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Gene expression changes in peripheral blood-derived dendritic cells following exposure to a contact allergen

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Abstract

A critical step in the induction of allergic contact allergy is the activation and subsequent migration of Langerhans cells (LC), an important antigen presenting dendritic cell (DC) of the skin. As the Langerhans cells migrate, they undergo a maturation process. It has been proposed that contact allergen exposure can induce DC maturation. While changes in DC gene expression profiles induced by various maturation stimuli have been explored, there are no published reports describing genomic-scale analysis of the changes induced by chemical allergen exposure. Therefore, to explore the concept of chemical allergen-induced DC maturation and to identify genes that are regulated by exposure to allergens we examined, at the transcriptional level, the effects of exposure to a contact allergen on DC. Peripheral blood-derived DC were exposed for 24 h to either 1 mM or 5 mM dinitrobenzenesulfonic acid (DNBS). Changes in gene expression were analyzed using Affymetrix U95Av2 GeneChip®. Comparison of mean signal values from replicate cultures revealed 173 genes that were significantly different ($P \leq 0.001$) between 1 mM DNBS treated and untreated control DC and 1249 significant gene changes between 5 mM DNBS treated and control DC. Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) was used to evaluate the observed transcript changes for selected genes in DC derived from a second donor. Comparison of the fold-changes in transcript levels between the two platforms and donors revealed a good correlation in both direction and magnitude. RT-PCR analysis was also used to assess the allergen specificity of a selected number of genes in DC derived from a third donor. Many of the gene expression changes were found to be induced only by exposure to the allergen, DNBS, and not by exposure to a structurally similar non-allergen, benzenesulfonic acid. A number of gene expression changes induced by allergen exposure were found to be consistent with what is known of the DC maturation process, and thus provide support for the theory of contact allergen-induced DC maturation. Additionally, it is hoped that some of the transcript changes identified through this approach will be shown to be suitable for use in the development of an in vitro predictive assay for contact sensitization.

Keywords: Contact allergy; Dendritic cells; Gene expression; In vitro method; Dinitrobenzenesulfonic acid

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1. Introduction

Dendritic cells (DC) are a distinct group of leukocytes characterized by their unique morphology and their ability to initiate immune responses by processing and presenting antigens. Subpopulations of DC reside both in the epidermis and dermis of normal skin (Maurer and Stingl, 1999; Shortman and Liu, 2002), each perhaps serving distinct antigen presenting functions (Allen et al., 2003). Langerhans cells (LC), the principal DC residing in the epidermis, were the first DC to be identified, and they typify the sentinel role of immature DC (Banchereau and Steinman, 1998). LC are known to play a key role in the development of allergic contact dermatitis (ACD). Following an encounter with a chemical allergen, LC become activated and subsequently migrate from the skin to the draining lymph nodes, undergoing a maturation process during the journey (reviewed in Kimber and Cumberbatch, 1992). Among the changes reported to occur in LC as a result of hapten exposure are the internalization of surface MHC class II molecules via endocytosis (Becker et al., 1992a,b; Girolomoni et al., 1990), the induction of tyrosine phosphorylation (Kühn et al., 1998) and the modulation of cell surface markers (Aiba and Katz, 1990; Schwarzenberger and Udey, 1996; Verrier et al., 1999) and cytokine expression (Enk and Katz, 1992; Wang et al., 2002). Since only limited numbers of LC can be obtained from skin for experimental use, many investigators have employed DC generated from peripheral blood mononuclear cells (PBMC) as surrogate LC in efforts to develop an in vitro model for contact sensitization (Aiba et al., 2000; Degwert et al., 1997). These PBMC-derived DC have been shown to respond to chemical allergens in a manner similar to LC and suggestive of maturation, such as demonstrating a modulation of receptor-mediated endocytosis (Becker et al., 1997), exhibiting changes in cell surface markers (Aiba et al., 1997; Coutant et al., 1999), and cytokine production (Aiba et al., 1997). While there is no doubt that contact allergens do induce important changes in the phenotype and function of DC, to date, there are no published reports describing genomic-scale analysis of these changes. Therefore, to explore further the concept of chemical allergen-induced DC maturation and perhaps identify novel genes that are regulated following the interaction of chemical allergens with cultured DC, we examined, at the transcriptional level, the effects of exposure to a contact allergen on DC. Additionally, information gained by transcript profiling may prove useful in the development of endpoint measures that can serve as the basis for an in vitro method to identify potential skin sensitizing chemicals.

2. Materials and methods

2.1. Generation of peripheral blood-derived dendritic cells

Enriched human leukocyte preparations were purchased from Sera-Tec Biologicals (North Brunswick, NJ). Following dilution with an equal part of complete medium (RPMI-1640 containing 1× L-glutamine, 1× penicillin–streptomycin antibiotic mixture (GIBCO, Rockville, MD), 30 μM 2-mercaptoethanol (GIBCO) and 10% heat inactivated fetal bovine serum (Hyclone Laboratories, Logan, UT) the leukocytes were separated over Ficoll-Paque gradient (Amersham Pharmacia Biotech, Uppsala, Sweden). The peripheral blood mononuclear cell fraction was collected, washed and counted using a Coulter Counter (Beckman-Coulter Inc., Miami, FL). The PBMC concentration was adjusted to 5×10^6 cells/ml with complete medium and 30 ml of the cell suspension was plated in T75 flasks, corresponding to a density of 2×10^6 cells/cm^2. Following a 2 h incubation at 37 °C/5% CO_2, the non-adherent cells were removed. Ten milliliter complete medium containing 10 ng/ml granulocyte-macrophage colony stimulating factor (GM-CSF; R&D Systems, Minneapolis, MN) and 10 ng/ml interleukin (IL)-4 (R&D Systems) was added to the remaining adherent cells in the flask. The cultures were incubated at 37 °C/5% CO_2 for 48 h during which the adherent cells became loosely or non-adherent. On day 2, the cells were collected, centrifuged, resuspended at a concentration of 1×10^6 cells/ml in fresh complete medium containing GM-CSF and IL-4 and re-plated. On day 5, residual T- and B-cells were removed from the cultures using two passages over CD2 (pan T-cell) and CD19 (pan B-cell) immunomagnetic beads (Dynal, Oslo, Norway) according to the manufacturer's directions. Following depletion of T- and B-cells, the remaining DC were re-suspended to concentration of

1×10^6 cells/ml in fresh complete medium containing GM-CSF and IL-4 and re-plated in a T75 culture flask. The cultures were incubated for another 48 h before experimental use. As previously described (Hulette et al., 2002), DC cultured for a total of 7 days in this manner express an immature DC phenotype: HLA-DR$^+$, CD1a$^+$, CD83$^-$, CD80^{lo}, and CD86^{lo} (data not shown). Alternatively, DC were derived from elutriated human monocytes (Advanced Biotechnology Inc., Columbia, MD) that were cultured in the same manner as the PBMC. The phenotype of these monocyte-derived DC at day 7 was found to be identical to that of the DC cultured from the enriched human leukocyte preparations (data not shown).

2.2. *Allergen treatment of DC*

2,4-Dinitrobenzenesulfonic acid, sodium salt (DNBS; 99%; Aldrich, Milwaukee, WI), a water soluble analog of the strong experimental contact sensitizer dinitrochlorobenzene (DNCB), was used as the test allergen. Working solutions of 3 mM and 15 mM DNBS were prepared in complete medium with GM-CSF and IL-4 and filter sterilized. After 1 week of culture, the DC were collected, washed and resuspended at a concentration of 1.5×10^6 cells/ml in complete medium containing GM-CSF (10 ng/ml) and IL-4 (10 ng/ml). Two milliliter of the cell suspension (3×10^6 DC) was plated in the wells of 6-well culture plates (Falcon; Becton Dickinson Labware, Franklin Lakes, NJ). Then, either 1 ml of complete medium with GM-CSF and IL-4 or 1 ml of one of the DNBS working solutions was added to the wells, resulting in final in-well concentrations of 1 mM and 5 mM DNBS. Replicate cultures were plated for each experimental group; control (untreated) and each allergen-treatment (1 mM and 5 mM DNBS). The cultures were incubated for 24 h at 37 °C/5% CO_2. DC viability following allergen treatment was assessed by propidium iodide dye exclusion using a Coulter Epics® XL flow cytometer (Beckman Coulter, Miami, FL). The viability of the control (untreated) DC and DC exposed to 1 mM DNBS was greater than 99% while exposure to 5 mM DNBS was slightly cytotoxic, resulting in a DC viability of approximately 85%. To determine whether or not some of the significant gene changes were differentially regulated by exposure to allergen, DC derived from elutriated human monocytes were exposed to medium alone, 1 mM and 5 mM DNBS, or 1 mM and 5 mM benzenesulfonic acid (sodium salt, 98%; BSAc; Aldrich), a non-allergen that is structurally similar to DNBS. Working solutions of BSAc were prepared and the DC treated in a manner similar to that of DNBS. The viability of DC exposed to either concentration of BSAc was greater than 97%.

2.3. *RNA isolation*

Following treatment, the DC were centrifuged and the resulting cell pellet was resuspended in TRIzol® reagent (GIBCO) for total RNA preparation following the manufacturer's instructions. Pellet Paint™ NF (Novagen, Madison, WI) was used as a co-precipitant to aid in the recovery of the RNA. The resulting total RNA was purified further using RNeasy Mini Kit (QIAGEN, Valencia,CA). The RNA content was determined spectrophotometrically and the quality of RNA was confirmed using the RNA 6000 Nano Assay (Agilent Technologies, Palo Alto, CA) with the Agilent 2100 bioanalyzer (Agilent Technologies). RNA samples were determined to be of high quality if the electropherograms generated by the bioanalyzer demonstrated distinct 18S and 28S ribosomal peaks with an absence of any small peaks between the two and a relatively flat baseline between 29 seconds and the 18S peak.

2.4. *GeneChip® microarray*

Total RNA from four replicate cultures of control and treated DC was used for gene expression profiling with Affymetrix Human Genome U95Av2 GeneChip® array. This array contains probe sets (oligos) designed to interrogate mainly full-length genes with previously identified functions, rather than expressed sequence tags, and represents approximately 12,000 characterized sequences. Briefly, total RNA from each replicate culture was reverse transcribed into double-stranded cDNA using SuperScript Choice cDNA Synthesis kit (GIBCO) with an oligo-dT primer containing a T7 RNA polymerase promoter. After purification by phenol/chloroform extraction, biotin-labeled cRNA was prepared from the double-stranded cDNA using an ENZO BioArray High Yield RNA Transcription Labeling Kit (Affymetrix Inc., Santa Clara, CA). The biotinylated

cRNA was cleaned-up using RNeasy kit (QIAGEN), then fragmented at 94 °C in fragmentation buffer (40 mM Tris-acetate pH 8.1, 100 mM KOAc, 30 mM MgOAc) for 35 min to generate hybridization targets. The overall quality of each labeled cRNA target was assessed using Affymetrix GeneChip® Test 3 array (Affymetrix). Parameters used to assess target quality included the 3′/5′ ratios of housekeeping/maintenance gene controls such as GAPDH and beta actin, the performance of target synthesis spikes, background fluorescence, noise and gene absent/present ratios (for details on assessing target quality see the Affymetrix GeneChip® Expression Analysis Technical Manual; http://www.affymetrix.com/support/technical/manual/expression_manual.affx). Based on the results of the test chips, cRNA targets that were determined to be of sufficiently high quality were hybridized for 16 h to HG-U95Av2 high-density microarrays. The microarrays were washed and bound cRNA was detected by staining with streptavidin-phycoerythrin. The signal intensity of the fluorescent staining was read using a Hewlett-Packard G2500A Gene Array Scanner using a randomized scanning order for the GeneChip®. As a quality control check on the hybridization, images of the microarrays were examined visually prior to analysis using Affymetrix Microarray Suite (version 5.0). Signal values for each transcript were generated by the Microarray Suite analysis and were used for statistical evaluation of the data.

2.5. *Analysis of microarray data*

The signal values used in analysis of the microarray data were those computed by the Affymetrix algorithm (Lockhart et al., 1996) using Affymetrix Microarray Suite (version 5.0). In order to obtain a general view of how the individual samples compared to one another, principal components analysis (PCA) was used in order to reduce the dimensionality of the data. Instead of examining the signal values of individual genes, linear combinations of these signal values (across all genes) were examined. PCA finds a set of standardized linear combinations called the principal components, which, taken together, explain all of the variance of the original data. The first principal component (PC) explains the largest proportion of the variance, the second PC explains the largest proportion of variance uncorrelated with the first principal component, and so forth. By looking at plots of the principal components, it can be determined whether variability among samples (chips) is due to experimental group (i.e. treatment) or some other factor (e.g. GeneChip® scanning order).

Algorithms in the microarray analysis suite (Affymetrix) were used to determine whether the expression of each transcript was considered to be present, absent or marginal in the sample. Transcripts designated as absent in all samples, both treated and control, were excluded from further analysis. Mean signal values were computed by experimental group, and the differences between the mean signal value of each treated group and the control group was calculated (i.e. treated group mean signal value minus control group mean signal value). Fold-change values for genes were calculated as the ratio of the mean signal values of allergen treated group over the control. Two-sample *t*-tests were used to compare the signal values of each treated group to the control group signal values. Only those changes in gene expression with a two-sided significance level of ≤ 0.001 were considered to be due to allergen treatment. This very low significance level was used in order to control the overall Type I error rate (probability of incorrectly declaring a significant difference in signal values) for a very large number of statistical tests. Using a level of significance of 0.001, one would expect 1 out of 1000 genes on average to be called statistically significant just by random chance. All analyses were conducted using S-PLUS 6.1 and StatServer 6.1 (Insightful Corporation, Seattle, WA).

2.6. *Reverse transcription and real-time PCR*

For a select number of genes identified as being significantly altered by concentrations of DNBS, the relative changes in gene expression that were observed in the microarrays were evaluated using reverse transcription followed by real-time PCR. In addition, real-time PCR was also conducted to determine whether or not those gene changes were differentially regulated by exposure to allergen. Primer pairs were designed for each of the selected genes using either the Affymetrix target sequence or the Genbank sequence (Table 1). Reverse transcription of RNA was performed using the Omniscript RT kit (Qiagen) under

Table 1
Primer sequences used for real-time PCR

Gene symbol	Sequence 5′ → 3′ forward primer	Sequence 5′ → 3′ reverse primer
ABCA6*	GAT CA TTG GCT CCA TCC	GTA AAC GTC TGC CAC GGG
ABCC1	GGC ACT CAA AAG CTG GGA	GCC CTG AGA CAA TCC CCT
AKR1C2	ATA TTT TTG CTG GCC CCC	GTC ACC ATC CAC ACG CAG
ARHGDIB	AAA CGC TGC TGG GAG ATG	TCC ATG GTG ATT GGT CCC
ATP6V1F	TCG AAG ACA CTT TCC GGC	GTG CTC CTT GGA GGG GAT
CTSH	ACT GGG ACC CCC AAC ATT	AGG CAC ATG GCT GGT GAT
CTSL*	GTA AGC CCA GGA AGG GGA	AAG CCT CCC AGT TTT CCG
CCL2*	CTT CAT TCC CCA AGG GCT	GGG ACA CTT GCT GCT GGT
CCL4*	GCT CTC CAG CGC TCT CAG	GCA GAG GCT GCT GGT CTC
CD86*	TAT GGG CCG CAC AAG TTT	TTC ATC TGG TGG ATG CGA
EPB41L2	TGA GGA TGC TGG GGA GAG	GGG TCA AAG GCA AAG GAA A
FCER1A*	TCT CCA GCA TCC TCC ACC	GCC ATC TGG AGC GAA GAA
GLA	GAT GTT GCT GGA CCA GGG	CTT TGG CTT GAG GGC TGA
HML2	GAC GGC AGG TGG AT GAC	CCA CCA AAG GCA GCT CAG
IL-3RAX	GGG GGT CTG CCT CAA TCT	TTC TTC CTG GCA GCT TCG
IL8*	GGT GCA GTT TTG CCA AGG	TTC CTT GGG GTC CAG ACA
NOTCH3*	GGG CAT GAA GAA CAT GGC	TGA GTC CAC TGA CGG CAA
PLAUR*	GAA GGG CGT CCA AAG GAT	AGC TGT AAC ACT GGC GGC
QPRT	GGA GGG CTG GTG ATG TTG	GGC TGC TGC ATT CCA CTT
TGIF	GTT GCA CTC AAA CGG GCT	CCC GGC AAT CAT GAC ATT
ICaBP (rat)	ATC CAA ACC AGC TGT CCA A	TGT CGG AGC TCC TTC TTC

Sequences were derived from either the Affymetrix target sequences or on the Genbank database sequence (indicated by asterisk (*)).

conditions described by the manufacturer. In all reactions, cDNA was synthesized in 20 μl using 1.5 μg of human total RNA from DC and 10 μM Oligo-dt primers (Ambion Inc., Austin, TX) All reactions contained 10 units of RNase Out RNAse inhibitor (Invitrogen, Carlsbad, CA). The resulting cDNA was stored frozen (−20 °C) until assayed by real-time PCR using an iCycler thermal cycler with the iCycler iQ™ real-time PCR detection system (Bio-Rad, Hercules, CA). An equivalent of 50 ng of total RNA was used in 25 μl of QuantiTect SYBR Green PCR Master Mix (Qiagen). The real-time PCR reaction mixture included 5 μl of sample, forward and reverse primers (0.3 μM each), and the PCR master mix containing probe (2× stock). The 96-well real-time PCR format included seven, 10-fold dilutions of a PCR purified DNA standard (2×10^{-2} ng to 2×10^{-8} ng), a PCR negative control (template), and up to seven genes independently analyzed on eight samples per plate. The positive control and PCR standard was a rat intestinal calcium binding protein (ICaBP) PCR product that was gel purified away from the primers. Test samples analyzed were biological triplicates performed individually within a given PCR run.

2.7. Analysis of real-time PCR data

Optical data obtained by real-time PCR was analyzed using the default and variable parameters available in the software provided with the iCycler iQ™ real-time PCR detection system. The PCR threshold cycle number (C_T), which is the point at which fluorescence exceeds the threshold limit (10× mean of the standard deviation of fluorescent reading of well over baseline cycles 2–10), and starting quantity (SQ) of test RNA samples was calculated after PCR baseline subtraction and C_T determination had been carried out on the standards. Standard curve equations were calculated by regression analysis of the log of the copy number (starting quantity) versus threshold cycle. The standard curve equations (R^2 usually greater than 0.96) were used to calculate quantities of test RNA. The mean relative fluorescence units (RFU) was calculated using individual well readings within a given PCR run on biological triplicate samples and then converted to mean fold-change comparing mean RFU of control samples versus mean RFU of treated samples. For each gene described in Table 5, the data presented represent the combined mean fold-change from multiple

PCR reactions (2–4) within a single run. For the genes described in Table 6, the data shown are the mean of fold-changes calculated from single real-time RT-PCR reactions run on each of three replicate cultures from a single donor.

3. Results

3.1. Exposure to contact allergen induces changes in gene expression

Dendritic cells generated from the adherent fraction of PBMC by 7 days of culture in the presence of GM-CSF and IL-4 were exposed for 24 h to DNBS, the water soluble analog of DNCB. Based on the results of the test chips, the fragmented cRNA target sample for one of the control culture replicates was found to be of poor quality with average background and noise values well above the other chips. Therefore, it was excluded from further analysis. High quality targets obtained from three replicate control cultures and four replicate cultures of each DNBS concentration were hybridized to HG-U95Av2 high-density microarrays for transcript profiling.

PCA, a multivariate analysis technique frequently used to identify sources of variability and detect outliers in microarray studies, was conducted to examine the variation amongst the 11 samples from the three experimental groups. Using PCA, the large number of (possibly) correlated variables (i.e. the measurements of thousands of genes measured across the various treatments) was mathematically reduced to a smaller number of uncorrelated variables, the principal components. The PCs were then examined to identify factors that introduced variability in the dataset. The first PC, which explains the largest proportion of variance, was found to account for 80% of the variance between the samples. The plot of the coefficient of PC 1 versus the 11 individual chips of each experimental group indicated that the four replicates of the 5 mM DNBS treated cultures were distinctly different from both the control and 1 mM DNBS treated chips (Fig. 1A). When just the 1 mM DNBS and vehicle chips were examined, the four allergen treated samples were identified as different from control (Fig. 1B). Therefore, results of the PCA clearly demonstrated that allergen treatment, rather than some other factor such as chip scanning order, had the greatest influence on the dataset. In addition, no outlier chips were identified, as all replicates within each treatment group were similar.

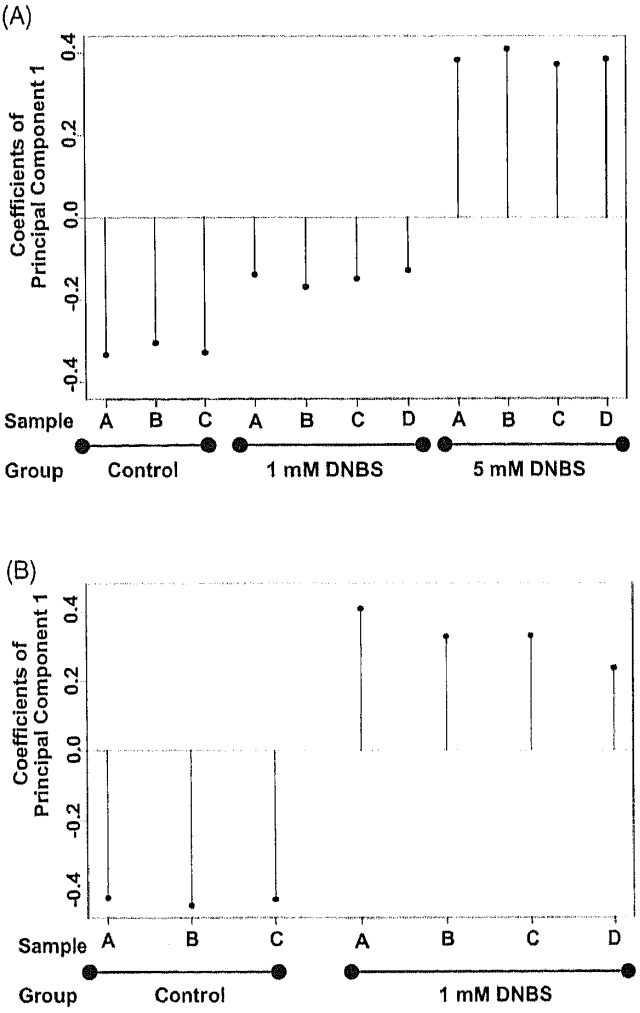

Fig. 1. Principal component analysis of the microarray-derived data. Principal component analysis demonstrated that allergen exposure was the greatest source of variability between the samples. In comparing all samples, the 5 mM DBNS exposed cultures were clearly different from the 1 mM DNBS exposed cultures and the control cultures (A). Comparison of 1 mM DNBS exposed cultures to control cultures also demonstrated a treatment effect (B).

Pairwise comparisons of the mean signal value of each DNBS treated group to the control group were conducted using two-sample *t*-tests. The numbers of gene changes that were found to be statistically different at the various levels of significance are shown in Table 2. Only those changes in gene expression with a two-sided significance level of ≤ 0.001 were considered to be caused by allergen treatment. In comparing the 1 mM DNBS treated DC to the control

Table 2
Number of statistically significant gene changes based on pairwise comparison to untreated control of mean signal value (*t*-test)

P-value	1 mM DNBS vs. control	5 mM DNBS vs. control
≤0.00001	14	189
≤0.0001	42	551
≤0.001	173	1249
≤0.01	598	2288

DC, 97 genes were significantly up-regulated with fold-changes of +1.1 to 29.4 and 76 genes were significantly down-regulated with fold decreases of −1.1 to −7.4. Of the 1249 genes that were found to be significantly different in the 5 mM DNBS treated DC, 586 genes had fold increases in expression over control ranging from +1.2 to +160.1 and 663 genes demonstrated a down-regulation of −1.2 to −98.2 times control. Sixty genes were significantly up-regulated and 58 were significantly down-regulated by both concentrations of DNBS (Fig. 2A and B). Of the total number of genes represented on the GeneChip®, only 1.6% were changed by treatment with 1 mM DNBS while 10.9% were influenced by 5 mM DNBS exposure. The significant gene changes observed represented a wide range of cellular processes including signal transduction, protein modification/synthesis, transcription and small molecule transport. The full transcript profile with signal values for each probe set on each of the 11 GeneChip® is available in an Excel spreadsheet provided as electronic supplementary material (see DC full profile.xls).

A list of genes which were significantly up-regulated in DC after 24 h of exposure to both 1 mM and 5 mM DNBS are presented in Table 3 along with their accession number and fold-change relative to the untreated control DC cultures. The gene with the largest fold-change in the 5 mM DNBS treated DC (+160.1), dihydropyrimidase like-3 (gene symbol DPYSL3), was not found to be significantly changed in the 1 mM DNBS-treated DC (data not shown). Genes that were significantly down-regulated ($P \leq 0.001$) are presented in Table 4.

3.2. Evaluation of gene expression changes by real-time RT-PCR–comparison to microarray data

In order to determine the reproducibility of the gene expression changes observed using the Affymetrix

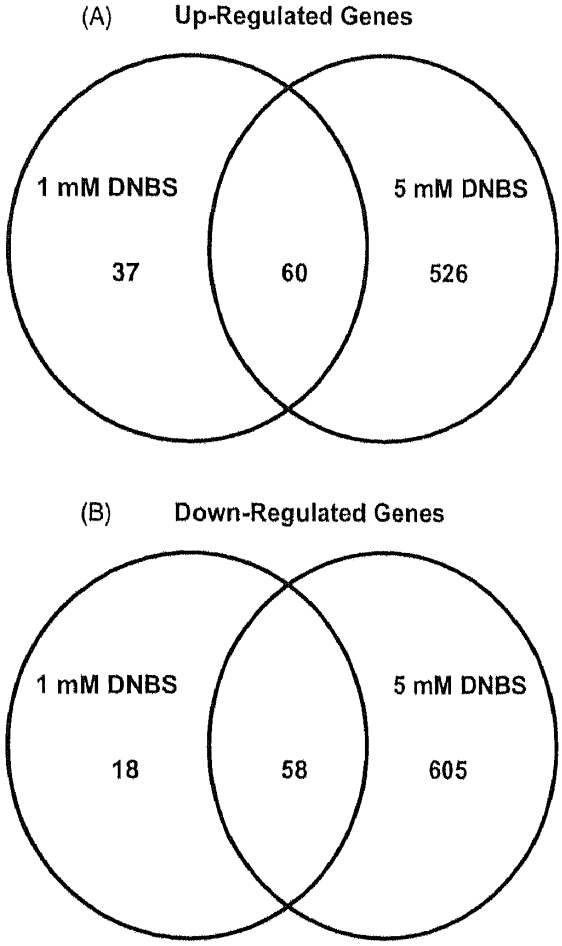

Fig. 2. Distribution of statistically significant genes. The number of genes that were significantly ($P \leq 0.001$) up-regulated (A) and down-regulated (B) at the concentrations of DNBS tested.

GeneChip®, a set of genes was selected for evaluation by real-time RT-PCR. The genes chosen were among those found to be significantly different from control at both concentrations of DNBS tested. Total RNA used for real-time analysis was obtained from DC derived from a second donor following 24 h exposure to 1 mM and 5 mM DNBS. Therefore, in addition to demonstrating the reproducibility of the gene expression changes observed in the microarrays, the real-time data also provided insight into the variability of those changes amongst different donors. For all of the genes examined thus far, there was agreement between the GeneChip® data and the real-time data in terms of the direction of the change in gene expression, either up- or down-regulated, relative to control (Table 5). In addition, the magnitude of the change observed was comparable across platforms for a number of the genes. However, for some

Table 3
List of genes that were significantly up-regulated ($P \leq 0.001$) by 24 h exposure to both concentrations of DNBS

Accession no.	Gene name	Gene symbol	Fold-change induced by DNBS (mM)	
			1	5
Cytokine/chemokine				
X52015	Interleukin-1 receptor antagonist	IL1RN	1.9	3.2
AF014958	Chemokine (C–C motif) receptor-like 2	CCRL2	1.8	9.5
L06797	Chemokine (C–X–C motif) receptor 4	CXCR4	2.3	4.7
Protein modification/synthesis				
X04371	2′,5′-Oligoadenylate synthase 1	OAS1	2.1	4.1
M55153	Transglutaminase 2	TGM2	3.1	2.7
X04741	Ubiquitin carboxyl-terminal esterase 1	UCHL1	10.3	23.2
M91670	Ubiquitin carrier protein	E2-EPF	1.7	2.7
M88279	Hsp binding immunophilin	FKBP4	1.9	3.3
AB013382	Dual specificity phosphatase 6	DUSP6	3.3	8.5
AF043325	*N*-myristoyltransferase 2	NMT2	4.8	5.5
U48296	Protein tyrosine phosphatase type IVA, member 1	PTP4A1	1.7	4.5
U09510	Glycyl-t RNA synthase	GARS	1.5	5.1
Signal transduction				
Y07566	Ras-like without CAAX1	RIT1	3.3	13.6
AF036927	Adenylate cyclase 9	ADCY9	2.3	2.1
AB005047	SH3 binding protein	SH3BP5	2.3	6.9
NM003329	Thioredoxin	TXN	2.1	4.0
U43077	Cell division cycle homolog 37	CDC37	1.7	2.2
Cell surface receptors/membrane proteins				
D11428	Peripheral myelin protein 22	PMP22	2.8	9.8
J02939	CD98	SLC3A2	2.9	4.6
AB026891	Solute carrier family 7, member 11	SLC7A11	2.9	5.0
X01060	Transferrin receptor, CD71	TFRC	2.0	3.1
AL080164	Low-density lipoprotein receptor-related protein 10	LRP10	1.4	1.5
Y00285	Insulin-like growth factor 2 receptor	IGF2R	1.6	2.0
X81817	Accessory protein BAP 31	BCAP31	1.3	2.0
Transcription factors/activators/repressors				
AF096870	Tripartite motif-containing 16/(estrogen-responsive B box protein)	TRIM16	2.2	35.7
AF019214	HMG-box containing protein 1	HBP1	1.8	3.3
J04102	Erythroblastosis virus oncogene homolog 2	ETS2	2.9	6.5
D13891	Inhibitor of DNA binding 2	ID2	1.4	1.2
Small molecule transport				
U79745	Monocarboxylate transporter 6	SLC16A6	3.2	4.3
U83461	Copper transporter 2	SLC31A2	1.9	3.3
U53347	Neutral amino acid transporter	SLC1A5	1.8	3.7
U45285	ATPase, H+ transporting, lysosomal V0 protein a isoform 3	TCIRG1	1.6	1.8
AA877795	ATPase, H+ transporting, lysosomal 34 kDa, V1subunit D	ATP6V1D	1.9	6.8
U39412	*N*-ethylmaleimide-sensitive factor attachment protein	NAPA	1.8	3.6
Cell cycle, proliferation, death				
Z23115	BCL2-like 1 isoform 1	BCL2L1	1.2	3.0
U03106	Cyclin-dependent kinase inhibitor 1A	CDKN1A	3.0	4.0
Miscellaneous				
D17793	3-Alpha hydroxysteroid dehydrogenase, type II	AKR1C3	9.7	71.2
X59812	Cytochrome P450, 27A	CYP27A1	29.4	43.0
AF010400	Transaldolase 1	TALDO1	2.4	3.4

Table 3 (*Continued*)

Accession no.	Gene name	Gene symbol	Fold-change induced by DNBS (mM)	
			1	5
D86961	Lipoma HMGIC fusion partner-like 2	LHFPL2	2.9	5.3
U43944	Malic enzyme 1	ME1	2.8	3.1
U24577	Phospholipase A2	PLA2G7	3.5	3.5
X91247	Thioredoxin reductase 1	TXNRD1	4.0	12.4
M13755	Interferon-stimulated protein, 15 kDa	G1P2	2.4	1.6
U30255	Phosphogluconate dehydrogenase	PGD	2.4	3.9
L13943	Glycerol kinase	GK	2.4	5.2
Y15521	Acetylserotonin *O*-methyltransferase-like	ASMTL	1.6	2.6
X66436	Guanine nucleotide binding protein-like 1	GNL1	1.7	3.1
U18009	Vesicle amine transport protein 1 homolog	VAT1	1.4	3.1

genes with higher expression levels as determined by the GeneChip® mean signal values, such as IL8, AKR1C2, and HML2, the real-time PCR treatment versus control fold-change values tended to be larger than the Affymetrix fold-changes.

3.3. Evaluation of gene expression changes by real-time RT-PCR—examination of allergen specificity of gene regulation

To determine whether any of the significant gene changes previously observed were due to the reactive nature of the contact allergen, DC derived from elutriated human monocytes were exposed to medium alone, 1 mM and 5 mM DNBS, or 1 mM and 5 mM BSAc, a non-allergen that is structurally similar to DNBS. Gene expression changes were examined by real-time RT-PCR for the same 20 genes that were used to evaluate the reproducibility of the microarray data. It was found that approximately half of those genes appeared to be differentially regulated by exposure to the contact allergen (Table 6). Of the genes that demonstrated some evidence of allergen specificity, the fold-changes (relative to the untreated control) observed for the DNBS-treated DC were greater in magnitude compared to the fold-changes observed for the BSAc-treated DC and, in some cases, were directionally different.

4. Discussion

Many differences exist between immature and mature DC (reviewed Banchereau et al., 2000) and it has been proposed that exposure to contact allergens can induce the maturation of DC (Manome et al., 1999). Indeed, many of the changes reported to occur in DC following treatment with contact allergens such as increases in cell surface expression of class II MHC, co-stimulatory and adhesion molecules (Aiba et al., 1997; Tuschl et al., 2000) are also observed with known maturation stimuli such as lipopolysaccharide (LPS) (Verhasselt et al., 1997). While changes in the DC gene expression profile due to maturation stimuli including viral and bacterial pathogens (Huang et al., 2001), lipopolysacharide (Chen et al., 2002; Messmer et al., 2003), cytokines (Dietz et al., 2000; Le Naour et al., 2001; Messmer et al., 2003; Moschella et al., 2001), and soluble CD40 ligand (Messmer et al., 2003; Moschella et al., 2001) have been explored, there are currently no published reports describing genomic-scale analysis of the early changes induced in DC resulting from chemical allergen exposure. In this study, we have examined changes in gene expression in cultured PBMC-derived DC that are induced by exposure to two different concentrations of a contact allergen using Affymetrix GeneChip® microarrays. Based on statistical analysis of the microarray data, the expression of many genes was found to be augmented significantly by 24 h treatment with either 1 mM or 5 mM DNBS, with a greater number of gene expression changes observed at the higher concentration. Since treatment with the higher concentration of DNBS resulted in a low level of cytotoxicity, we focused our attention only on those genes for which expression was found to be significantly different from control at both allergen concentrations.

Table 4
List of genes that were significantly down-regulated ($P \leq 0.001$) by 24 h exposure to both concentrations of DNBS

Accession no.	Gene name	Gene symbol	Fold-change induced by DNBS (mM)	
			1	5
Cytokine/chemokine				
M21121	RANTES; SCYA5	CCL5	−2.3	−4.7
	(two probe sets for the same gene)		−1.6	−2.9
M28225	Chemokine (C–C motif) ligand 2; MCP-1[a]	CCL2	−2.8	−10.1
D43767	Chemokine (C–C motif) ligand 17; TARC	CCL17	−2.8	−5.6
U95626	Chemokine (C–C motif) receptor 2	CCR2	−7.4	−9.4
Protein modification/synthesis				
X71125	Glutaminyl cyclase	QPCT	−2.0	−5.8
D45248	Proteasome activator subunit 2	PSME2	−1.5	−2.2
Signal transduction				
AI828880	Protein tyrosine phosphatase non-receptor type 2	PTPN2	−1.4	−3.4
NM001386	Collapsin response mediator protein hCRMP-2	DPYSL2	−1.8	−3.7
L77730	Adenosine A3 receptor	ADORA3	−2.2	−7.7
Cell surface receptors/membrane proteins				
M28827	CD1c	CD1C	−1.4	−7.4
U33017	Signaling lymphocytic activation molecule	SLAM	−4.2	−13.0
M15395	Integrin beta 2 subunit, CD18, LFA-1	ITGB2	−1.5	−2.8
U97145	GDNF family receptor alpha 2	GRAF2	−2.2	−9.5
	(two probe sets for the same gene)		−2.4	−7.7
M34064	*N*-cadherin	CDH2	−1.8	−4.4
X16863	Low affinity receptor IgG, IIIa (CD16)	FCGR3A	−1.9	−6.0
	(two probe sets for the same gene)		−1.9	−5.0
AF016266	TNF-related apoptosis-inducing ligand receptor 2	TNFRSF10B	−1.3	−3.0
Z22555	CD36 antigen-like 1	SCARB1	−1.7	−2.9
Transcription factors/activators/repressors				
J03161	Serum response factor	SRF	−1.3	−1.6
AF030227	Vav1 oncogene	VAV1	−1.4	−3.3
Small molecule transport				
NM004925	Aquaporin 3	AQP3	−2.8	−16.5
	(two probe sets for the same gene)		−3.8	−14.4
W72186	S100 calcium binding protein A4	S100A4	−1.7	−1.4
NM013306	Sorting nexin 15	SNX15	−1.4	−1.8
Cell cycle, proliferation, death				
M87507	Interleukin 1 beta convertase; caspase 1	CASP1	−2.8	−5.6
Miscellaneous				
U83116	Absent In melanoma 1	AIM1	−1.9	−5.8
M80261	Apurinic/apyrimidinic endonuclease	APEX1	−1.5	−5.7
U21931	Fructose-1,6-biphosphatase 1	FBP1	−1.5	−4.6
D31766	Glucosamine-6-phosphate isomerase	GNPI	−1.5	−1.4
X15331	Phosphoribosyl pyrophosphate synthetase 1	PRPS1	−1.8	−3.5
X04098	Actin, gamma 1	ACTG1	−1.4	−2.7
V00599	Beta-5-tubulin	OK/SW-cl.56	−1.7	−2.9
U03057	Facin homolog 1, actin-bundling protein	FSCN1	−1.3	−1.6
D13643	24-Dehydrocholesterol reductase	DHCR24	−1.9	−2.8
U41767	A disintegrin and metalloproteinase domain 15	ADAM15	−1.7	−2.2
M14539	Coagulation factor XIII, A1 polypeptide	F13A1	−2.0	−1.6
AL080121	DKFZp564O0823		−1.7	−6.6
J05070	Matrix metalloproteinase 9	MMP9	−1.8	−5.5

Table 4 (*Continued*)

Accession no.	Gene name	Gene symbol	Fold-change induced by DNBS (mM)	
			1	5
D14664	C-type lectin BIMLEC precursor	BIMLEC	−1.9	−6.5
J00287	Similar to pepsin A precursor	NA	−1.9	−4.2
AL023653	Chromosome X open reading frame 9	CXorf9	−1.4	−5.9
AA977580	Fatty-acid-coenzyme A ligase, long chain 3	FACL3	−1.7	−2.5
U11863	Amiloride binding protein 1	ABP1	−2.0	−2.5
AF089814	Tumor suppressor deleted in oral cancer-related 1	DOC-1R	−1.1	−2.0
Y14768	Allograft inflammatory factor 1	AIF1	−1.6	−1.8
X05409	Aldehyde dehydrogenase 2 family (mitochondrial)	ALDH2	−1.8	−1.8
AF054996	snoRNP protein 4 homolog	IMP4	−1.3	−1.7

[a] Second probe set representing CCL2, see Table 5 for other CCL2 probe set results.

We observed a number of gene expression changes induced by allergen exposure that are consistent with what is known of the DC maturation process including up-regulation of transcripts for the co-stimulatory molecule CD86 (Ozawa et al., 1996) and the constitutive chemokine receptor CXCR4 (Sallusto et al., 1998, 1999) and down-regulation of genes encoding molecules involved in antigen uptake such as the high affinity IgE receptor (Maurer and Stingl, 1999), aquaporin 3 (De Baey and Lanzavecchia, 2000) and the C-type lectin HML2 (Higashi et al., 2002). CCR2 or monocyte chemotactic protein 1 receptor (MCP-1-R) is classified as an inducible/inflammatory chemokine receptor and has been shown to be expressed on immature DC (Sallusto et al., 1998). As DC mature in response to extrinsic signals such as TNF-α, IL-1β and LPS, they lose surface expression of inflammatory chemokine receptors, including CCR2 (McColl, 2002). In this study, down-regulation in message for CCR2 in DNBS treated DC versus control was observed. Consistent with our findings, Messmer et al. (2003) observed a down-regulation of transcripts for the CCR2 ligand CCL2 (also known as monocyte chemoattractant protein 1, alternate gene symbol SCYA2) after 48 h maturation with either LPS or pro-inflammatory cytokines. Our data also suggest that the observed decrease in CCL2 transcripts may be the result of differential regulation by allergen as exposure of DC to the non-allergen, BSAc, failed to induce such changes. However, using semiquantitative RT-PCR, Sallusto et al. (1998) reported a strong up-regulation in mRNA expression for MCP-1 in monocyte-derived DC following 40 h stimulation with LPS. Following allergen exposure, one of the most up-regulated genes seen by GeneChip® was IL-8, with 11.9- and 32.7-fold increases over control for the 1 mM and 5 mM DNBS treated DC, respectively. And, while IL-8 was found to also be up-regulated by exposure to the non-allergen BSAc, the magnitude of the change resulting from exposure to the allergen DNBS was far greater, owing perhaps to the more reactive nature of the allergen molecule. Aiba et al. (2003) have shown previously that treatment of monocyte-derived DC with the contact allergens DNCB and nickel chloride induced an up-regulation in both IL-8 mRNA expression and protein production. Increases in IL-8 gene expression have also been reported for PBMC-DC following exposure to LPS and pro-inflammatory cytokines (Messmer et al., 2003), soluble CD40 ligand trimer (Moschella et al., 2001) and different pathogens (Huang et al., 2001), in agreement with our findings.

We also observed changes in gene expression that are contrary to what has been reported to occur during DC activation/maturation. Signaling lymphocytic activation molecule (SLAM) was first identified on activated T and B cells. Recently, mRNA encoding for both membrane-bound and soluble secreted forms of SLAM were detected in CD40 ligand-activated monocyte-derived DC (Bleharski et al., 2001). Membrane-bound SLAM protein was shown to be highly expressed on the surface of DC activated by CD40L, LPS, or poly(I:C) with peak expression occurring at 12 h post-stimulation. SLAM up-regulation appeared to be a direct result of the induction of DC maturation. Additional evidence

Table 5
Selected gene expression changes verified by real-time RT-PCR

Accession no.	Gene name	Gene symbol	Fold-change induced by DNBS (mM Affy[a] RT[b])	
			1	5
Cytokine/chemokine				
M28130	Interleukin-8	IL8	+11.9 +118.0	+32.7 +65.1
D49410	Interleukin-3 receptor α	IL3RAX	+1.6 +1.6	+2.2 +3.5
J04130	Chemokine (C–C motif) ligand 4; MIP1-β	CCL4	−2.7 −4.4	−98.2 −96.4
M26683	Chemokine (C–C motif) ligand 2; MCP1	CCL2	−2.7 −4.2	−12.5 −60.2
Protein modification/synthesis				
X12451	Cathepsin L	CTSL	+3.5 +7.7	+5.6 +10.7
X16832	Cathepsin H	CTSH	−2.0 −3.9	−4.8 −11.0
Signal transduction				
X69549	Rho GDP dissociation inhibitor (GDI) beta	ARHGDIB	−2.0 −2.9	−7.9 −18.1
Cell surface receptors/membrane proteins				
U97669	Notch homolog 3	NOTCH3	+13.4 +76.3	+27.8 +83.2
X74039	Plasminogen activator, urokinase receptor	PLAUR	+2.0 +1.2	+3.1 +1.6
U04343	CD86	CD86	+2.0 +1.7	+2.0 +1.0
D50532	Macrophage lectin 2	HML2	−6.9 −7.5	−18.2 −203.6
X06948	High affinity IgE receptor, alpha-subunit	FCER1A	−2.3 −1.5	−9.6 −12.4
Transcription factors/activators/repressors				
X89750	TGFB-induced factor	TGIF	+1.8 +1.4	+3.9 +1.9
Small molecule transport				
D49400	ATPase, H+ transporting, lysosomal subunit F	ATP6V1F	+1.3 +2.0	+1.9 +1.7
NM004996	ATP-binding cassette, sub-family C, member 1	ABCC1	+2.2 +9.5	+2.5 +4.0
U66680	ATP-binding cassette, sub-family A, member 6	ABCA6	−3.2 −5.0	−10.3 −55.2
Miscellaneous				
NM001354	3-Alpha hydroxysteroid dehydrogenase, type III	AKR1C2	+18.1 +29.8	+73.9 +348.4
NM000169	Galactosidase, alpha	GLA	+1.6 +2.1	+2.5 +3.4
D78177	Quinolate phosphoribosyl transferase	QPRT	−1.7 −0.5	−4.8 −16.5
AF027299	Erythrocyte membrane protein band 4.1-like 2	EPB41L2	−1.6 −2.2	−3.5 −5.2

Comparison to microarray results.

[a] Affymetrix fold-changes shown were based on comparison of the mean signal values of replicate allergen treated and control cultures (GeneChip®) from a single donor. All gene changes shown were statistically significant ($P \leq 0.001$).

[b] Real-time RT-PCR data shown are the mean of fold-changes calculated from repeated real-time RT-PCR reactions ($n = 2$–4) on triplicate cultures from a single donor.

in support of this idea is reported by Kruse et al. (2001). SLAM expression was found in mature, but not immature DC and surface expression of SLAM was shown to be up-regulated by IL-1β. In contrast, we observed significant down-regulation in SLAM with 4.2- and 13.0-fold decreases in expression relative to control following 24 h exposure to 1 mM and 5 mM DNBS, respectively. CCL17 (alternate gene symbol SYCA17), also known as thymus and activation-regulated chemokine (TARC), is a T-cell chemoattractant (Imai et al., 1996) and has been shown to be induced in DC upon maturation with LPS (Messmer et al., 2003; Sallusto et al., 1998), proinflammatory cytokines (Messmer et al., 2003) and CD40L (Moschella et al., 2001). Exposure to the chemical allergen DNBS induced a down-regulation in CCL17 expression. The observed discrepancies in gene expression changes in response to chemical allergen exposure versus 'traditional' methods of DC maturation may be due simply to differences in the kinetics of the response between the different stimuli. However, a more interesting hypothesis is that the gene expression changes which result from DC encounter with allergen represent only a portion of

Table 6
RT-PCR analysis of changes in gene expression induced by exposure to an allergen (DNBS) or a structurally similar irritant (benzenesulfonic acid; BSAc) in a single donor[a]

Accession no.	Gene name	Gene symbol	Fold-change induced by DNBS (mM)		Fold-change induced by BSAc (mM)	
			1	5	1	5
Cytokine/chemokine						
M28130	Interleukin-8	IL8	+30.4	+261.0	+2.3	+7.1
D49410	Interleukin-3 receptor α	IL3RAX	+3.2	+7.9	+3.0	+4.7
J04130	Chemokine (C–C motif) ligand 4; MIP1-β	CCL4	−3.7	−21.7	−2.3	+1.1
M26683	Chemokine (C–C motif) ligand 2; MCP1	CCL2	−4.8	−8.2	−1.7	+2.0
Protein modification/synthesis						
X12451	Cathepsin L	CTSL	+3.9	+3.8	+2.7	+3.0
X16832	Cathepsin H	CTSH	+1.3	−1.9	+2.2	+2.5
Signal transduction						
X69549	Rho GDP dissociation inhibitor (GDI) beta	ARHGDIB	−1.9	−21.5	+1.0	+1.2
Cell surface receptors/membrane proteins						
U97669	Notch homolog 3	NOTCH3	+13.8	+34.0	+1.0	+1.5
X74039	Plasminogen activator, urokinase receptor	PLAUR	−1.1	+1.3	−1.3	−1.2
U04343	CD86	CD86	+1.8	+1.6	+1.7	+1.9
D50532	Macrophage lectin 2	HML2	−3.0	−52.8	+1.0	−1.3
X06948	High affinity IgE receptor, alpha-subunit	FCER1A	−1.7	−10.0	+1.1	+1.1
Transcription factors/activators/repressors						
X89750	TGFB-induced factor	TGIF	−2.1	+1.1	−1.7	−1.8
Small molecule transport						
D49400	ATPase, H+ transporting, lysosomal subunit F	ATP6V1F	+1.6	+2.2	+1.0	+1.9
NM004996	ATP-binding cassette, sub-family C, member 1	ABCC1	+1.0	+2.0	−1.5	−1.3
U66680	ATP-binding cassette, sub-family A, member 6	ABCA6	−2.4	−11.0	+1.2	+1.4
Miscellaneous						
NM001354	3-Alpha hydroxysteroid dehydrogenase, type III	AKR1C2	+25.0	+1752.0	+2.4	+3.1
NM000169	Galactosidase, alpha	GLA	+1.7	+1.6	−1.1	+1.3
D78177	Quinolate phosphoribosyl transferase	QPRT	+1.0	−12.0	+1.7	+2.3
AF027299	Erythrocyte membrane protein band 4.1-like 2	EPB41L2	−1.4	−8.5	−1.1	+1.3

[a] Data shown are the mean of fold-changes calculated from a single real-time RT-PCR reaction on triplicate cultures from a single donor.

the changes that occur as part of the maturation process. And, we may find that, with further exploration, some of the observed gene expression changes may be chemical-specific.

Further evaluation of a selected set of genes that demonstrated significant changes in the microarray was conducted using reverse transcription followed by real-time PCR. A good correlation was found between the microarray and real-time PCR platforms when comparing the relative fold-changes in transcript levels induced by allergen exposure. The direction of the change in gene expression, either up- or down-regulated, was the same for all 19 genes that were examined by real-time PCR. In addition, the order of magnitude of the relative fold-changes-derived by real-time PCR was similar to that obtained using the microarrays. In some cases, the results were almost identical. For example, CCL4 expression in DC treated with 5 mM DNBS was down-regulated 98.2-fold by microarray and 96.4 fold by real-time PCR; macrophage lectin 2 (HML2) expression in 1 mM DNBS treated DC was down-regulated 6.9-fold by microarray and 7.5-fold by real-time PCR. Other investigators also have reported concordance between expression profiles determined by Affymetrix microarrays and real-time PCR (Naciff et al., 2003). Donor to donor variability has been reported in DC responses to allergen treatment for both cell

surface marker expression (Aiba et al., 1997; Coutant et al., 1999; Tuschl et al., 2000) and gene expression (Pichowski et al., 2001). However, we observed a high degree of inter-individual reproducibility of the transcriptional changes in our comparison of microarray and real-time PCR data for 20 genes which utilized DC derived from two different donors. Perhaps as we expand our investigations to include other gene sets and DC derived from additional donors, we may encounter donor variability in the response to allergen treatment.

To examine whether or not the significant expression changes observed in the microarray data set could be attributed specifically to allergen exposure, RT-PCR was conducted for the same selected set of genes. Elutriated monocyte-derived DC from a third donor were exposed to either the allergen, DNBS, or the structurally similar non-allergen BSAc. Of the 20 genes examined, about half were, to some degree, differentially regulated by the allergen (as compared to the non-allergen) as evidenced by either the magnitude of the response, the direction (up or down-regulation), or both. In addition, many of the gene changes observed in the DNBS-treated DC from this donor were quite similar to the changes seen in DC derived from the two other donors used in the microarray and previous real-time PCR experiments; again, demonstrating donor-to-donor reproducibility of many of the transcriptional changes.

In summary, the results of our examination, at the transcriptional level, of the effects of contact allergen on DC revealed a number of gene expression changes known to be associated with the DC maturation process. These data, in part, provide support to the theory that exposure to contact allergens can induce the maturation of DC. Additionally, it is hoped that some of the transcript changes identified through this approach will be shown to be sufficiently robust, sensitive and selective such that they are suitable for use in the development of an in vitro predictive assay for contact sensitization.

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References

Aiba, S., Katz, S.I., 1990. Phenotypical and functional characteristics of in vivo-activated Langerhans cells. J. Immunol. 145, 2791–2796.

Aiba, S., Terunuma, A., Manome, H., Tagami, H., 1997. Dendritic cells differently respond to haptens and irritants by their production of cytokines and expression of co-stimulatory molecules. Eur. J. Immunol. 27, 3031–3038.

Aiba, S., Manome, H., Yoshino, Y., Tagami, H., 2000. In vitro treatment of human transforming growth factor-β_1-trated monocyte-derived dendritic cells with haptens can induce the phenotypic and functional changes similar to epidermal Langerhans cells in the initiation phase of allergic contact sensitivity reaction. Immunology 101, 68–75.

Aiba, S., Manome, H., Nakagawa, S., Mollah, Z.U.A., Mizuashi, M., Ohtani, T., Yoshino, Y., Tagami, H., 2003. P38 Mitogen-activated protein kinase and extracellular signal-related kinases play distinct roles in the activation of dendritic cells by two representative haptens, $NiCl_2$ and 2,4-dinitrochlorobenzene. J. Invest. Dermatol. 120, 390–399.

Allen, R.S., Smith, C.M., Belz, G.T., van Lint, A.L., Wakim, L.M., Heath, W.R., Carbone, F.R., 2003. Epidermal viral immunity induced by $CD8\alpha^+$ dendritic cells but not by Langerhans cells. Science 301, 1925–1928.

Banchereau, J., Steinman, R.M., 1998. Dendritic cells and the control of immunity. Nature 392, 245–252.

Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y.-J., Pulendran, B., Palucka, K., 2000. Immunobiology of dendritic cells. Ann. Rev. Immunol. 18, 767–811.

Becker, D., Mohamadzadeh, M., Reske, K., Knop, J., 1992a. Increased level of intracellular MHC class II molecules in murine Langerhans cells following in vivo and in vitro administration of contact allergens. J. Invest. Dermatol. 99, 545–549.

Becker, D., Neiß, U., Neis, S., Reske, K., Knop, J., 1992b. Contact allergens modulate the expression of MHC class II molecules on murine epidermal Langerhans cells by endocytic mechanisms. J. Invest. Dermatol. 98, 700–705.

Becker, D., Kuhn, U., Lempertz, U., Enk, A., Saloga, J., Knop, J., 1997. Flow-cytometric screening for the modulation of receptor-mediated endocytosis in human dendritic cells: implications for the development of an in vitro technique for predictive testing of contact sensitizers. J. Immunol. Methods 203, 171–180.

Bleharski, J.R., Niazi, K.R., Sieling, P.A., Cheng, G., Modlin, R.L., 2001. Signaling lymphocytic activation molecule is expressed on CD40 ligand-activated dendritic cells and directly augments

production of inflammatory cytokines. J. Immunol. 167, 3174–3181.

Chen, Z., Gordon, J.R., Zhang, X., Xiang, J., 2002. Analysis of the gene expression profiles of immature versus mature bone marrow-derived dendritic cells using DNA arrays. Biochem. Biophys. Res. Comm. 290, 66–72.

Coutant, K.D., de Brugerolle de Fraissinette, A., Cordier, A., Ulrich, P., 1999. Modulation of the activity of human monocyte-derived dendritic cells by chemical haptens, a metal allergen, and a staphylococcal superantigen. Toxicol. Sci. 52, 189–198.

De Baey, A., Lanzavecchia, A., 2000. The role of aquaporins in dendritic cell macropinocytosis. J. Exp. Med. 191, 743–747.

Degwert, J., Steckel, F., Hoppe, U., Kligman, H., 1997. In vitro model for contact sensitization. I. Stimulatory capacities of human blood-derived dendritic cells and their phenotypical alterations in the presence of contact sensitizers. Tox. In Vitro 11, 613–618.

Dietz, A.B., Bulur, P.A., Knutson, G.J., Matasić, R., Vuk-Pavlović, S., 2000. Maturation of human monocyte-derived dendritic cells studied by microarray hybridization. Biochem. Biophys. Res. Comm. 275, 731–738.

Enk, A.H., Katz, S.I., 1992. Early molecular events in the induction phase of contact sensitivity. Proc. Natl. Acad. Sci. U.S.A. 89, 1398–1402.

Girolomoni, G., Cruz, P.D., Bergstresser, P.R., 1990. Internalization and acidification of surface HLA-DR molecules by epidermal Langerhans cells: a paradigm for antigen processing. J. Invest. Dermatol. 94, 753–760.

Higashi, N., Fujioka, K., Denda-Nagai, K., Hashimoto, S., Nagai, S., Sato, T., Fujita, Y., Morikawa, A., Tsuiji, M., Miyata-Takeuchi, M., Sano, Y., Suzuki, N., Yamamoto, K., Matsushima, K., Irimura, T., 2002. The macrophage C-type lectin specific for galactose/*N*-acetylgalactosamine is an endocytic receptor expressed on monocyte-derived immature dendritic cells. J. Biol. Chem. 277, 20686–20693.

Huang, Q., Liu, D., Majewski, P., Schulte, L.C., Korn, J.M., Young, R.A., Lander, E.S., Hacohen, N., 2001. The plasticity of dendritic cell responses to pathogens and their components. Science 294, 870–875.

Hulette, B.C., Ryan, C.A., Gerberick, G.F., 2002. Elucidating changes in surface marker expression of dendritic cells following chemical allergen treatment. Toxicol. Appl. Pharmacol. 182, 226–233.

Imai, T., Yoshida, T., Baba, M., Nishimura, M., Kakizaki, M., Yoshie, O., 1996. Molecular cloning of a novel T cell-directed CC chemokine expressed in thymus by signal sequence trap using Epstein–Barr virus vector. J. Biol. Chem. 271, 21514–21521.

Kimber, I., Cumberbatch, M., 1992. Dendritic cells and cutaneous immune responses to chemical allergens. Toxicol. Appl. Pharm. 117, 137–146.

Kruse, M., Meinl, E., Henning, G., Kuhnt, C., Berchtold, S., Berger, T., Schuler, G., Steinkasserer, A., 2001. Signaling lymphocytic activation molecule is expressed on mature CD83+ dendritic cells and is up-regulated by IL-1β. J. Immunol. 167, 1989–1995.

Kühn, U., Brand, P., Willemsen, J., Jonuleit, H., Enk, A.H., van Brandwijk-Petershans, R., Saloga, J., Knop, J., Becker, D., 1998. Induction of tyrosine phosphorylation in human MHC class II-positive antigen-presenting cells by stimulation with contact sensitizers. J. Immunol. 160, 667–673.

Le Naour, F., Hohenkirk, L., Grolleau, A., Misek, D.E., Lescure, P., Geiger, J.D., Hanash, S., Beretta, L., 2001. Profiling changes in gene expression during differentiation and maturation of monocyte-derived dendritic cells using both oligonucleotide microarrays and proteomics. J. Biol. Chem. 276, 17920–17931.

Lockhart, D.J., Dong, H., Byrne, M.C., Follettie, M.T., Gallo, M.V., Chee, M.S., Mittmann, M., Wang, C., Kobayashi, M., Horton, H., Brown, E.L., 1996. Expression monitoring by hybridization to high-density oligonucleotide arrays. Nat. Biotech. 14, 1675–1680.

Manome, H., Aiba, S., Tagami, H., 1999. Simple chemicals can induce maturation and apoptosis of dendritic cells. Immunology 98, 481–490.

Maurer, D., Stingl, G., 1999. Dendritic cells and skin immunity. In: Lotze, M.T., Thomson, A.W. (Eds.), Dendritic Cells. Academic Press, San Diego, pp. 111–122.

McColl, S.R., 2002. Chemokines and dendritic cells: a crucial alliance. Immunol. Cell Biol. 80, 489–496.

Messmer, D., Messmer, B., Chiorazz, N., 2003. The global transcriptional maturation program and stimuli-specific gene expression profiles of human myeloid dendritic cells. Int. Immunol. 15, 491–503.

Moschella, F., Maffei, A., Catanzaro, R.P., Papadopoulos, K.P., Skerrett, D., Hesdorffer, C.S., Harris, P.E., 2001. Transcript profiling of human dendritic cells maturation induced under defined culture conditions: comparison of the effects of tumor necrosis factor alpha, soluble CD40 ligand trimer and interferon gamma. Br. J. Haematol. 114, 444–457.

Naciff, J., Overmann, G., Torontali, S., Carr, G., Tiesman, J., Richardson, B., Daston, G., 2003. Gene expression profile induced by 17 β-ethynyl estradiol in the prepubertal female reproductive system of the rat. Tox. Sci. 72, 314–330.

Ozawa, H., Nakagawa, S., Tagami, H., Aiba, S., 1996. Interleukin-1β and granulocyte-macrophage colony-stimulating factor mediate Langerhans cell maturation differently. J. Invest. Dermatol. 106, 441–445.

Pichowski, J.S., Cumberbatch, M., Dearman, R.J., Basketter, D.A., Kimber, I., 2001. Allergen-induced changes in interleukin 1 beta (IL-1 beta) mRNA expression by human blood-derived dendritic cells: inter-individual differences and relevance for sensitization testing. J. Appl. Toxicol. 21, 115–121.

Sallusto, F., Schaerli, P., Loetscher, P., Schaniel, C., Lenig, D., Mackay, C.R., Qin, S., Lanzavecchia, A., 1998. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. Eur. J. Immunol. 28, 2760–2769.

Sallusto, F., Palermo, B., Lenig, D., Miettinen, M., Matikainen, S., Julkunen, I., Forster, R., Burgstahler, R., Lipp, M., Lanzavecchia, A., 1999. Distinct patterns and kinetics of chemokine production regulate dendritic cell function. Eur. J. Immunol. 29, 1617–1625.

Schwarzenberger, K., Udey, M.C., 1996. Contact allergens and epidermal proinflammatory cytokines modulate Langerhans cell

E-cadherin expression in situ. J. Invest. Dermatol. 106, 553–558.

Shortman, K., Liu, Y.J., 2002. Mouse and human dendritic cell subtypes. Nat. Rev. Immunol. 2, 151–161.

Tuschl, H., Kovac, R., Weber, E., 2000. The expression of surface markers on dendritic cells as indicators for the sensitizing potential of chemicals. Toxic. In Vitro 14, 541–549.

Verhasselt, V., Buelens, C., Willems, F., Groote, D., Haeffner-Cavaillon, N., Goldman, M., 1997. Bacterial lipopolysaccharide stimulates the production of cytokines and the expression of costimulatory molecules by human peripheral blood dendritic cells. J. Immunol. 158, 2919–2925.

Verrier, A.C., Schmitt, D., Staquet, M.J., 1999. Fragrance and contact allergens in vitro modulate the HLA-DR and E-Cadherin expression on human epidermal Langerhans cells. Int. Arch. Allergy Immunol. 120, 56–62.

Wang, B., Feliciani, C., Howell, B.G., Freed, I., Cai, Q., Watanabe, H., Sauder, D.N., 2002. Contribution of Langerhans cell-derived IL-18 to contact hypersensitivity. J. Immunol. 168, 3303–3308.