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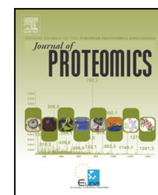

Seasonal proteome changes of nasal mucus reflect perennial inflammatory response and reduced defence mechanisms and plasticity in allergic rhinitis

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ABSTRACT

Introduction: Nasal mucus and its proteins are a defence against allergens. We sought to investigate dynamic proteome changes in allergic rhinitis upon environmental allergen provocation.
Methods: Nasal mucus was collected in and out of pollen season from allergic rhinitis patients (N = 10) and healthy controls (N = 12). Liquid chromatography–tandem mass spectrometry was performed. Proteins were identified by SwissProt database search and quantified from normalized areas under curve of precursor ion chromatograms. Gene enrichment analysis was performed with Cytoscape/BINGO software.
Results: In total 430 different proteins were detected in both groups, 203 (47.2%) were newly identified. In allergics CLU and IGKC were significantly more abundant in season (2.2 and 2.1-fold respectively). GSTP1 (0.5-fold), ELANE (0.4-fold), HIST1H2BK (0.3-fold), S100A8 (0.2-fold), S100A12 (0.2-fold) and ARHGDIB (0.1-fold) were significantly less abundant in season. In healthy controls UBC, TUBA1B, HBB and FABP5 were only present in season. Ig kappa chain V-I region DEE (5.3-fold), CLU (5.0-fold), TXN (4.3-fold), MSMB (3.2-fold) and Ig heavy chain V-III region BRO (2.7-fold) were significantly more abundant in season. MUC5B (0.5-fold), SLPI (0.2-fold) and S100P (0.2-fold) were significantly less abundant in season.
Conclusion: Contrary to their symptoms allergic rhinitis patients show perennial inflammatory response lacking adequate reaction to allergens in season.
Biological significance: Many studies dealing with allergic rhinitis are focused on the nasal epithelium. This is the first study to analyse the nasal mucus as primary defence barrier on a proteomic level in and out of pollen season and contrary to the leading opinion shows that allergic patients show a perennial inflammatory response with reduced reaction to allergens whereas healthy controls react on proteome basis towards enhanced defence in season despite lacking allergic sensitization.
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1. Introduction

Allergic rhinitis is a global health problem affecting up to 40% of the population in some regions regardless of gender or age [1]. The pathophysiology of allergic rhinitis is well understood with affected subjects being sensitized to otherwise harmless inhaled allergens. Upon contact with the patients' immune system immune cells produce IgE instead of IgG which causes mast cell degranulation and the classical symptoms of nasal congestion and rhinorrhea [1,2]. Nevertheless the process of sensitization is unclear. Environmental and many other factors have been proposed to explain why certain individuals develop allergy and some not [3]. Nasal mucus is the first line defence barrier against allergens and its proteins might play a role in the sensitization process and allergen interaction with the underlying epithelium [4,5].

Abbreviations: AUC [%], mean normalized areas under the curve; CID, collision induced dissociation; DTT, dithiothreitol; Fw-rev, forward-reverse score; IAA, iodoacetamide; ICR, ion cyclotron resonance; LC–MS/MS, liquid chromatography–tandem mass spectrometry; nano-HPLC, nano-flow-high performance liquid chromatography; SD, standard deviation; SEM, standard error of mean; %SPI, Scored Peak Intensity Percent; SPT, skin prick test.

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A possible role of nasal mucus could be to block pollen proteases with innate antiproteases and to prevent subsequent epithelial damage and allergen transport through the mucosa [6–9]. Proteins exudated from plasma could have immunomodulatory functions and influence disease severity [10,11]. To understand the nasal mucus proteome proteomic studies are feasible detecting a large range of proteins and facilitating analysis of their origin and function [12].

We recently showed that there are significant differences in nasal mucus proteome between allergic rhinitis patients and healthy controls, and that some proteins might be novel biomarkers and lead to better understanding of the disease [13,14] Moreover, we validated our methodology and confirmed our mass spectrometry results by Western Blotting of selected proteins [13,14]. In these previous studies patients were analyzed at a single time point solely and thus seasonal differences of the proteome could not be assessed. The present study is aimed at resolving seasonal proteome changes in allergic rhinitis patients and healthy controls to reveal whether pollen exposure leads to changes of the nasal mucus proteome in dependence of disease. We therefore collected nasal mucus of allergic rhinitis patients and healthy controls at two distinct time points, i.e. in and out of pollen season. The obtained paired dynamic proteome profiles were examined with respect to how either group reacts to natural pollen exposure on a proteome level. Our results further stratify and identify novel key proteins playing a role in allergic rhinitis.

2. Material and methods

2.1. Patients

Sixty-eight individuals were enrolled in the study, with a drop out number of 46, because they did not appear to the follow-up controls or did not meet the inclusion criteria at the follow-up visit anymore, like usage of topical and/or systemic corticosteroids, antihistamines or any other immunomodulatory drugs. The remaining twenty-two individuals (7 males, 15 females) with a mean age of 33 years (SD: 9.7 years) were included in the study group comprising 10 (45%) allergic rhinitis patients and 12 (55%) healthy controls. Allergy status was verified by skin prick tests (SPT, Allergopharma GmbH & Co. KG, Reinbek, Germany) and specific IgE (ImmunoCAP, Thermo Fisher Scientific Inc., Vienna, Austria) in all patients and controls. Patients sensitized to house dust mite or animals solely were excluded to avoid bias due to small sample size (Table 1 and supplementary Table E1). Thus, only patients sensitized to pollen and also showing symptoms during the pollen season were considered for evaluation. Patients with acute and/or chronic sinusitis as defined by the EPOS [15] guidelines were also excluded, as were patients with malignant tumors and any infectious or cardiopulmonary disease, or who had been treated with systemic or topical drugs including antihistamines, corticosteroids, antibiotics, antifungals or any other immunomodulatory drugs in the four weeks prior to the study. The same exclusion criteria applied to the controls, who were healthy volunteers recruited from the hospital staff. Informed consent was obtained from all participants (allergics and controls) before enrolment. The study was approved by the institutional review board of the Medical University of Graz.

Table 1
Summarized epidemiologic data with distribution of gender, age, group as well as skin prick test (SPT) for clinically relevant allergens, symptoms present and total IgE.

Parameter	Allergic rhinitis	Healthy controls
Number of patients	10	12
Demographics		
Mean age, years (SD)	30.4 (8.4)	36.3 (10.1)
Women, %	75%	40%
Clinically relevant positive SPT		
Alder/Hasel/Birchpollen	8	0
Grasspollenmix	9	0
Ragweedpollen	3	0
Symptoms during pollenseason		
Allergic rhinitis	10	0
Allergic rhinitis and asthma	0	0
Total IgE (kU/l), (SEM)	136.1 (122.1)	42 (37.5)

SD: standard deviation.
SPT: skin prick test.
SEM: standard error of mean.

2.2. Sample collection

In pollen season (with clinical symptoms present in allergic rhinitis patients) and out of pollen season (without clinical symptoms in allergic rhinitis patients) nasal mucus was collected with a special suction device (Sinus Secretion Collector, Medtronic Xomed Inc., Jacksonville, Florida, USA). Healthy controls' samples were collected on the same day as allergic rhinitis patients' samples. Without previous interventions (decongestants, local anesthetics) untreated mucus was obtained under endoscopic control from the nasal cavity and middle meatus with meticulous care taken not to touch the mucosa. The mucus volume obtained was equal in both groups. Then, mucus was deep-frozen at −92 °C before processing for LC–MS/MS mass spectrometry.

For sample preparation and mass spectrometric analysis please see methods supplement in the online repository. Proteomic experiments were performed according to MIAPE (minimum information about a proteomic experiment) [14,16].

2.3. Data analysis

The LC–MS/MS data were analyzed by searching the human SwissProt public database (downloaded on March 10th 2012) with Proteome Discoverer 1.4 (Thermo Scientific) and Mascot 2.2 (Matrix Science, London, UK). Detailed settings: Enzyme: trypsin, max. missed cleavage sites: 2, N-terminus: hydrogen, C-terminus: free acid, carbamidomethylation on lysine as fixed modification, oxidized methionine as variable modification, maximum precursor charge 3; precursor mass tolerance +/− 0.05 Da, product mass tolerance +/− 0.7 Da; acceptance parameters were 2 or more identified distinct peptides after automatic validation (decoy search, FDR < 5%). Identified proteins were annotated using data from Uniprot (www.uniprot.org). Areas under the curve (AUCs) (i.e. mean areas of extracted ion chromatograms of the individual peptides matched to a protein) normalized on the total AUC of all proteins in each sample were used to compare relative protein abundances of the same protein between groups [17]. Data are reported as means and standard errors of mean (SEM). Statistical analysis by Mann Whitney U test for seasonal or group differences and multivariate analysis for seasonal and group differences were performed with SPSS 18.0 software (Chicago, Illinois, USA). A p-value of <0.05 was considered significant.

Enrichment analysis was performed with BINGO 2.44 [18] in Cytoscape 2.81 software (www.cytoscape.org) [18]. For statistical analysis of enrichment data created with BINGO/Cytoscape hypergeometric tests were performed and corrected by Benjamini & Hochberg False Discovery Rate (FDR) correction at a significance level of 0.05.

3. Results

The mean total protein concentration over all samples was 0.58 mg/ml (SD: 0.66). Allergic rhinitis patients (AR) had a higher mean protein concentration (0.78 mg/ml (SD: 0.87)) than healthy controls (HC) (0.42 mg/ml (SD: 0.35)). However, this difference was not significant ($p = 0.377$). Overall 430 different proteins were detected in both groups, while 327 were detected in AR and 366 in HC respectively. Of these 203 proteins (47.2%) were newly identified as nasal mucus proteins (supplementary Table E2). The most abundant proteins were Serum albumin (ALB), Lysozyme C (LYZ), Ig alpha-1 chain C region (IGHA1) and Ig alpha-2 chain C region (IGHA2) independent of disease

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