mRNA and protein levels in normal human bronchial epithelial cells (337, 338) and in the respiratory cell line NCI-H292 (221, 222). In addition, some studies reported a decrease in steady-state MUC5AC mRNA levels after incubation with dexamethasone in A549 and NCI-H292 cells (322, 324), and on bacteria-induced Calu-3 cells and explanted human nasal mucosa (339). In this way, Chen et al. reported dexamethasone to transciptionally repress the MUC5AC promoter (324). Althought we reported for the first time the downregulation on IL-1β-induced MUC5AC expression caused by dexamethasone, Kim et al. demonstrated budesonide to attenuate IL-1β-induced MUC2 gene and protein production levels (340).

From all these results, we can speculate that the potential mechanisms by which glucocorticoids could exert their regulatory action on MUC5AC expression are: a) directly through attaching to its receptor and binding to GRE sites in the MUC5AC gene promoter (324); b) and/or indirectly through inhibiting the induction caused by proinflammatory cytokines [306 (not published yet), 340]. Additionally, glucocorticoids might regulate gene expression and/or protein production by acting on mRNA and protein stability (Fig. 16).

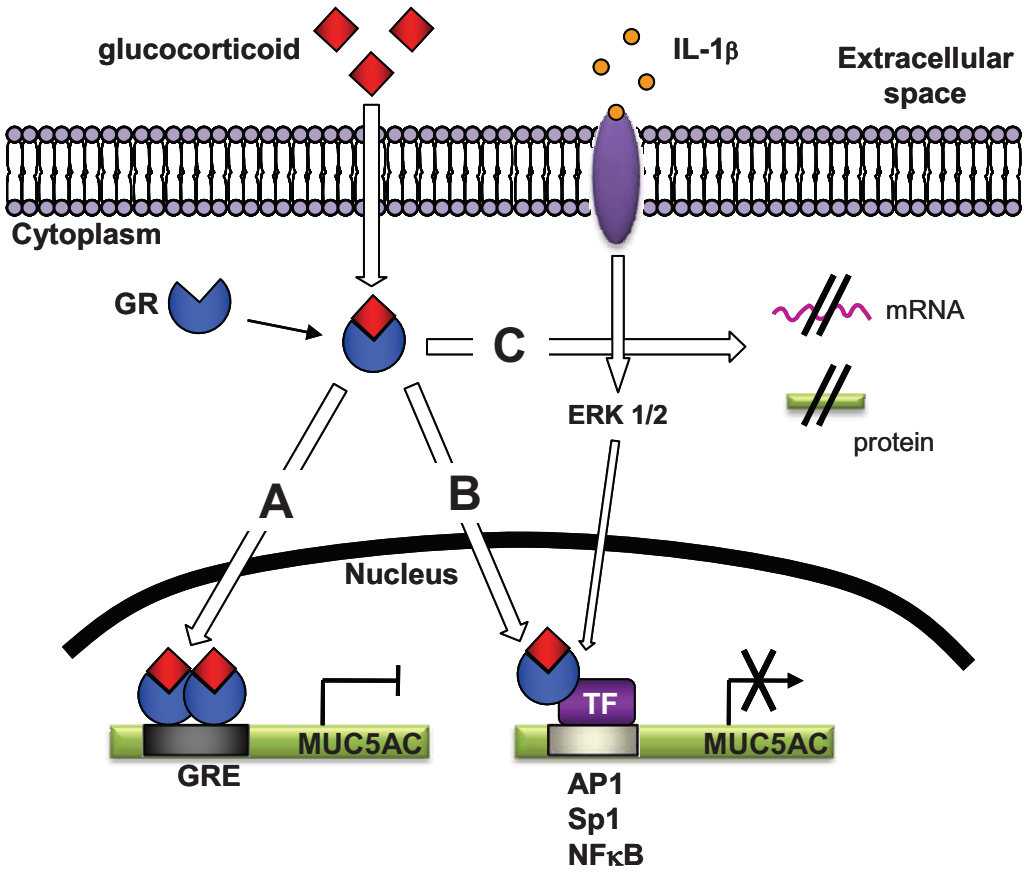


Figure 16. Potential mechanism of action of glucocorticoids (GC) on basal and IL-1β-induced MUC5AC expression. In the cellular cytoplasm GC bind the glucocorticoid receptor (GR) where, after activation, the dimer form translocates to the nucleus. In the nucleus: A) a dimer of activated GR can bind glucocorticoid response elements (GRE), located in the promoter region of the MUC5AC gene, inhibiting its transcription; B) the induction of MUC5AC transcription by IL-1β via the extracellular signal-regulated kinase 1/2 (ERK 1/2), can be inhibited by the transrepression of a monomer of activated GR with specific transcription factors (TF) binding regions [activator protein 1 (AP1), specificity protein 1 (Sp1), nuclear factor κB (NFκB)]. C) The activated form of GR can modify the stability of both MUC5AC mRNA and protein.

5. CONCLUSIONS

Conclusions

1. MUC1, MUC4, MUC5AC, and MUC8 are the main mucins found expressed in the epithelium whereas MUC5B and MUC8 in submucosal glands of both, healthy nasal mucosa and nasal polyps.

2. Nasal polyps show a different mucin expression pattern, with MUC1 and MUC4 increased and MUC5AC decreased, compared to healthy nasal mucosa.

3. Among the different groups of nasal polyps,

    a. Nasal polyps from cystic fibrosis patients, with an increase in MUC5B, and antrochoanal polyps, with a decrease in MUC2, also show a differential expression pattern than bilateral nasal polyposis.

    b. Nasal polyps from asthmatic patients present an increased MUC5AC and MUC8 mucin expression compared to non-asthmatic patients.

4. Corticosteroid treatment,

    a. Increases membrane-tethered (MUC1 and MUC4) while decreases secreted (MUC5AC and MUC5B) mucin expression in nasal polyps, especially from aspirin-tolerant asthmatic patients. Aspirin-sensitive asthmatics seem to show a trend of resistance to corticosteroid therapy.

    b. Decreases the number of mucin-producing cells, goblet cells in the epithelium and mucous cells in submucosal glands, this decrease correlating with the reduction in major secreted mucin (MUC5AC and MUC5B) expression after corticosteroid treatment.

c. Reduces nasal obstruction and rhinorrhea symptoms in nasal polyposis patients, the rhinorrhea fall correlating with the reduction of major secreted mucin (MUC5AC and MUC5B) expression after corticosteroid treatment.

5. Dexamethasone is able to decrease both basal and IL-1β-induced MUC5AC mRNA expression and protein secretion as well as basal MUC5B mRNA expression in A549 cells.

Final conclusions

1. Different mucin expression profiles are found between healthy and inflamed sinunasal mucosa, being these differences partly responsible for the secretion of mucus with altered viscoelatic properties in nasal polyp patients.

2. Corticosteroids are able to directly or indirectly decrease mucin expression and/or secretion both, *in vivo* and *in vitro*, under inflammatory conditions. *In vivo*, the downregulation of secreted mucins could result from the ability of corticosteroids to reduce mucin-producing cells, and could account for the reduction of mucus production and rhinorrhea in nasal polyps. In vitro, dexamethasone is able to directly decrease basal and induced mucin expression and secretion.

3. These findings together with further studies on mucin expression and regulation in health and disease may help to establish disease-specific differential diagnostics, and to improve current and explore further therapies for the treatment of mucus hypersecretion in upper and lower respiratory inflammatory diseases.

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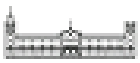

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