

Gene transcripts as potential diagnostic markers for allergic contact dermatitis

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The standard procedure for diagnosing allergic contact dermatitis is to perform a patch test. Because this has several disadvantages, the development of a new *in vitro* test system would be of immense value. Gene transcripts that distinguish allergics from non-allergics may have the potential to serve as the molecular basis for such a diagnostic tool. In this study, we use the microarray technology in the identification of differentially expressed genes in allergen-stimulated peripheral blood mononuclear cells (PBMCs) from 3 chromium-allergic patients versus 3 healthy controls. Using an Affymetrix GeneChip[®], the gene expression was analysed in PBMC cultures grown with 100 µg/ml CrCl₃ or in media alone for 24 hr. A total of 26 genes were differentially expressed by more than twofold ($P < 0.01$) in allergen-activated PBMCs from patients compared with controls. 18 of these were upregulated, whereas 8 were downregulated. The expression of 1 downregulated gene, CASP8, was also found specifically and significantly reduced in an expanded population including 4 additional chromium allergic patients and 1 additional control subject by real-time reverse transcriptase polymerase chain reaction (RT-PCR) analysis. The expression of 2 upregulated genes, ETS2 and CISH, correlated with a high-proliferative response following CrCl₃ exposure. Additionally, real-time RT-PCR analysis indicated that the same gene expression changes are valid for nickel allergics, potentially making the expression profile more widely available. The 26 differentially expressed genes identified in this study may potentially function as diagnostic markers for contact sensitivity.

Key words: allergic contact dermatitis; diagnostic gene transcripts; PBMCs. © Blackwell Munksgaard, 2005.

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Allergic contact dermatitis (ACD), both occupational and non-occupational, comprises a major health problem in the industrial world. The disease is characterized by local inflammation in the skin with particular involvement of hands, feet and face. Common allergenic substances are low-molecular weight compounds such as preservatives, dyes and metals.

Patients are diagnosed on the basis of exposure assessment and a positive epicutaneous patch test (1). However, because there are several drawbacks to this rather cumbersome method including risk for patient sensitization and subjective interpretation of the test result (2), the development of a fast and reliable *in vitro* test would be of good use.

The lymphocyte proliferation test has been proposed to comprise such an *in vitro* diagnostic method (3). However, there seem to be frequent overlap in test results between allergics and non-

allergics, and its value as a reliable diagnostic tool remains still to be proved (4). Interestingly, some studies show that the production and secretion of different cytokines in allergen-stimulated cultures is more useful, providing a better separation between allergic and non-allergic individuals. For example, it has been proposed that the allergen-induced *in vitro* production of interleukin-2 (IL-2), IL-5, IL-10 and interferon- γ may be used as diagnostic markers for contact allergy (5–8). In addition to cytokines, T cells are thought to express a wide range of other genes in response to allergen activation. In this study, we aim to identify unique gene transcripts in allergics versus non-allergics as a new approach in the development of an *in vitro* diagnostic test for ACD.

We introduce the use of microarrays in the investigation of allergen-activated peripheral blood mononuclear cells (PBMCs). The

technique is based on the hybridization process where labelled mRNA (or more correctly cRNA) is binding to complementary oligonucleotide sequences (probes) immobilized onto a solid support. By selecting probes that correspond to the genes of interest, the expression level of specific genes can be measured in a sample. An Affymetrix GeneChip contains a vast array of probes making it possible to measure the expression level of thousands of genes simultaneously, allowing an almost complete transcript profiling (9). Recently, microarrays have been used for the identification of transcript changes during the induction phase of allergic contact dermatitis (10).

In order to identify gene transcripts that distinguish chromium allergics from non-allergics, we stimulated PBMCs isolated from chromium allergic patients and controls with CrCl_3 for 24 hr followed by array analysis. In addition, we compared the differential expression of 3 selected genes in PBMCs from the chromium allergics to that of a nickel allergic.

Materials and Methods

Patients and controls

7 chromium allergic patients with chronic eczema and 7 volunteers with no history of contact allergy participated in the study after informed consent had been obtained. Both groups were equally represented by gender. All 14 participants were patch tested with 0.5% potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$; Sigma-Aldrich, Broendby, Denmark) and 13% chromium trichloride (CrCl_3 ; Sigma-Aldrich) in water.

Additionally, 1 nickel allergic patient and 2 controls were included in the study. All 3 participants were patch tested with 5% nickel sulphate (NiSO_4 ; Sigma-Aldrich) in water.

All patch tests were performed using 15 μl of each test solution in 8 mm Finn chambers[®]. The patch tests were applied on the back of the subject with Scanpor[®] tape and left for 48 hr, and readings were noted on day 2, 3 and 7. The reactions were scored according to the ICDRG scale (11). The study was approved by the local Ethical Committee.

Preparation of PBMCs

To avoid the impact of any positive patch test, blood samples were obtained before patch testing.

PBMCs were isolated from venous blood by density centrifugation on Ficoll-Paque[™] PLUS solution (Amersham Biosciences, Hilleroed, Denmark). The tubes were centrifuged at 800 *g* for 15 min at room temperature. After centrifugation, the granulocytes and erythrocytes were trapped in the lower

part of the tube, and a distinct layer of mononuclear cells was found in the upper part. The mononuclear cells were collected and washed 3 times with RPMI (Cambrex Bio Sciences, Vallensbaek, Denmark) at 250 *g* for 10 min.

Lymphocyte proliferation test

PBMCs (8×10^3 cells well⁻¹ when using PHA and 3×10^6 cells well⁻¹ when using CrCl_3) from patients and controls were stimulated with 2 $\mu\text{g}/\text{ml}$ phytohaemagglutinine (PHA) (Murex Diagnostics Limited, Dartford, England) and grown for 3–4 days or with 100 $\mu\text{g}/\text{ml}$ CrCl_3 and grown for 7 days. Corresponding control cultures in media alone were grown for 3–4 and 7 days. PHA is a non-specific mitogen included in the study to control that all cell cultures were viable and capable of proliferating. For the nickel study, PBMCs from the nickel allergic patient and the 2 controls were stimulated with 0.05 mM nickel sulphate and grown for 7 days.

T-cell proliferation was estimated by ³H-thymidine incorporation (5 $\mu\text{Ci}/\text{well}$ were added for the last 24 hr of incubation; Amersham Biosciences), which was measured by liquid scintillation counting. The proliferative response was reported as a stimulation index (SI), determined by dividing the counts per minute (cpm) of the stimulated cultures by the cpm of control cultures (PBMCs in media alone). A SI value of at least 2 indicates T-cell responsiveness.

Cell cultures for gene expression studies

For each subject, three 3×10^6 mill cells/well cultures were grown for 24 hr in 24-well plates in RPMI-1640 with antibiotics, 1% glutamine and 10% heat-inactivated autologous serum with either 100 $\mu\text{g}/\text{ml}$ CrCl_3 , 0.05 mM NiSO_4 or media alone. The cells were harvested and stored at -80°C .

DNA microarray

Total RNA was isolated using the GenElute[™] Mammalian Total RNA Kit from Sigma-Aldrich followed by DNase I (Amersham Biosciences) digestion, phenol/chloroform extraction and S-200 microspin purification. First strand cDNA was synthesized from 5 μg total RNA by incubation (42°C , 1 hr) in a 20 μl reaction volume containing 2.5 μM T7-(dT)₂₄ primer, 50 mM Tris-HCl pH 8.3, 75 mM KCL, 3 mM MgCl_2 , 10 mM DTT and 500 μM dNTP, 10 U/ μl Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Second strand cDNA was synthesized directly by adding 91 μl RNase free water, 30 μl 5 times second strand reaction buffer

(Invitrogen), 3 µl 10 mM dNTP, 1 µl *Escherichia coli* DNA ligase (10 U/µl), 4 µl *E. coli* DNA polymerase I (10 U/µl), 1 µl *E. coli* RNase H (2 U/µl) followed by incubation (2 hr, 16°C). The ends of the double-stranded cDNA were polished using T4 DNA polymerase (20 U, 5 min, 16°C). The cDNA was purified and concentrated by phenol/chloroform extraction and ethanol precipitation. Generation of biotin-labelled RNA was accomplished by *in vitro* transcription with the T7 RNA polymerase using the BioArray High Yield RNA transcript labelling kit (Enzo LifeSciences, Farmingdale, NY, USA). Biotin-labelled cRNA was subsequently purified from the transcription reaction using the RNeasy system (Qiagen, Hilden, Germany). Hybridization of biotin-labelled cRNA to HG-U133a GeneChips, washing, staining and scanning were performed according to the protocols published by the manufacturer (Affymetrix, Santa Clara, CA, USA). The HG-U133a GeneChip comprises more than 22000 probe sets corresponding to 14613 different human genes.

Real-time RT-PCR

Primers for CISH, CASP8 and ETS2 transcripts were planned using the PRIMER3 programme (12). The CISH primers (5'-CCCTCAACAAGG GGTCACTA-3' and 5'-CCTGGCCACCTGAA CTGTAT-3') amplify a 152 bp fragment of the CISH transcript defined by the GenBank entry NM_145071. The CASP8 primers (5'-GGGGCT TGATCTCAAAATGA-3' and 5'-AAGCAAAC CTCGGGGGATACT-3') amplify a 164 bp fragment of the CASP8 transcript defined by the GenBank entry NM_033355. The ETS2 primers (5'-CCTCTGCAGATTTCAGTTCA-3' and 5'-CAACAGGCTTGGATTCCATT-3') amplify a 236 bp fragment of the ETS2 transcript defined by the GenBank entry NM_005239.

For normalization of the data, GAPDH was measured using primers (5'-GGTTGAGCACAG GGTA CTTT-3' and 5'-GAGAGAGACCCTCA CTGCTG-3') amplifying a 160 bp fragment of the GAPDH transcript defined by the GenBank entry NM_002046.

1 µg total RNA was used for first strand cDNA synthesis in a 20 µl reaction containing 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 500 mM each of dATP, dCTP, dGTP, dTTP, 0.5 µg oligo-dT17, 10 U superscript reverse transcriptase (Invitrogen). The reaction was incubated at 42°C for 1 hr.

For real-time RT-PCR, the LightCycler FastStart DNA Masterplus SYBR green I system (Roche Diagnostica, Basel, Switzerland) was

applied. Reactions were assembled in LightCycler capillary tubes (Roche) and 1 µl of first strand cDNA synthesis reaction was used as template. For standardization, 1 µl of serial dilutions of the gel purified and sequence verified PCR products were used as templates in separate reactions.

Before each PCR fragment was used to quantify mRNA levels in unknown samples an optimization procedure was carried out. PCR was performed on first strand cDNA (1 µl) with the planned primers using 1 U Taq DNA polymerase (Fermentas UAB, Vilnius, Lithuania) in a 50 µl reaction volume. The products were analysed on a 3% agarose gel and their expected sizes confirmed. The correct sized DNA fragments were subsequently extracted from the gel by PerfectPrep DNA extraction kit (Eppendorf, Hamburg, Germany) and their identities confirmed by sequencing using the BigDye[®] Terminator v3.1 Cycle Sequencing Kit and the Avant 3100 Genetic analyser (Applied Biosystems, Foster City, CA, USA). These sequence verified and gelpurified DNA preparations were then used as templates in the LightCycler procedure. Initially 10-fold serial dilutions were used to determine the range where no primer – dimer products were produced for each fragment. Dissociation curves were performed, and the products from the LightCycler capillaries were spun out and analysed on a 3% agarose gel to verify their sizes again, also in the LightCycler settings. This optimization procedure allowed determination of the lower detection limit for each PCR fragment.

The significance of differences was analysed on log-transformed data with a two-sample *t*-test.

Analysis of microarray data

Normalized expression measures from the scanned GeneChips were obtained using the robust multiarray analysis (RMA) procedure (13), which has been demonstrated to be very efficient in detecting small differences in expression levels. The calculations were performed using the implementation of RMA provided by the open source bioconductor project (<http://www.bioconductor.org>).

Before performing an unpaired *t*-test comparing patients and controls, the expression measures in the unstimulated cell cultures were subtracted from the CrCl₃-activated cultures to correct for any background gene expression. It has been demonstrated that 50% of all genes display less than 1.14-fold difference in expression level when applying the RMA procedure to replicate GeneChip experiments using the same mRNA for probe synthesis (13). As a safety measure, we therefore considered all genes that displayed less than a

twofold average difference as unchanged. A list of 234 genes with more than a twofold difference in their average induction between patients and controls was generated, and *t*-tests subsequently performed. None of the *t*-tests resulted in *P*-values that could be considered significant if a conservative bonferroni procedure for multiple tests was applied. It is, however, generally accepted that the bonferroni procedure is overly restrictive for use in microarray data (14). As an alternative, we calculated *q*-values using the procedure described by Storey (15, 16). The *q*-value gives a measure of the maximal false discovery rate for the significant *t*-tests at a specified significance level. 26 genes had a significantly different ($P < 0.01$) fold induction. The *q*-value for the 26 genes was less than 9%, thus more than 90% of the detected differences are likely to be true.

Results

Patch test results

The 7 chromium allergic patients all elicited an allergic reaction (minimum 1+) in response to both chromium compounds. In 3 of the volunteers, an irritative reaction to 1 or both chromium compounds was observed and these were therefore excluded from the study.

The nickel allergic patient had a 2+ reaction to the 5% nickel sulphate, whereas no reactions were observed in the 2 controls.

Lymphocyte proliferation test

The degree of chromium-stimulated DNA synthesis by PBMCs obtained from chromium allergies and corresponding controls is shown in Table 1. In 4 patients, the PBMCs had a strong proliferative response to chromium stimulation (SI: 8–281). In 3 patients, only a tendency towards proliferation was observed in the presence of chromium (SI: 1.5–4). In the control group, none of the 4 participants had any proliferative response in the presence of chromium (SI = 1).

PBMCs from both patients and controls had a stimulation index of at least 2 when stimulated with the non-specific mitogen PHA. This showed

that the cell cultures were viable and capable of proliferating *in vitro*. The high degree of variation in the proliferative response to PHA is due to differences in incubation time (3 or 4 days) and big variations in the background proliferation in the unstimulated cell cultures.

PBMCs from the nickel allergic patient responded strongly (SI = 22), whereas no response were observed in cultures from the controls (SI = 0.5–1).

Subjects qualifying for the Microarray analysis

The 3 most chromium-responsive PBMC cultures were selected among the patients (P1, P2 and P5) to be used in the microarray analysis. 3 of the 4 controls with no clinical reaction, and no *in vitro* chromium response were included in the microarray analysis (C1, C2 and C3).

Microarray

A total of 26 genes exhibited a more than twofold difference in expression in CrCl₃-activated PBMCs from patients compared with controls ($P < 0.01$). Among the 26 genes, 18 displayed increased activity and 8 displayed decreased activity. The 26 genes are separated into 5 functional groups according to the Gene Ontology Consortium (<http://www.geneontology.org/>) (Table 2). 1 gene, ETS2, was detected with more than 1 probe set. The expression measures derived from the different probe sets for ETS2 were in good agreement (Table 2).

Even though the lymphocyte transformation test showed no activation of the PBMCs from controls, CrCl₃ may still have some subtle effect on the cells, which might be reflected in the gene expression profile. However, when performing a paired *t*-test comparing CrCl₃-stimulated and unstimulated PBMCs from controls with a significance level of 0.01, no genes was identified as being differentially expressed based on the twofold change criteria. Thus, the PBMCs from controls grown in the presence of CrCl₃ may be considered to reflect unstimulated cells.

Table 1. Stimulation index for CrCl₃- and phytohaemagglutinine (PHA)-stimulated peripheral blood mononuclear cells from chromium allergic patients (P1–P7) and control subjects (C1–C4)

	P1	P2	P3	P4	P5	P6	P7	C1	C2	C3	C4
SI (CrCl ₃ -stimulated cells)	281	12	1.5	4	28	2	8	1	1	1	1
SI (PHA-stimulated cells)	6	352	4	22	44	2	ND	6	3	7	3

CrCl₃- or PHA-induced lymphocyte DNA synthesis in PBMCs cultures. DNA synthesis was measured by [³H]thymidine incorporation and expressed as a stimulation index (SI), which was obtained by dividing the mean counts per minute (cpm) in stimulated cells by the mean cpm in unstimulated cells. The subjects indicated with bold are included in the microarray study. ND = Not done.

Table 2. List of genes that exhibited a more than twofold difference in expression ($P < 0.01$) in CrCl_3 -stimulated peripheral blood mononuclear cells (PBMCs) from patients compared with controls

GenBank accession number	Gene name	Gene symbol	Direction of regulation	Fold change
Immune response/inflammatory response				
NM_007115	Tumor necrosis factor, alpha-induced protein 6	TNF, AIP6	↑	3.64
NM_005849	Immunoglobulin superfamily, member 6	IGSF6	↑	2.09
NM_000417	Interleukin-2 receptor, alpha	IL-2RA	↑	2.03
NM_001465	FYN-binding protein (FYB-120/130)	FYB	↓	-2.10
NM_016562	Toll-like receptor 7	TLR 7	↓	-2.30
NM_000570	Fc fragment of IgG, low-affinity IIIb, receptor for (CD16)	FCGR3B	↓	-3.60
Cell growth/maintenance				
NM_002438	Mannose receptor, C type 1	MRC1	↑	4.21
NM_002648	Pim-1 oncogene	PIM1	↑	2.97
NM_005239	v-ets erythroblastosis virus E26 oncogene homologue 2	ETS2	↑	2.23
NM_005239	v-ets erythroblastosis virus E26 oncogene homologue 2	ETS2	↑	2.18
NM_006185	Nuclear mitotic apparatus protein 1	NUMA1	↓	-2.80
Apoptosis				
NM_000657	B-cell CL lymphoma 2	BCL2	↑	2.23
NM_001228	Caspase 8, apoptosis-related cysteine protease	CASP8	↓	-2.10
Metabolism and synthesis				
NM_005525	Hydroxysteroid (11-beta) dehydrogenase 1	HSD11B1	↑	3.03
NM_003364	Uridine phosphorylase 1	UPP1	↑	2.92
NM_004527	Mesenchyme homeo box 1	MEOX1	↑	2.91
NM_012413	Glutaminyl-peptide cyclotransferase (glutaminyl cyclase)	QPCT	↑	2.88
NM_003679	Kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	KMO	↑	2.80
NM_006406	Peroxiredoxin 4	PRDX4	↑	2.00
NM_003032	Sialyltransferase 1	SIAT1	↓	-2.00
NM_025080	Asparaginase-like 1	ASRGL 1	↓	-2.40
Cell communication/signal transduction				
NM_145071	Cytokine inducible SH2-containing protein	CISH	↑	4.20
NM_003877	Suppressor of cytokine signalling 2	SOCS2	↑	3.80
NM_000632	Integrin, alpha M, CD11b	ITGAM	↑	2.45
NM_003149	src homology three (SH3) and cysteine rich domain	STAC	↑	2.22
NM_003745	Suppressor of cytokine signalling 1	SOCS1	↑	2.13
NM_001619	Adrenergic, beta, receptor kinase 1	ADRBK1	↓	-2.10

26 genes were identified as being differentially expressed ($P < 0.01$) in allergen-activated PBMCs from chromium-allergic patients compared with healthy controls. The genes have a fold change equal to or higher than 2. The genes are segregated into 5 functional groups according to the Gene Ontology Consortium (<http://www.geneontology.org/>).

CASP8 mRNA expression is significantly decreased in PBMCs from 7 allergics following chromium exposure

To confirm the expression data obtained using the microarray technology in a larger patient population, we included 4 additional patients (P3, P4, P6 and P7) and 1 additional control subject (C4) for analysis by real-time RT-PCR. 3 genes were selected for the real-time RT-PCR analysis: ETS2 and CISH, which were found upregulated and CASP8, which was found down-regulated in the microarray study. A

housekeeping gene, GAPDH, was also measured for normalization of the data. The CASP8 mRNA level was the most sensitive marker for CrCl_3 exposure as it demonstrated a significant ($P = 0.02$) reduction in the mean expression level in the allergic patients compared with control subjects (Fig. 1). There was a non-significant increase in the mean expression for the ETS2 and CISH in allergic patients following CrCl_3 exposure compared with controls. Interestingly, for the ETS2 and CISH transcripts the expression level correlated with the SI value (correlation coefficient: CISH: $r = 0.94$

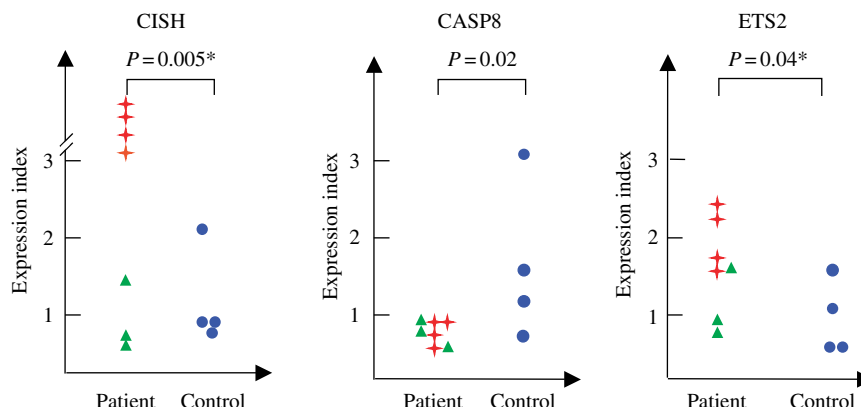


Fig. 1. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) of CISH, CASP8 and ETS2. The expression level of each gene is given as an expression index (the mRNA level in CrCl_3 -stimulated cell cultures divided by the mRNA level in unstimulated cell cultures). The patient group is divided into (+) patients showing a high response in the proliferation test (SI: 8–281) and ($\blacktriangle$) patients showing a low response in the proliferation test (SI: 1.5–4). The 4 non-allergic controls are represented by ($\bullet$) CASP8 is significantly downregulated when comparing all 7 chromium allergic patients to the 4 controls ($p = 0.02$).

*For CISH and ETS2, the differential expression between the patient and control group is significant only when excluding the three patients with a low-proliferative response.

($P = 0.056$); ETS2: $r = 0.69$ ($P = 0.31$)). Accordingly, if only the patients with a SI above 4 are included in the analysis, the mean expression levels of ETS2 and CISH are significantly ($P < 0.05$) increased in allergic patients compared with controls (Fig. 1).

The expression changes of all 3 selected genes, CASP8, ETS2, CISH, were also valid for the nickel allergic patient compared with the 2 controls (Fig. 2).

Discussion

The diagnosis of allergic contact dermatitis is presently based on a positive patch test and a

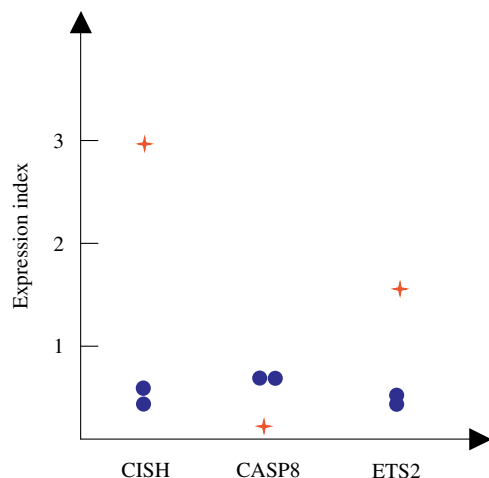


Fig. 2. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) of CISH, CASP8 and ETS2 in peripheral blood mononuclear cells (PBMCs) from 1 nickel allergic patient (+) and 2 controls ($\bullet$). The gene expression in the nickel allergic patient follow that of chromium allergies in that CISH and ETS2 is upregulated and CASP8 is down regulated when compared with controls.

clinical history. The patch test, however, holds distinct disadvantages including both false-negative and false-positive reactions, risk for sensitization and interpretative difficulties (2). The development of a new *in vitro* diagnostic assay would be of considerable value and may potentially, in combination with exposure assessment, supplement or even replace the epicutaneous test.

The *in vitro* test most often used to detect T-cell sensitization to allergens is the proliferation test, which measures ^3H -thymidine uptake by dividing cells. However, the usefulness of this test for diagnosing contact allergy has been debated because many groups have reported a high degree of subclinical reactivity in healthy controls (4, 17). We hypothesized that the identification of various gene transcripts, expressed in allergen-activated PBMCs from patients, might form the basis for the development of a sensitive and specific *in vitro* diagnostic assay.

In this study, unique gene transcripts in allergen-activated PBMCs from allergics versus non-allergics were identified after 24 hr. The identified differential transcriptome is in correlation with what is expected because it reflects processes coupled to PBMC activation such as components of the immune system, cell division and increased metabolism. In contrast, early pro-inflammatory cytokines have peaked at an earlier time-point and are therefore not identified in this study. Instead, the gene expression profile begins to reflect some of the repressive mechanisms that ultimately result in the termination of the inflammatory response. This is demonstrated by the upregulation of 3 members of the SOCS family (SOCS1, SOCS2 and CISH) (Table 2).

Using real-time RT-PCR, transcript changes were confirmed in the patients and controls participating in the array study. The selected genes were also investigated in PBMCs from one additional chromium allergic with a high-proliferative response, 3 chromium allergics with a low-proliferative response and a new control. The CASP8 transcript was found to be downregulated in PBMCs from all allergic subjects following CrCl₃ exposure. This reduction was significant and specific to allergic patients. It also appears that for at least PBMCs showing a high proliferative response (SI above 4), the expression of the 2 other investigated genes, ETS2 and CISH, was significantly increased following CrCl₃ exposure.

Additionally, real-time RT-PCR analysis indicated that the same gene expression changes were valid for nickel allergics, potentially making the expression profile more widely available.

The 26 differentially expressed genes identified in this study may potentially function as diagnostic markers for contact sensitivity. Even though their individual expression pattern may not provide an entirely consistent outcome, the combination of expression changes for numerous candidate genes may enable reliable discrimination between allergics and non-allergics. Through further studies, the genes providing the highest degree of diagnostic sensitivity and specificity should be identified. Also, in addition to chromium and nickel, other allergens need to be thoroughly assayed to see whether the transcript changes is valid for other contact sensitizers. Whether the gene changes are due to allergen-specific transcription or to the general processes activated in relation to increased lymphocyte turnover cannot be determined with the current experimental set-up. Distinguishing between the 2 possibilities is, however, not of major importance in relation to the diagnostic potential of the gene transcripts. Both unspecific and allergen-specific gene changes induced only in allergen-stimulated PBMCs from patients may allow separation between allergics and non-allergics.

Finally, an important issue arising in the discussion of *in vitro* tests is the fact that they, in contrast to *in vivo* tests, only detect a sensitization but do not predict whether the sensitization leads to clinical reactions. Thus, the clinical relevance of detecting allergen-induced expression changes should be thoroughly investigated.

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