

Gene profiling reveals decreased expression of uteroglobin and other anti-inflammatory genes in nasal fluid cells from patients with intermittent allergic rhinitis

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Summary

Background Intermittent allergic rhinitis (IAR) results from interactions between a large number of pro- and anti-inflammatory mediators. Little is known about anti-inflammatory mediators in IAR. DNA microarrays allow simultaneous analysis of the whole transcriptome in a sample.

Objective To identify anti-inflammatory transcripts in nasal fluid cells from patients with IAR during season and from healthy controls.

Methods Nasal lavage fluids were obtained from 15 patients with symptomatic birch/and or grass pollen-induced IAR and 28 healthy controls. RNA was extracted from the nasal fluid cells and pooled into one patient- and one control pool. These were analysed with DNA microarrays containing more than 44 927 genes and variants.

Results Seventeen thousand three hundred and fifty three genes were expressed in the controls and 17 928 in the patients. One thousand five hundred and seventy nine of the genes had higher expression in patients than in controls, and 1570 had lower expression in patients. Out of 189 up-regulated inflammatory genes, 187 were pro-inflammatory and two were anti-inflammatory. These genes regulated key steps of inflammation, ranging from influx of leukocytes to immunoglobulin production. By comparison, out of 49 down-regulated inflammatory genes, 36 were pro-inflammatory and 13 were anti-inflammatory. The anti-inflammatory gene that decreased most in expression in the patients was uteroglobin (also known as Clara Cell protein 16, CC16). The nasal fluid concentrations of uteroglobin protein were significantly lower in patients than in controls, 5.43 ± 1.53 and 12.93 ± 2.53 ng/mL, respectively ($P < 0.05$).

Conclusion IAR is associated with decreased expression of uteroglobin and other anti-inflammatory genes.

Keywords DNA microarrays, gene expression

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Introduction

Release of cytokines from type 2 T-helper (Th2) cells contributes to the symptoms of intermittent allergic rhinitis (IAR) [1]. These cytokines induce IgE-synthesis, as well as degranulation of eosinophils and mast cells. However, an increasing number of other cells and their products have been implicated in the pathogenesis of allergic rhinitis; e.g. other cytokines and immunoglobulins may contribute to degranulation of eosinophils and growth factors may protect the mucosa by maintaining epithelial integrity [2]. DNA microarrays consist of a matrix with attached DNA sequences that allow simultaneous analysis of expression of the whole human genome [3]. With the help of DNA microarrays a great diversity of gene expression has been shown in nasal

biopsies from patients with IAR [4]. Interestingly, decreased activity of the anti-inflammatory gene TGF was found [5]. Moreover, in a recent microarray study of nasal polyps decreased expression of other anti-inflammatory genes, such as uteroglobin was found (also known as Clara cell protein 16 or CC16) [6]. These findings suggest that a decreased expression of anti-inflammatory genes could contribute to airway inflammation.

While many mediators have been described in biopsies from the nasal mucosa it is not clear to what extent they are produced by cells in nasal airway secretions. This distinction is not trivial since inflammatory cells are not equally distributed between the nasal mucosa and lumen. Lymphocytes predominate in the mucosa and granulocytes in the lumen [7]. Thus, the two compartments may have distinct immunological characteristics. The aim of this study was to examine cells in nasal fluids from patients with IAR and healthy controls with DNA microarrays, with particular emphasis on anti-inflammatory transcripts.

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Materials and methods

Subjects

The study included 15 patients with symptomatic birch and/or grass pollen-induced allergic rhinitis and 28 healthy controls. The median (range) age of the patients and the controls were 37 (18–61) years and 33 (18–51) years, respectively. Eight patients and 17 controls were women.

The diagnosis of birch and grass pollen induced allergic rhinitis was based on a positive history of seasonal allergic rhinitis, presenting itself with symptoms like nasal congestion, clear watery discharge, nasal itching and paroxysmal sneezing and positive skin prick tests (SPT) against birch pollen and/or grass. All subjects had suffered from allergic rhinitis during at least two seasons before the present one. During previous seasons the patients suffering have been moderate-to-severe, requiring treatment with antihistamines and local steroids often in combination with antihistamine or cromoglycate eye drops. Two of the patients suffered from asthma that required occasional treatment with terbutaline during season.

The eight patients sampled during the birch pollen season all exhibited positive SPT towards birch (either 3+ or 4+), three of these were also positive for timothy (2+ or 3+) and one of these was also positive for mugwort (2+). All of them were negative for house dust mite (*Dermatophagoides pteronyssinus* and *D. farinae*), horse, and molds (*Cladosporium* and *Alternaria*). The seven patients sampled during the grass pollen season all exhibited positive SPT towards timothy (either 3+ or 4+) and two of these were also positive for birch (3+ or 4+). All of them were negative for mugwort, house dust mite (*D. pteronyssinus* and *D. farinae*), horse, dog, cat and molds (*Cladosporium* and *Alternaria*).

The controls were all symptom-free, had no history of allergic rhinitis or any other atopic disease, and had negative SPT against a panel of allergens, including birch, timothy, mugwort, house dust mite (*D. pteronyssinus* and *D. farinae*), horse, dog, cat, molds (*Cladosporium* and *Alternaria*).

Before inclusion, all subjects, patients as well as controls, were evaluated by an experienced ear-, nose-, and throat-consultant, performing nasoscopy. Individuals with signs or symptoms of chronic rhinitis, nasal polyposis or recurrent sinusitis were excluded along with subjects with a history of concurrent purulent nasal infection within 6 weeks before the study or nasal surgery during the last year. Subjects treated with local or systemic corticosteroids for the last 2 months before the present investigation were also omitted.

Study design

The patients were seen during either birch (eight patients) or grass pollen season (seven patients). They were included when they had experienced substantial symptoms of rhinoconjunctivitis (itchy nose and eyes, sneezing, nasal secretion and nasal blockage) during at least three consecutive days. The majority of patients were seen within 5–10 days after the first appearance of symptoms and a local pollen count confirmed the presence of relevant pollen during this period. The controls were seen during the same period. All anti-allergic medication, except the occasional use of cromoglycate eye drops (not used during the last 24 h), was withheld until the nasal lavage (NAL) was performed.

Skin prick tests

SPT were performed with a standard panel of 10 common airborne allergens (ALK, Copenhagen, Denmark) including pollen (birch, timothy, artemisia), house dust mites (*D. Pteronyssinus* and *D. Farinae*), molds (*Cladosporium* and *Alternaria*) and animal allergens (cat, dog and horse). The tests were performed on the volar side of the forearm with saline buffer as negative and histamine chloride (10 mg/mL) as positive controls. The weal reactions were measured after 20 min and designated as 4+, 3+, 2+, 1+ or 0 depending on the size in relation to histamine and saline.

Nasal lavage fluids were obtained as previously described [8]; after clearing excess mucous by forceful exsufflation, sterile normal saline solution of normal room temperature was aerosolized into each nostril, while alternately clearing the other. The nasal fluids were allowed to return passively and collected in a graded test tube that was submerged in iced water, until 5 mL were recovered. The fluids were then centrifuged for 10 min at 1334 g in 4 °C. The supernatant was separated from the pellet and saved in RNeasy (Qiagen, Valencia, CA, USA).

The study was approved by the Ethics Committee of the Medical Faculty, Lund University.

Preparation of cRNA

RNA was isolated using the RNeasy kit (Qiagen). RNA concentrations were determined spectrophotometrically. The RNA was pooled into one patient pool and one control pool and used for target preparation. Each pool was divided and analysed according to the following procedure. Double-stranded cDNA was prepared using Life Technologies Superscript Choice system (Life Technologies, Paisley, UK) and an oligo(dT)24-anchored T7 primer. Biotin-labelled cRNA was synthesized from the total amount of cDNA by *in vitro* transcription with biotin-labelled nucleotides and T7 RNA-polymerase, Enzo BioArray High Yield RNA Transcription Labeling Kit, according to the manufacturers instructions (Enzo Diagnostics, Farmingdale, NY, USA). One round of amplification was performed. Labelled cRNA was purified using RNeasy columns (Qiagen) and 20 µg of biotinylated cRNA was fragmented at 94 °C for 35 min with 1 × fragmentation buffer (40 mM Tris-acetate pH 8.1, 100 mM KOAc, 30 mM MgOAc) in a final volume of 40 µL. Gel electrophoresis was performed to verify expected size distribution of cDNA, cRNA and fragmented cRNA. Hybridization cocktails were prepared by mixing 15 µg fragmented cRNA (adjusted for RNA originating from the starting material), 50 pmol/L B2 control oligonucleotide (Genset, Paris, France), 1.5, 5, 25 and 100 pmol/L, respectively, of BioB, BioC, BioD and Cre control transcripts generated by *in vitro* transcription using the plasmids pGIKS-BioB, pGIKS-BioC, pGIKS-BioD and pGIKS-Cre as template (ATCC, Manassas, VA, USA), 0.1 mg/mL herring sperm, 0.5 mg/mL acetylated BSA, 1 × MES hybridization buffer (100 mmol/L MES, 1 M NaCl, 20 mmol/L ethylenediamine tetraacetic acid (EDTA), 0.01% Tween 20) in a final volume of 300 µL.

In vitro amplification

The quantity and quality of RNA was analysed with Nanodrop ND-1000 UV Spectrophotometer (Nanodrop

Technologies, Wilmington, DE, USA). The amount was 96 and 64 ng for controls and allergic patients, respectively. As a result of the small amount of RNA an *in vitro* amplification of two cycles was performed in accordance with protocol for Small Sample Target Labeling Assay Version II (Affymetrix, Santa Clara, CA, USA) before the hybridization. Briefly for the first cycle, first strand cDNA synthesis for each sample was performed by initially mixing total RNA (1 µL: 24 and 16 µg for controls and allergic patients, respectively) with T7-Oligo(dT) promotor primer (1 µL; 5 µM) for 6 min incubation at 70 °C, and thereafter adding 3 µL RT_Premix_1 for incubation 1 h at 42 °C. Second strand cDNA synthesis was performed by adding the above mixture to 32.5 µL SS_Premix_1 for 2 h incubation at 16 °C then adding 1 µL T4 DNA polymerase (5 U/µL) for 10 min further incubation at 16 °C. The following double stranded cDNA cleanup was performed by ethanol precipitation overnight at -20 °C. Subsequently, cRNA was *in vitro* transcribed at 37 °C for 5 h using Ambion MEGascript T7 Kit (5 µL/reaction) and thereafter cleaned up with RNeasy columns (QIAGEN). This step gave 1.00 and 0.80 µg cRNA for controls and allergic patients, respectively. For the second cycle and the first strand cDNA synthesis of each sample, 1 µL random primers (0.2 µg/mL) was added to the cRNA (4 µL) for 10 min incubation at 70 °C. Thereafter the sample (400 ng of each group) was mixed with 5 µL RT_Premix_2 for 1 h incubation at 42 °C followed by 20 min treatment with 1 µL RNase H (2 U/µL) at 37 °C to remove the RNA template. Second strand cDNA synthesis was performed by mixing the sample with 2 µL T7-Oligo(dT) promotor primer (5 µM) for 6 min incubation at 70 °C then adding 62 µL SS_Premix_2 for incubation 2 h at 16 °C and further adding 2 µL T4 DNA polymerase (5 U/µL) for 10 min incubation at 16 °C. After that, double-strand cleanup was performed similarly as described above. Subsequent *in vitro* transcription and labelling at 37 °C for 4 h was performed using ENZO BioArray HighYield RNA Transcript Labeling Kit (40 µL/reaction) and thereafter cleaned up with RNeasy columns. This step gave 35 and 47 µg cRNA for controls and allergic patients, respectively.

Oligonucleotide array hybridization and scanning

Hybridization cocktails were prepared by mixing 15 µg fragmented cRNA, 50 pmol/L B2 control oligonucleotide (Genset), 1.5, 5, 25 and 100 pmol/L, respectively, of BioB, BioC, BioD and Cre control transcripts generated by *in vitro* transcription using the plasmids pGIKS-BioB, pGIKS-BioC, pGIKS-BioD and pGIKS-Cre as template (ATCC, Manassas, VA, USA), 0.1 mg/mL herring sperm, 0.5 mg/mL acetylated BSA, 1 × MES hybridization buffer (100 mmol/L MES, 1 M NaCl, 20 mmol/L EDTA, 0.01% Tween 20) in a final volume of 300 µL. The hybridization cocktails were denaturated at 99 °C for 5 min, transferred to 45 °C, incubated for 5 min and then centrifuged for 5 min to pellet debris. One HuGe133A and HuGe133B array was hybridized for each sample (Affymetrix). Pre-hybridization solution was exchanged with hybridization cocktail and hybridization was done for 16 h at 45 °C. Washing and staining was done using a fluidics station and a confocal scanner essentially according to the manufacturers instructions (Affymetrix). In brief, the hybridized probe array was washed and stained with

streptavidin phycoerythrin conjugate and scanned by the Hewlett-Packard (HP) GeneArray™ Scanner at the excitation wavelength of 488 nm. The amount of light emitted at 570 nm is proportional to bound target at each location on the probe array. A signal-amplification step was performed using biotinylated anti-streptavidin antibody and the array was scanned again.

Analysis of DNA microarray data

Scanned output files were visually inspected for hybridization artifacts and then analysed with Microsoft Array Suite (MAS) version 5.0 (Affymetrix). To allow comparison of gene expression between patients and controls the arrays were scaled to an average intensity of 500 (ref as usually). RNA expression was quantitatively estimated by computing the average difference between a set of oligonucleotides that perfectly matched the gene and a set of mismatch control oligonucleotides. The average difference value will subsequently be referred to as the gene expression level and given in arbitrary units. In addition, a qualitative estimate of gene expression was given by 'the absolute call'. This was obtained by an algorithm based on the signal intensity and quality of the average difference (Affymetrix). With the absolute call gene expression is classified as absent, marginal or present. Each probe set on the patient array was compared with its counterpart on the control array, and a Change *P*-value was calculated indicating an increase, decrease or no change in gene expression (Affymetrix).

A quantitative estimate of the difference in gene expression is given by the signal log ratio. The log scale used is 2 base. Thus, a signal log ratio of 1.0 indicates an increase of the transcript level by twofold and -1.0 indicates a decrease by twofold. A signal log ratio of zero would indicate no change.

Analysis of hybridization-, cDNA synthesis- and *in vitro* transcription quality of the DNA microarrays

To examine variability in hybridization quality in the DNA microarrays four control bacterial and phage gene cRNAs, BioB, BioC, BioD and Cre were analysed on each DNA microarray. Bio-B, C, D and Cre were all present according to the absolute call criterion. *In vitro* transcription- and cDNA synthesis quality were assessed by comparing 3' and 5' expression levels of actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the four DNA microarrays. Actin and GAPDH expression levels were checked and differed less than 30% between 3' and 5' regions of the transcripts.

Nasal fluid mediator assays

Uteroglobin levels were determined with ELISA according to the manufactures protocol (Biovendor, Brno, Czech Republic).

Statistics

The levels of nasal fluid uteroglobin in nasal fluids from patients and controls were compared using the non-parametric Mann-Whitney *U*-test.

Results

Analysis of gene expression in nasal biopsies from patients and controls

In this study expression of 44 927 genes were analysed. The total numbers of genes that were present according to the absolute call criterion were 17 353 in the controls and 17 928 in the patients. One thousand five hundred and seventy nine of the genes had higher expression in patients than in controls, and 1570 had lower expression in patients than in controls. Out of 189 up-regulated inflammatory genes, 187 were pro-inflammatory and two were anti-inflammatory. By comparison, out of 49 down-regulated genes, 36 were pro-inflammatory and 13 were anti-inflammatory (a complete list of regulated inflammatory genes is given in the online suppository: <http://www.oron.mas.lu.se/klinik/allergi.html>).

Up-regulated genes

The majority of these were pro-inflammatory, i.e. genes regulating the influx of Th2 cells, eosinophils and mast cells (chemokine receptors CXCR4 and CCR3), antigen presentation (MHC class I and II), the general activation of inflammatory cells (e.g. phosphodiesterase 4B), and of specific cells; T-lymphocytes (T-cell differentiation protein), B-lymphocytes (CD83, immunoglobulins), mast cells (the high-affinity IgE receptor, tryptase), eosinophils (Charcot–Leyden crystal protein) and neutrophils (defensin). Moreover, several Toll-like receptors were up-regulated indicating activation of the innate immune system. Cytokines and chemokines constituted the largest group, comprising 59 genes. These indicated increased activity of IL-1, TNF and interferons. The up-regulated genes also included genes with anti-inflammatory activity, i.e. two leucocyte immunoglobulin-like receptors (LIR), i.e. LIR subfamily B and paired LIR- α . (Table 1).

Down-regulated genes

Out of 235 differentially expressed inflammatory genes, only 49 were down-regulated. The largest single functional group among the down-regulated inflammatory genes were anti-inflammatory (Table 1). In this group uteroglobin differed most in expression. This gene encodes a protein that is abundant in airway secretions and has wide-ranging anti-inflammatory actions. It is also known as CC16. This protein was analysed in nasal fluids from the same patients and controls that were included in this microarray study. In controls, the amount of uteroglobin in nasal fluids was 12.93 ± 2.53 ng/mL ($n = 28$). The level of uteroglobin in nasal fluids from allergic patient was significantly lower 5.43 ± 1.53 ($n = 15$), $P < 0.05$ (Fig. 1). Mammaglobin B, another member of the uteroglobin family also had a lower expression in the patients.

Other down-regulated anti-inflammatory genes included several glutathione-S-transferases, and genes related to the transforming growth factor (TGF) family (i.e. TGF- β receptor III, activin A receptor, prostate differentiation factor and SMAD5).

Discussion

Allergic inflammation results from altered interactions between a large number of genes. It is not known how many of the 30 000–40 000 human genes that are involved in allergic disease. DNA microarray technology allows analysis of expression of all these genes. DNA microarray studies of cells or biopsies from patients with IAR, asthma and eczema have implicated several pro-inflammatory genes [4, 9, 10]. It is, however, also possible that decreased expression of anti-inflammatory genes may be involved. In a DNA microarray study of nasal biopsies from patients with IAR, decreased

Table 1. Differentially expressed anti-inflammatory genes

		Gene expression levels†		
Accession number*	Description	Patients	Controls	Log-ratio‡
Anti-inflammatory genes with higher expression levels in patients than in controls				
NM_005874.1	Immunoglobulin-like receptor, subfamily B	343	423	0.3
AJ400843.1	Paired immunoglobulin-like receptor-α	248	308	0.3
Anti-inflammatory genes with lower expression levels in patients than in controls				
NM_003357.1	Uteroglobin	271	630	− 1.1
NM_002407.1	Mammaglobin 2	79	147	− 0.8
AF010601.1	SMAD5	11	4	− 1.1
AW193698	Transforming growth factor-β receptor III	309	186	− 0.8
AF003934.1	Prostate differentiation factor	851	520	− 0.8
NM_001616.2	Activin A receptor, type II	168	98	− 0.4
NM_001512.1	Glutathione S-transferase (GST) A4	284	143	− 0.9
NM_000846.1	GST A2	642	222	− 0.8
BC001453.1	GST-ζ 1	81	59	− 0.7
NM_000852.2	GST-π	918	580	− 0.5
X08020.1	GST subunit 4	70	51	− 0.4
AI220117	GST 1	2104	1627	− 0.3

*Accession number to LocusLink (<http://www.ncbi.nlm.nih.gov/LocusLink/>).

†Gene expression levels are given in arbitrary units, based on the differences in signal intensities between of a set of perfect and mismatch probes for each gene.

‡Log-ratio between patient and control expression levels. The log scale used is 2 base. Thus, a signal log ratio of 1.0 indicates an increase of the transcript level by twofold and –1.0 indicates a decrease by twofold. A signal log ratio of zero would indicate no change.

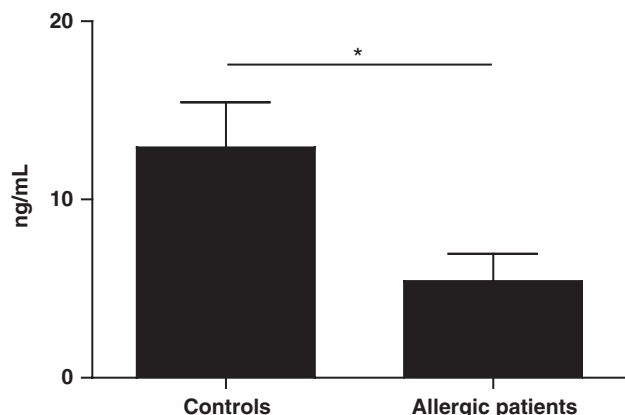


Fig. 1. Detection of uteroglobin levels in nasal lavage fluids from controls and allergic patients with ELISA ($n = 28$ and 15 , respectively). Data are expressed as mean with error bars representing SE mean. $*P < 0.05$.

activity of the anti-inflammatory gene TGF- β was found [5]. IAR is a common, accessible and well-defined disease that allows local and *in vivo* analysis of allergic inflammation. In this study, expression of some 44 000 genes were analysed in cells collected from nasal fluids from patients with active IAR and healthy controls. The aim was to identify new inflammatory genes with particular emphasis on anti-inflammatory genes.

There are several methodological concerns in such a study. Because of low amounts of RNA we could not examine individual samples. Therefore this study was performed in pooled RNA samples. Pooling implies that valuable information on individual variation is lost. Moreover, very large datasets are generated. These could include genes of both known and unknown relevance, and potentially new information about pathogenic mechanisms. On the other hand, the risk of spurious identification is great given the large number of genes. To some extent this may be compensated for by looking for groups of related genes that show coordinated changes in expression. The rationale behind this is that if many genes with similar function change in the same direction the likelihood of biological relevance increases. Another possibility is to analyse protein expression of selected genes in nasal fluids from individual patients and controls. This allows examination of individual samples and statistical analysis. The overall performance of the DNA microarrays were assessed by different control experiments; the hybridization quality was supported by the four control bacterial and phage gene cRNAs being present on all DNA microarrays; the quality of *in vitro* transcription- and cDNA synthesis were indicated by the 5' expression levels of actin and GAPDH being more than 70% of the 3' intensities. Although DNA microarrays have been validated with other methods [11] it should be emphasized that transcriptional responses may not be representative of protein expression patterns. Given these reservations our data still enable us to make some general observations regarding gene expression in nasal fluid cells. About 17 000 genes were expressed in these cells. Two hundred and thirty seven genes related to inflammation differed between patients and controls. The majority of these genes were pro-inflammatory and increased in expression. By contrast, the predominant group of down-regulated genes

was anti-inflammatory. The up-regulated genes regulated key steps of inflammation, ranging from leukocyte adhesion to immunoglobulin production. Some of the up-regulated pro-inflammatory genes were specifically related to allergy e.g. the high-affinity IgE receptor and tryptase that are expressed by mast cells, or the Charcot-Leyden crystal protein which is expressed by eosinophils. Moreover, the chemokine receptors CCR3 and CXCR4 that are expressed on Th2 cells, eosinophils and mast cells were up-regulated [12]. This is consistent with a Th2 like IgE mediated reaction. However, a large number of interferon-induced genes also showed increased expression. Similar observations have been made in asthma and eczema [13, 14], suggesting that natural allergic inflammation may not exclusively be of the Th2 phenotype.

The main focus of this work was to find anti-inflammatory genes that differed in expression between patients with allergic rhinitis and healthy controls. Interestingly, the main group of down-regulated genes had anti-inflammatory effects. This suggests that decreased activity of anti-inflammatory genes may contribute to the pathogenesis of IAR. This is the first study to show this on a genome wide scale. The gene that differed most in expression was uteroglobin, or CC16. This protein is secreted by Clara cells in the lungs and by nasal mucosal epithelial cells. Uteroglobin has wide-ranging anti-inflammatory effects, i.e. the inhibition of leukocyte chemotaxis, phospholipase A2 and pro-inflammatory cytokines as well as protease activity [15]. Peptides derived from uteroglobin are among the most potent anti-inflammatory agents identified to date [16]. Recently, a peptide of this kind was successfully tested in an animal model of allergic conjunctivitis [17]. The relevance of these anti-inflammatory effects is supported by decreased local expression of uteroglobin protein in asthma [18]. In a previous DNA microarray study we found lower expression in nasal polyps than in healthy nasal mucosa [6]. Nasal polyps have immunohistochemical similarities with allergic rhinitis and Lindahl et al. [19] have previously shown lower uteroglobin in nasal fluids from four children with allergic rhinitis compared with healthy controls. It was therefore of interest that we could confirm decreased levels of uteroglobin protein in nasal fluids from patients with allergic rhinitis. Mammaglobin B, another member of the uteroglobin family also had lower expression in the patients.

Other down-regulated anti-inflammatory genes included several glutathione-S-transferases, as well as genes related to the TGF family (i.e. TGF- β receptor III, activin A receptor, prostate differentiation factor and SMAD5). The glutathione-s-transferases protect the airways against toxic damage from reactive oxygen species during inflammation. These genes are highly polymorphic. Sequence variations in these genes have been associated with asthma, IAR and susceptibility for respiratory tract infections [20–23]. Decreased expression has previously been shown in a microarray study of nasal mucosa [24]. Interestingly, local treatment with glutathione improved symptoms in patients with allergic rhinitis [25].

The TGF family comprises several molecules that are constitutively expressed in human airways. TGF- β inhibits IgE synthesis, mast cell- and eosinophil proliferation. TGF- β may therefore have an important role in allergic inflammation [26, 27]. Decreased expression of TGF-related genes in this

study is consistent with data from a microarray study of nasal biopsies from patients with IAR [5].

In summary, DNA microarray analysis of nasal fluid cells indicate that decreased expression of uteroglobin and other anti-inflammatory genes may contribute to the pathogenesis of IAR.

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