

DNA microarrays to study gene expression in allergic airways

M. Benson, P. A. Svensson*, B. Carlsson*, M. Jernås*, J. Reinholdt†, L. O. Cardell and L. Carlsson*

Allergy Laboratory, Department of Oto-Rhino-Laryngology, Malmö University Hospital, Malmö, *Research Centre for Endocrinology and Metabolism, Sahlgrenska University Hospital, Gothenburg, Sweden, and †Department of Medical Microbiology and Clinical Immunology, Aarhus University Hospital, Aarhus, Denmark

Summary

Background Allergic rhinitis results from interactions between a large number of cells and mediators in different compartments of the body. DNA microarrays allow simultaneous measurement of expression of thousands of genes in the same tissue sample.

Objective To study gene expression in nasal mucosal biopsies from patients with allergic rhinitis using DNA micro-arrays.

Methods Nasal biopsies were obtained from 14 patients with symptomatic birch pollen-induced allergic rhinitis and five healthy controls. RNA was extracted from the biopsies and pooled into one patient pool and one control pool. These were analysed in duplicate with DNA micro-arrays containing more than 12000 known genes.

Results Approximately half of the genes were expressed in the patient and control samples. Guided by the current literature we chose 32 genes of possible relevance to allergic airway inflammation and investigated their relative expression. Among these, transcripts encoding immunoglobulins and their receptors were most abundant. The expression of cytokines and growth factors was low, whereas their corresponding receptors and cell surface markers displayed higher expression levels. IgA had the highest expression of all 12626 genes. RT-PCR showed that IgA1 was the predominant subclass. This was confirmed by the protein level in nasal fluids. Allergen-specific IgA was significantly higher in patients than in controls and correlated significantly with eosinophil granulae proteins.

Conclusion DNA micro-array analysis can be used to identify genes of possible relevance to allergic airway inflammation. In this study, the expression profile in the nasal mucosa was quantitatively dominated by immunoglobulins, particularly IgA. Protein analyses in nasal fluids indicated a role for allergen-specific IgA in eosinophil degranulation.

Keywords allergic rhinitis, DNA microarrays, gene expression

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Introduction

In recent years detailed understanding of mucosal responses in allergic rhinitis has been gained [1,2]. Cytokines from type 2 Th2 cells induce IgE-synthesis, as well as degranulation of eosinophils and mast cells. However, an increasing number of other cells and their products could be implicated in the pathogenesis of allergic rhinitis, e.g. other cytokines and immunoglobulins may contribute to degranulation of eosinophils, and growth factors protect the mucosa by maintaining epithelial integrity [3,4].

While most of the mediators have been described in the mucosa it is not clear to what extent they are produced locally, e.g. mucosal immunoglobulins may be synthesized locally but also be serum-derived [5–8]. Moreover, many mediators are synthesized when inflammatory cells differentiate in the bone marrow. It is also of note that inflammatory cells are not equally distributed between the nasal mucosa and lumen. Lymphocytes

predominate in the mucosa and eosinophils in the lumen [9]. Thus, the two compartments may have distinct immunological characteristics. Examination of gene expression in the nasal mucosa could contribute to understanding of the role of this compartment.

DNA micro-arrays consist of a matrix with attached DNA sequences that allow simultaneous analysis of expression of thousands of genes [10]. With the help of DNA microarrays great diversity of gene expression has been shown in activated T cells [11]. There are few DNA micro-array studies of human tissues. Using surgical material transcriptional responses in rheumatoid arthritis and colon cancer have been examined [12,13]. This could result in identification of novel disease-related genes. The most common approach is to look for genes with a twofold or higher difference in expression levels between two groups [14]. However, smaller differences can have pathogenetic relevance. Conversely, higher differences can be due to methodological or non-disease-related biological variation. Bioinformatic methods to interpret data from DNA microarray analyses are continuously developed, and have been described in large studies of malignant diseases [15,16]. As of yet, there are no published DNA microarray studies of

Correspondence: Mikael Benson, Allergy Laboratory, Department of Oto-Rhino-Laryngology, Malmö University Hospital, S-205 02 Malmö, Sweden.

tissues from patients with allergic diseases. Such studies are complicated by the difficulties in obtaining sufficient material. However, the recent development of protocols that use only a few micrograms of total RNA instead of mRNA may allow DNA microarray analysis of small biopsies obtained in clinical settings [17,18].

The aims of this study were to: (i) determine if DNA microarrays can be used to study gene expression in biopsies of nasal mucosa from patients with allergic rhinitis and healthy controls; (ii) obtain an estimate of the relative transcriptional levels of cytokines, growth factors, their receptors, cell surface markers, eosinophil and neutrophil granulae proteins, immunoglobulins and their receptors in the nasal mucosa from patients with allergic rhinitis and healthy controls; and (iii) compare transcriptional responses in the mucosa with proteins in nasal fluids.

Materials and methods

The study included 14 patients with symptomatic birch pollen-induced allergic rhinitis and five healthy controls. Both patients and controls were seen during the birch pollen season. The median (range) age of the patients and the controls were 40 (18–53) years and 35 (29–50) years, respectively. Eight patients and four controls were women. The diagnosis of birch pollen-induced allergic rhinitis was based on a positive history of seasonal allergic rhinitis and positive skin prick tests against birch pollen. All patients were symptomatic at the time of inclusion in the study. The controls were all symptom-free, had no history of allergic rhinitis or any other atopic disease, and had negative skin prick tests against a panel of allergens, including birch, timothy, mugwort, house dust mite (*Dermatophagoides pteronyssinus* and *D. farinae*), horse, dog, cat and moulds (*Cladosporium* and *Alternaria*). The study was approved by the Ethics Committee of the Medical Faculty, Lund University.

Biopsies were obtained as described from the nasal inferior turbinate, frozen immediately in liquid nitrogen, and then stored at -70°C [19].

Nasal lavage fluids were obtained as previously described [20]; 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 until 4 mL were recovered. The total number of cells/mL was computed in a Bürker chamber. The fluids were then centrifuged for 10 min at 1334g at 4°C . The supernatant was separated from the pellet. The pellet was used to prepare slides that were stained according to the May-Grünwald and Giemsa method for morphological assessment of cells in the fluid by two independent microscopists. The percentages of epithelial cells, eosinophils, neutrophils and mononuclear cells out of a total of 100 cells/slide were computed, and then the total number of each cell type/mL.

Preparation of cRNA

RNA was isolated using the RNeasy kit (Quiagen, Valencia, CA, USA). RNA concentrations were determined spectrophotometrically. The sample-by-sample weights of RNA in the patients and controls were 2.9, 3.2, 0.9, 1.3, 1.9, 1.2, 0.9, 2.3, 0.8, 1.4, 1.5, 1.0, 0.75, 0.9 µg and 1.5, 3.1, 2.7, 3.1, 4.6 µg, respectively. The RNA was pooled into one patient pool and

one control pool and used for target preparation. Each pool was divided and analysed in duplicates 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 Transcript Labelling 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 $1\times$ 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, and $1\times$ 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.

Oligonucleotide array hybridization and scanning

The hybridization cocktails were denatured at 99°C for 5 min, transferred to 45°C , incubated for 5 min and then centrifuged for 5 min to pellet debris. One HuGeU95A array was hybridized for each duplicate sample (Affymetrix, Santa Clara, CA, USA). Pre-hybridization solution was exchanged with hybridization cocktail and hybridization was carried out for 16 h at 45°C . Washing and staining was carried out 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) GeneArrayTM 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 antistreptavidin 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 GENECHIP 3.1 software (Affymetrix). To allow comparison of gene expression between patients and controls the arrays were scaled to an average intensity of 500 [10,11]. 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 (Fig. 1). 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.

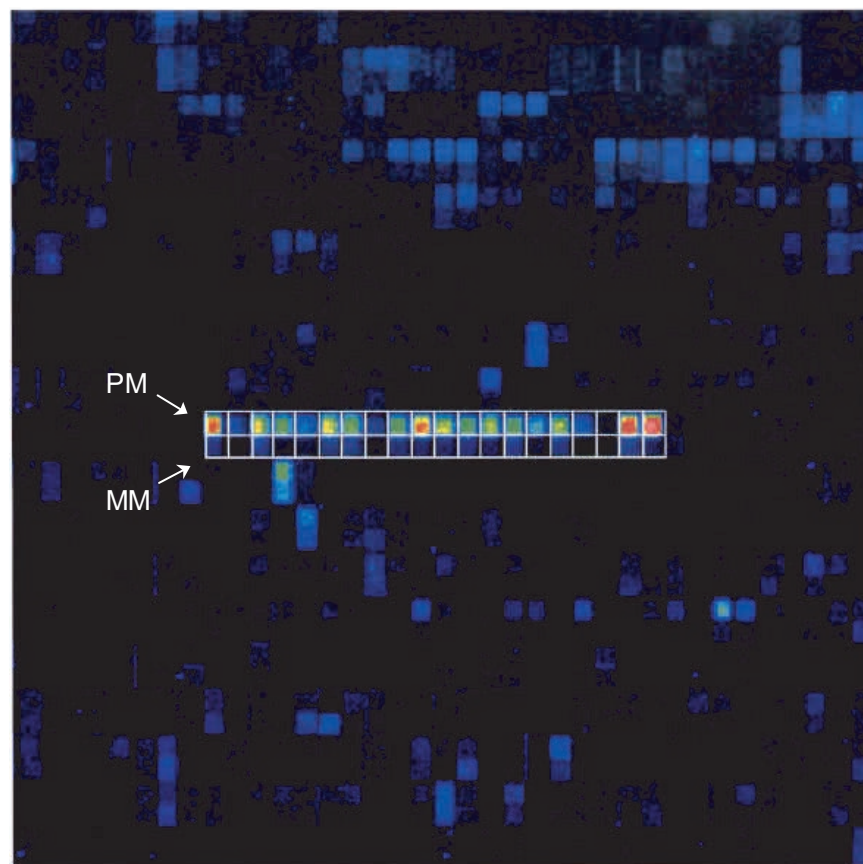


Fig. 1. Magnification of a DNA microarray after hybridization with RNA. Each cell represents an oligonucleotide. These are arranged in pairs consisting of one perfect match (PM) and one control mismatch (MM) oligonucleotide. A gene transcript is usually represented by 16–20 different probe cells. One such transcript is marked with a grid. The size of a probe cell is 24 μm and each cell contains millions of oligonucleotides. The colour intensities of the probe cells are proportional to their hybridization intensities (HuGeneFL DNA microarray, derived from a pilot study).

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

To examine variability in hybridization quality in the DNA micro-arrays, four control bacterial and phage gene cRNAs, BioB, BioC, BioD and Cre, were analysed on each DNA microarray. BioB, BioC, BioD 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.

Data mining strategy

Guided by current literature, transcripts of possible relevance to mucosal inflammatory responses were analysed: cytokines (IL-2, IL-4, IFN- γ , TNF- α) [1,2,7,20–24], their receptors (IL-2 receptor(R) γ , IFN-R α , IL-4R α , TNF-R1) [2,25], growth factors (heparin-binding epidermal growth factor (HB-EGF), transforming growth factor (TGF)- α , amphiregulin, epiregulin, betacellulin), their receptors, all of which belong to the c-erbB family of receptors (epidermal growth factor (EGF)-R, c-erbB2, c-erbB3) [3], cell surface markers (CD3, CD4, CD45, CD8, CD68, HLA-DR β), eosinophil and neutrophil granulae proteins (eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), myeloperoxidase (MPO) [26]), immunoglobulins (IgA, IgD, IgE, IgG) and their receptors (polymeric Ig-R, IgE-R, IgG-R) [4–6,8]. The identities of the analysed

transcripts were verified by BLAST analysis (<http://www.ncbi.nlm.nih.gov/BLAST/>) and by database searches in ExPASy Molecular Biology Server (<http://www.expasy.ch>). With the blast analysis, the DNA sequences from the GeneChip were compared with all other sequences that are available on the world-wide web. The Molecular Biology server gives descriptions of the transcripts both on the molecular and protein level.

Analysis of IgA subclasses by RT-PCR

For first strand cDNA synthesis 0.5 μg RNA was heat denatured and reverse transcribed using 0.5 μg random hexamers (Roche Diagnostics, Mannheim, Germany) and 20U AMV-reverse transcriptase (Promega, Madison, WI, USA) in AMV-reverse transcriptase buffer (Promega). PCR was done in Taq buffer (Roche Diagnostics), 1 μM of primers IgA-808 (5'-CTCAGGTGGTCCTTGAAC-3'; Genset, Paris, France) and IgA-217 (5'-AGTGTGACCTGGAGCGAA-3'; Genset), 50ng cDNA, dNTP (0.2mm each), using GeneAmp PCR system 9600 (Perkin-Elmer, Foster City, CA, USA). The thermocycler was programmed for an initial denaturation at 94°C (1min), followed by 30 cycles with 30s denaturation at 94°C, 30s annealing at 60°C and 60s elongation at 72°C. The expected size of the IgA1 and IgA2 PCR fragments was 380bp. PCR products, Eco RI-digested PCR products, Hinf I-digested PCR and a 1Kb DNA-ladder (Life Technologies, Gaithersburg, MD, USA) were separated on a 2% agarose gel containing ethidium bromide.

Nasal fluid mediator assays

IgE, granulae proteins and albumin These assays were obtained from Pharmacia & Upjohn Diagnostics AB (Uppsala, Sweden). Total IgE was determined with a competitive radioimmunoassay. IgE concentrations are given in kU/L (1 kU = 2.42 µg). Bet v 1- and bet v 2-specific IgE as well as ECP levels were determined with fluoroenzyme immunoassays. EDN, MPO and albumin were determined using competitive radioimmunoassays.

IgA Levels of total IgA, IgA1 and IgA2 were estimated by titration in calibrated enzyme-linked immunosorbent assays (ELISA), essentially as previously described [27].

In an assay for quantification of nasal IgA antibodies to birch allergen, wells intended to receive serial dilutions of test samples were coated with recombinant bet v 1, kindly donated by ALK-ABELLØ, Hørsholm, Denmark. The assay was calibrated by titration of purified S-IgA2 standard in other wells of the plate coated with IgA2 subclass-specific monoclonal antibody (Nordic, Tilburg, the Netherlands). After incubation with samples and standard, the plate was developed with α -chain-specific, peroxidase-conjugated rabbit antibodies. The adopted method of calibration, involving the binding of S-IgA2 standard to a subclass-specific monoclonal antibody of relatively low affinity ($K_a = 3.7 \times 10^7$) [28], proved advantageous by generating a standard titration curve parallel to that for bet v 1-reactive IgA antibodies in most test samples. Besides, antibodies could be estimated in gravimetric units (µg/L).

Statistics

The levels of nasal fluid eosinophils, neutrophils, ECP, EDN, Total IgA, IgA1, IgA2 and albumin were compared using the non-parametric Mann–Whitney *U*-test. The Spearman rank correlation test was used to determine if IgA1, IgA2 and bet v 1-specific IgA were correlated with ECP and EDN. *P*-values <0.05 were considered significant. As the nasal biopsies were pooled into one patient pool and one control pool, differences in gene expression were not tested statistically.

Results

Analysis of gene expression in nasal biopsies from patients and controls

The total numbers of genes that were present according to the absolute call criterion in the duplicate DNA microarray analysis of the patient sample were 5773 and 6477, respectively. The corresponding numbers in the controls were 5944 and 6544. Ninety per cent of the transcripts had identical absolute calls in the duplicate chips of both controls and patients. The expression level of each gene transcript was estimated by computing the average difference in hybridization intensity between a set of perfect and mismatch oligonucleotides (Fig. 1). The expression levels of the transcripts ranged from 20 to 117813 arbitrary units. The majority of these, approximately 90%, had expression levels lower than 1000. Genes associated with expression levels greater than 1000 included known high abundance genes, such as tubulin and ubiquitin. By contrast, mRNA for albumin, which is not expected to be expressed in the nasal mucosa, was absent in both patient and control DNA microarrays. Four

hundred and eighty-six genes had expression levels that were more than twofold higher in the patients than in the controls, and 416 genes had more than twofold lower expression levels in the patients.

Analysis expression of genes related to mucosal responses in nasal biopsies from patients and controls

Guided by current literature, 32 transcripts of possible relevance to mucosal inflammatory responses were analysed: cytokines, growth factors, their receptors, cell surface markers, eosinophil and neutrophil granulae proteins, immunoglobulins and their receptors [1–8,20–26]. A graphic overview of the expression levels of the transcripts in patients and controls is given in Fig. 2. The levels varied from 20 to 117813 arbitrary units. IgA had the highest expression level in patients and controls, 117813 and 114000, respectively. IgD, IgG and IgM, the polymeric immunoglobulin receptor and the IgG receptor also had high expression levels. The expression levels of IgE were <100 in both patients and controls, i.e. more than 1000 times lower than IgA. Both IgE and its receptor were absent according to the absolute call criterion. Most cytokines and growth factors, except TNF- α , were also absent. By contrast, their receptors were generally present, as were CD markers. Transcripts for neutrophil and eosinophil granulae proteins were absent in both patients and controls. The differences in gene expression between patients and controls were generally modest.

Nasal fluid protein levels and cell counts

In order to compare immunological responses in the nasal mucosa and lumen, nasal fluid proteins and cells were analysed. The differences in mucosal gene expression of IgA and IgE were similar to the corresponding proteins in nasal fluids; IgA was found in mg/L and IgE in µg/L. Total IgE was higher in patients than in controls ($P < 0.01$), but bet v 1- and bet v 2-specific IgE were not detectable in either patients or controls (Table 1). The median eosinophil counts in the patients were $1.65 \times 10^4/\text{mL}$ (0.1–8.3) and 0.0/mL (0.0–0.0) in the controls ($P < 0.001$). The corresponding figures for neutrophils were $6.8 \times 10^4/\text{mL}$ (0.1–21.3) vs. $0.08 \times 10^4/\text{mL}$ (0–20.0) in the controls ($P < 0.05$). In contrast to gene expression in the nasal mucosa, albumin was found in high concentrations (mg/L) in nasal fluids, and was significantly higher in patients than controls ($P < 0.01$). ECP, EDN and MPO were found in µg/L. ECP and EDN, but not MPO, were significantly higher in the patients (both $P < 0.01$, Table 1).

IgA and its relation to eosinophil degranulation.

Because of the high expression of IgA in both nasal mucosa and fluids and its possible role in eosinophil degranulation, IgA [4] was further analysed. To obtain an estimate of the expression level of IgA relative to the other mucosal genes, IgA was compared with all 12626 genes on the DNA micro-array genechip; IgA was the transcript with the highest expression level. RT-PCR analysis was performed to distinguish between IgA1 and IgA2 in the patients. This analysis showed that IgA1 was the predominant isoform (Fig. 3). In nasal fluids IgA1 levels were significantly higher than IgA2 ($P < 0.001$), but there were no differences between patients and controls. The concentrations of bet v 1-specific IgA were significantly higher in patients than

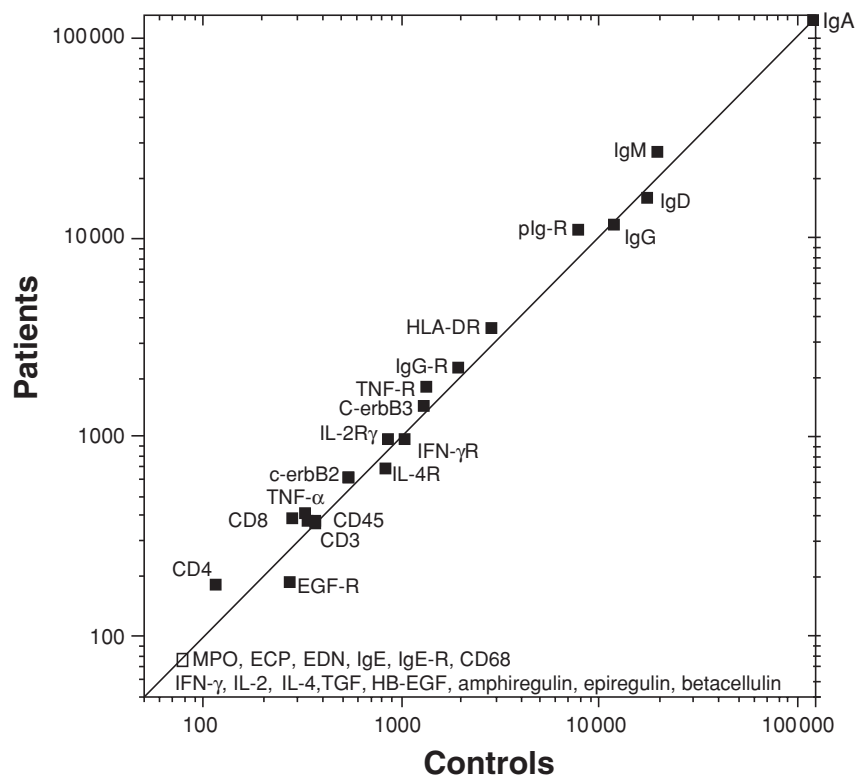


Fig. 2. Scattergram showing gene expression levels in patients and controls (filled squares). The open square indicates transcripts that were absent according to the absolute call criterion. The diagonal line is the line of identity. Abbreviations: ECP=eosinophil cationic protein, EDN=eosinophil-derived neurotoxin, EGF-R=epidermal growth factor receptor, HB-EGF=heparin-binding epidermal growth factor, IFN=interferon, IL=interleukin, IL-2R γ =IL-2 receptor γ , IFN- γ R=interferon- γ receptor, MPO=myeloperoxidase, pIg-R=polymeric immunoglobulin receptor, TGF=transforming growth factor, TNF-R=tumour necrosis factor receptor.

Table 1. Nasal fluid levels of MPO, ECP, EDN, IgE and IgA in patients and controls

	Controls	Patients
ECP (μ g/L)	5.0 (0–8)	31.7 (3–83)**
EDN (μ g/L)	12.8 (0–24)	158.0 (10–634)**
MPO (μ g/L)	66.2 (13–655)	167.5 (11–1000)
IgE (kU/L)	2.5 (2.3–2.8)	2.8 (2.4–5.2)**
Total IgA (mg/L)	31.7 (24.1–137)	79.6 (25.3–114)
IgA1(mg/L)	23.5 (19.8–111)	59.6 (23.1–116)
IgA2(mg/L)	5.5 (3.5–23.6)	8.4 (2.4–20.0)
Betv 1 IgA(μ g/L)	0.0 (0.0–0.0)	3.0 (0.0–32.6)**
Albumin (mg/L)	9.6 (5.2–114)	109.7 (10–393)**

Median (range) values are given. ** $P < 0.01$ compared with controls, Mann–Whitney U -test.

in controls ($P < 0.01$). To examine if IgA might contribute to eosinophil degranulation, correlation analyses between IgA1, IgA2, bet ν 1-specific IgA and ECP, EDN were performed. IgA1, but not IgA2 was significantly correlated to ECP ($r = 0.64$, $P < 0.01$) and to EDN ($r = 0.55$, $P < 0.05$). Significant correlations between bet ν 1-specific IgA and ECP ($r = 0.61$, $P < 0.01$) and EDN ($r = 0.55$, $P < 0.05$) were also found.

Discussion

Allergic rhinitis results from interactions between a large number of cells and mediators. These are synthesized in different compartments of the body, e.g. the bone marrow, lymph nodes and the nasal mucosa. These compartments could, by

inference, have different pathogenetic roles. The aim of this study was to examine gene expression in the nasal mucosa using DNA microarrays. This technique allows analysis of thousands of genes in the same sample. DNA micro-array analyses have been used to study gene expression in tissues from patients with rheumatoid arthritis and cancer [12,13], but not in allergic diseases. The studies of arthritis and cancer were performed on surgically removed specimens. Recent methodological improvements permit DNA microarray analysis of small biopsies obtained in clinical settings [17,18]. This led us to examine whether gene expression could be determined in biopsies of nasal mucosa from patients with allergic rhinitis and healthy controls. However, with current protocols the amounts of RNA obtained from our biopsies did not allow individual samples to be analysed. Therefore this study was performed in pooled RNA samples. It is possible that methodological alterations, e.g. adding a second round of *in vitro* transcription, would have increased the amount of RNA. Such alterations have to be balanced against every procedure in the DNA micro-array technology being a potential source of fluctuation. Further research is likely to provide improved protocols for cRNA synthesis and DNA micro-arrays with higher sensitivity [29,30].

Pooling of the samples might lead to the signals being confounded by mixed cell populations and individuals. However, the range and distribution of expression level values were similar to those found in DNA microarray studies of single cell lines [11,31]. Known high-abundance genes, such as β -tubulin and ubiquitin, had high expression levels in patients and controls, whereas cytokines had low values. The overall performance of the DNA microarrays was assessed by different control experiments: the hybridization quality was supported by the

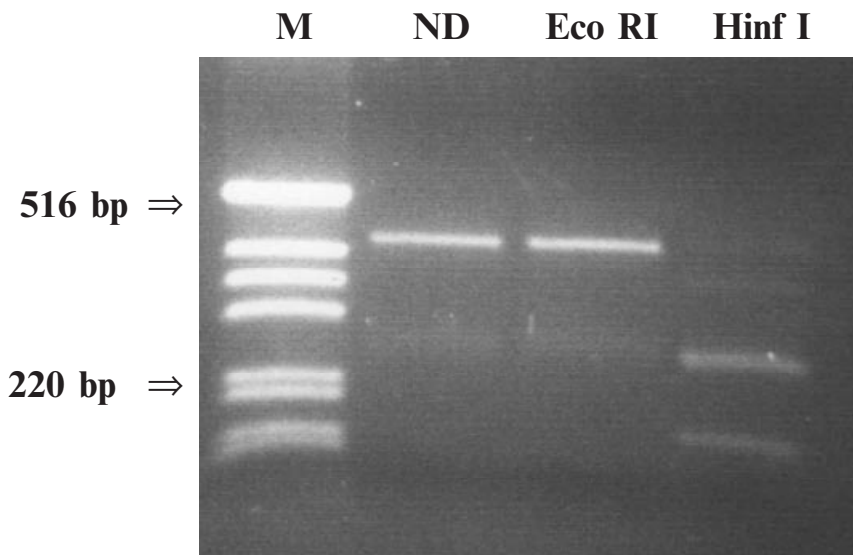


Fig. 3. RT-PCR analysis of IgA isoforms in pooled nasal biopsies from the patients with allergic rhinitis. The primers were designed to amplify both IgA1 and IgA2 transcripts (380bp). The restriction enzyme Eco RI specifically digests IgA2 PCR product and the restriction enzyme Hinf I specifically digests IgA1 PCR product. The analysis shows that IgA1 is the main expressed isoform. Abbreviations: M = molecular marker, ND = non-digested PCR product, Eco RI = Eco RI digested PCR product, Hinf I = Hinf I digested PCR product. The figures to the left of the DNA ladder indicate the 516 and 220 fragments of the molecular marker.

four control bacterial and phage gene cRNAs being present on all DNA microarrays; the quality of *in vitro* transcription and cDNA synthesis was 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 [11,31–33] it should be emphasized that transcriptional responses may not be representative of protein expression patterns. Moreover, very large datasets are generated. In this study approximately half of more than 12000 analysed genes were expressed. These could include genes of both known and unknown relevance, and potentially new information about pathogenetic mechanisms. Identification of such genes may be the most complicated part of DNA microarrays.

Three basic analytical steps have been described [10]. (i) Normalization or scaling of the data so that paired samples can be compared [11]. (ii) Identification of disease-related transcripts. The most common approach is to search for genes with twofold or higher differences in expression between two groups [14]. However, this is likely to result in many false negative and positive identifications. Pathogenetic genes can have lower differences in expression levels. Conversely, higher differences can be due to non-disease-related biological or methodological variation [14,34]. Thus, a search for pathogenetic genes among the 902 transcripts that differed more than twofold in this study would be likely to result in many fortuitous findings. Recently, statistical methods that separate disease-related and non-disease-related variation have been described based on a material with many individual samples [14]. (iii) Identification of gene expression patterns. This has been used for diagnostic classification of oncological materials [15,16]. Such applications could be very useful in allergy research, but also require many individual samples. To be able to do this, further improvement of protocols or sensitivity of the DNA microarrays are needed.

In this study the analysis was restricted to 32 genes considered relevant to mucosal responses [1–8,20–26]. It should be noted that this restriction involved exclusion of many genes of known relevance. However, the chosen genes allowed comparison with previous studies as well as with proteins in nasal fluids. This may be of particular interest for studies of allergic rhinitis. The

relevance of a transcript detected in pooled biopsies might be tried statistically after analysing the corresponding protein in a larger number of nasal fluid samples. In this report, group differences and correlations were analysed. In terms of biological effects it may also be important to make detailed studies of proteins, e.g. the allergen-specificity of immunoglobulins.

There were great quantitative variations in gene expression; the expression level of IgA in the patients was approximately 1000 times higher than that of IgE, which was not present according to the absolute call criterion. These proportions were similar to the concentrations of the corresponding proteins in nasal fluids. This could imply that mucosal gene expression and proteins in nasal fluids are closely associated. However, there were also differences between mRNA and protein expression; while albumin was absent in the mucosa, high concentrations were found in the nasal fluids from the patients. This can be explained by extravasation of albumin from the capillary bed to the nasal fluids [35]. Similarly, while transcripts for eosinophil and neutrophil granule proteins were absent in the mucosa, they were present in high protein concentrations in nasal fluids. This could depend on granule proteins being synthesized during differentiation of granulocytes in the bone marrow, rather than in the mucosa [36]. Moreover, granulocytes are more common in nasal fluids than in the mucosa, in which mononuclear cells predominate [9]. These findings highlight that allergic inflammation results from interactions between different compartments with different roles. The data in this study indicate that the nasal mucosa is an important source of immunoglobulins. This has been previously shown by immunohistochemical studies [5–8]. However, with the exception of IgE [5,7] this is the first report demonstrating local mRNA for immunoglobulins and their receptors.

Of all analysed transcripts IgA was the most highly expressed. The role of IgA in allergic inflammation is not clearly defined. Early studies indicated that atopy was associated with defective allergen elimination due to IgA deficiency [37]. This was not supported by later studies of IgA in serum, sputum and nasal secretions of allergic patients [38–40]. Moreover, IgA may induce eosinophil degranulation. This has been shown by

experimental studies and supported by correlations between IgA and ECP in nasal fluids from patients with allergic rhinitis, following allergen challenge [4,41]. Understanding of the possible role of IgA in allergic inflammation is complicated by the existence of two subclasses, IgA1 and IgA2. Immunohistochemical studies indicate that IgA1 predominates in the nasal mucosa [8]. This was confirmed in this study by RT-PCR. Recently this was also shown on the protein level, in nasal fluids from healthy subjects [42]. This is the first study to demonstrate that IgA1 also predominates in nasal fluids from patients with allergic rhinitis. It is not known if IgA1 and IgA2 have functional differences. In this study IgA1, but not IgA2, was significantly correlated with ECP and EDN. This could indicate a functional difference between the two subclasses in relation to eosinophils. However, this possibility is not supported by experimental data [43]. Moreover, the allergen specificity of the IgA is likely to be more important than the subclass. Bet v 1-specific IgA was significantly higher in nasal fluids from the patients than the controls, and correlated with both ECP and EDN. Given the abundance of IgA, the role of this antibody in activation of eosinophils and other granulocytes warrants further elucidation. It is also of note that this complexity highlights the need to combine molecular and protein studies to gain functional understanding of data derived from DNA microarrays.

The low expression of IgE mRNA in the nasal mucosa relative to other immunoglobulins probably reflects quantitative differences in immunoglobulin synthesis. Nasal fluid total IgE concentrations were low, and allergen-specific IgE not detectable. In addition to local synthesis, IgE may also be derived from serum or lymph nodes, and the relative contributions from these compartments remain to be defined [6]. Similarly, cytokines may be locally produced [1–3,7,21–24], or enter the nasal mucosa and fluids as proteins stored in granulocytes [44]. Expression of receptors for cytokines and growth factors in the mucosa was higher than their ligands. Similar proportions have been described previously, both in nasal mucosa and fluid [3,20]. The expression of cytokine receptors was similar in patients and controls. Previous studies of cytokine receptor expression have shown variable results [25,45,46]. A possible explanation could be that cytokine receptors are found on a wide variety of cells. The effects of the receptor may therefore depend more on its cellular distribution than its total quantity [2].

In summary, DNA microarray analysis may be used to analyse gene expression in the nasal mucosa. Quantitatively the transcriptional responses varied greatly, but were dominated by immunoglobulins and their receptors, particularly IgA. Complementary studies of nasal fluid proteins indicated that allergen-specific IgA might have a role in eosinophil degranulation. In combination with other methods, DNA microarray studies may yield new understanding of the pathogenetic programmes underlying allergic inflammation.

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