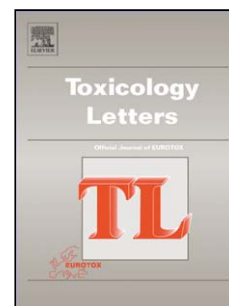


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Chemicals with weak skin sensitizing properties can be identified using low-density microarrays on immature dendritic cells

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Abbreviations: β2M: β2 microglobulin, DC: dendritic cell, DNBS: 2,4-Dinitrobenzenesulfonic acid, Eug: eugenol, Isoeug: isoeugenol, HC: hydroxycitronellal , SDS: sodium dodecyl sulfate

ABSTRACT

A critical step in the induction of allergic contact dermatitis is the interaction of haptens with immature dendritic cells (iDC) leading to their activation. Therefore iDC appear as suitable targets for the evaluation of the sensitizing properties of haptens with the aim of developing *in vitro* toxicologic methods. Here, using a low density cDNA-array, we analyzed the expression of 165 genes related to DC biology in human iDC following a 24 hrs incubation with 4 haptens representative of strong (DNBS), moderate (isoeugenol) and weak (eugenol, hydroxycitronellal) contact sensitizers and with one irritant (SDS). Results show that 21/165 iDC genes were significantly modulated by hapten treatment. Some genes were preferentially modulated by a given chemical. Thus, DNBS, Isoeugenol, Eugenol and Hydroxycitronellal consistently modulated CCR5, CCL27, CCL2 and CCR7, respectively, whereas the CXCL10 gene was regulated by SDS. When subjected to principal component analysis, the 21 target genes fell into 4 groups associated with a particular type of chemical endowed with distinct sensitizing or irritant properties. Thus, gene profiling of iDC using low density microarray allows, for screening of chemicals, the identification of weak haptens with potential skin sensitizing properties. These results suggest that gene profiling of iDC using low density microarrays may be useful to identify chemicals with weak skin sensitizing properties.

INTRODUCTION

Dendritic cells (DC) are the most effective antigen presenting cells and play a pivotal role in the induction of allergic contact dermatitis (Saint-Mezard et al., 2004). Following exposure to a chemical, immature DC (iDC) capture and process antigens and migrate to draining lymph nodes to prime naive T cell precursors. During this process, many biological changes occur in DC including: i) modification of cell surface markers such as CD80, CD86, CD54 and HLA DR (Aiba et al., 1997; Tuschl et al., 2000), ii) production of cytokines and chemokines such as IL1 β , IL6, TNF α (Aiba et al., 1997; Holliday et al., 1996; Lore et al., 1998; Reutter et al., 1997; Tuschl et al., 2001), iii) activation of signal transduction pathways such as the P38 MAPK pathway (Arrighi et al., 2001; Boisleve et al., 2004; Boisleve et al., 2005; Kuhn et al., 1999). Hapten-induced modification of DC phenotype and cytokine expression have been investigated by several groups in the last 15 years to define markers suitable for evaluation of the sensitizing properties of chemicals. Studies have identified IL1 β , CD86, MHC class II and CD54 as candidate molecules because their expression in DC as protein or mRNA transcripts could be modulated by contact sensitizers (Degwert et al., 1997; Pichowski et al., 2001; Pichowski et al., 2002; Reutter et al., 1997; Rougier et al., 2000; Staquet et al., 2004). More recently, Aeby et al., proposed the analysis of IL1 β and aquaporin 3 gene expression combined with flow cytometric analysis of CD86 to characterize the sensitizing potential of chemicals (Aeby et al., 2004). However, these phenotypic and functional studies, that were useful for characterizing strong experimental haptens such as trinitrophenyl (TNP), fluorescein isothiocyanate (FITC) (Guironnet et al., 2000; Rougier et al., 2000), were unable to detect the subtle modifications induced by weak haptens, including fragrances, dyes, and preservatives which represent the majority of haptens relevant to human allergic contact dermatitis (Saint-Mezard et al., 2004).

Gene profiling studies of DC are a more recently available strategy to better characterize the changes occurring in DC following interaction with antigens, since they offer the opportunity to identify the expression of hundreds of individual genes simultaneously. This approach has been used successfully to analyze various biological responses in DC, such as those associated with activation and differentiation (Tang et al., 2004) and the induction of immune responses (Granucci et al., 2001) and tolerogenicity (Griffin et al., 2004; Messmer et al., 2003; Suciu-Foca Cortesini et al., 2001). Studies in contact allergy have explored the transcriptional changes in hapten-exposed DC using pan-genomic microarrays with the ability to identify thousands of

genes and have demonstrated that haptens could potentially activate iDC (Gildea et al., 2006; Ryan et al., 2004a; Schoeters et al., 2005; Schoeters, et al., 2006). Indeed, the moderate/strong hapten DNBS was able to modulate up to 2880 DC genes (Gildea et al., 2006; Ryan et al., 2004a), whereas the moderate hapten Nickel induced several hundred DC gene modifications (Schoeters et al., 2006). These studies confirmed the hypothesis that DC gene profiling could easily detect chemicals endowed with moderate to weak sensitizing properties. However, the pan-genomic technology appears unsuitable for routine toxicological analysis since it requires large amount of mRNA, it is time consuming and has a high cost.

Because haptens can induce a very high number of gene changes in iDC, we postulated that a low density microarray composed of only hundreds of selected gene probes could be sufficient for routine *in vitro* assessment of the sensitizing properties of chemicals. In this study, we used a sensitive, rapid and quantitative low-density transcriptomic assay OLISA™ (OLigo Sorbent Array) (Pichon, 2006), previously reported for its reliability and specificity for gene profiling studies in human keratinocytes (Bonnet-Duquennoy et al., 2006). We selected 165 genes involved in DC biology (proliferation, maturation, migration, antigen presentation, transcription and signalling) and we monitored the changes in gene expression following incubation of iDC with a panel of 4 chemicals representative of strong/moderate (DNBS), moderate (isoeugenol, Isoeug) and weak (eugenol, Eug, hydroxycitronellal, HC) haptens. Our results show that all chemicals induced iDC gene modulation in unrelated donors, involving a limited number of hapten-specific genes. This study confirms the interest of the genomic approach and extends its utilization for the characterization of the sensitizing properties of weak haptens.

MATERIALS AND METHODS

Selection of chemicals

The contact sensitizers used in this study comprised haptens belonging to different classes of chemicals endowed with weak to strong sensitizing properties, as determined by the local lymph node assay (LLNA) (Gerberick. et al., 2005). 2,4-Dinitrobenzenesulfonic acid, sodium salt (DNBS), a water soluble analog of the strong contact allergen 2,4-Dinitrochlorobenzene (DNCB), is endowed with moderate to strong sensitizing properties (Ryan et al., 2002). Isoeugenol (Isoeug) is a moderate allergen whereas Eugenol (Eug) and Hydroxycitronellal (HC) behave as weak allergens (Gerberick et al., 2005). Sodium dodecyl sulfate (SDS) was used as a typical non sensitizing irritant chemical. Lipopolysaccharide S (LPS) was used as a positive control for DC activation and maturation in FACS analysis only.

Generation of MDDC

DC were obtained from monocytes and referred to as “monocyte-derived dendritic cells” (MDDC). Monocytes were isolated from leukocyte-enriched buffy coats from healthy donors using Pancoll (Pan Biotech, Aidenbach, Germany) and Percoll (Pharmacia Bioscience, Uppsala, Sweden) density gradient centrifugation. Monocytes were purified with the negative monocyte isolation kit using magnetic microbeads (Dynal, Invitrogen Corporation, Carlsbad, USA). To obtain MDDC, monocytes were plated ($3 \times 10^6/3$ ml per well) onto 6-well culture plates (Falcon, Becton Dickinson, Franklin Lakes, NJ, USA) in RPMI 1640 medium supplemented with 2 mM glutamine, 50 μ M 2-mercaptoethanol, 100 IU/ml penicillin/streptomycin (Invitrogen Corporation, Carlsbad, USA) and cultured for six days in 800 IU/ml GM-CSF (R&D Systems, Minneapolis, MN, USA) and 1000 IU/ml IL-4 (R&D Systems).

Chemical treatment of DC

After 6 days of differentiation, MDDC were then cultured for an additional 24 hrs either with 1 μ g/ml LPS (*Salmonella minnesota*, Sigma, St Louis, MO, USA) or with the test chemical directly added to the cells without medium renewal (final volume 3 ml / well). Chemicals were initially dissolved in either complete medium or 100% DMSO and subsequent dilutions were performed in cytokine-containing media. All chemicals were used at the highest non-toxic concentration, i.e. the concentration responsible for a mortality of iDC < 20%, as assessed by trypan blue exclusion dye, after a 24 hrs incubation time with each chemical, in 3 iDC cultures

from distinct donors. For those chemicals that were initially dissolved in 100% DMSO, the final in-well DMSO concentration was 0.1%. DNBS (CAS-no.885-62-1) tested at 270 µg/ml and Isoeugenol (CAS-no.97-54-1) tested at 100 µg/ml were purchased from Fluka (Buchs, Switzerland). Eugenol, (CAS-no.97-53-0) tested at 100 µg/ml and Hydroxycitronellal, (CAS-no.107-75-5) tested at 100 µg/ml were purchased from Aldrich (Milwaukee, WI). SDS, (CAS-no.151-21-3) tested at 10 µg/ml was purchased from Sigma (St Louis, MO).

Flow cytometric measurement of cell surface molecule expression

The expression of cell surface molecules was measured by flow cytometry using saturating concentrations of the following fluorochrome-conjugated monoclonal antibodies supplied by BD PharMingen, (San Diego, CA corporation): CD86-fluorescein isothiocyanate (FITC) (clone FUN-1), CD83-FITC (clone HB15e) CD80-FITC (L307.4), CD1a-FITC (HI149), and HLA-DR-FITC (L243). Appropriate conjugated isotype-matched antibodies were used as controls. The fluorescence intensities of the surface markers were measured by using a Coulter Epics® XL2 flow cytometer (Beckman Coulter, Miami, FL). Cut off values were established to define high, intermediate and low expression of markers. Instrument alignment was validated by using flow-check™ beads (Beckman Coulter Inc., Fullerton, CA). Listmode data were analyzed by using EXPO32™ software (Beckman Coulter). Single parameter histograms were produced for at least 10,000 cells and the histograms for each of the cell surface markers were analysed for changes in the Mean Fluorescence Intensity (MFI) of iDC compared to treated iDC. MFI was determined using a 4-decade log-scale beginning at 10⁰. For each treatment, and for a given marker, the phenotypic alterations were quantified by the ratio of the MFI in the presence of the hapten to the MFI in the absence of the hapten. The differences in the MFI were considered as significant when the ratio exceeded 1.25 or was below 0.75 for up-regulated or down-regulated markers respectively.

Nucleic acid extraction and purification

Total RNA extraction was performed with 1 ml of the commercially available RNA extraction mixture, RNAlplus (Q-BIOgene, Illkirch, France), in accordance with the manufacturer's instructions. The RNA template was qualitatively assessed and quantified using an Agilent 2100 Bioanalyser with the RNA 6000 Nano LabChip® Kit (Agilent Technologies,

Palo Alto, CA, USA). The RNA stock solutions were stored at -80°C . Total extracted RNA was treated with RNase-free RQ1 DNase (Promega Corp, Madison, USA) at 37°C for 30 minutes.

OLISA microarray technology

Design, synthesis and spotting of probes

All 60-mer probes for the 165 genes (Table 1), identified by their gene accession number (NCBI source), were optimized based on bioMérieux (Grenoble, France) specific criteria, chemically synthesized for highly efficient coupling oligonucleotides (purity > 95%), and then spotted onto a 96-well microtitre plate (OLISA™) at bioMérieux in a circle format as previously described (Pichon, 2006)

Synthesis of labelled cDNA

10 μg of total RNA were retro-transcribed with the Superscript II Reverse-Transcription kit (Invitrogen Corporation, Carlsbad, USA) in the presence of dNTP-biotin. cDNA were purified on microcon filters following the manufacturer's recommendations (Millipore Corp., Canton, USA).

Principle of OLISA™ technology

The OLigo Sorbent Array (OLISA) is a quantitative low-density transcriptomic assay (Cuzin, 2001) previously described in detail (Pichon et al., 2006). Biotin-labelled targets were denatured with 0.2 N NaOH for 5 minutes, then hybridized to oligonucleotide probes in the presence of a hybridization standard for one hour at 37°C . Wells were washed at room temperature four times with 200 μl of the washing buffer in a fully automated washing system. The detection solution using streptavidin-coupled horseradish peroxidase (Streptavidine-HRP, Immunotech, Marseille, France) was added to each well and incubated at 25°C for 20 minutes. The wells were washed again as described above and 50 μl of the detection solution containing HRP substrate (BioFX Laboratories, MD, USA) were added for 15 minutes at 25°C . Hybridized arrays were then read using Apimager® (bioMérieux, Grenoble, France), a densitometry reading system, and the density ratio measurement was determined by taking into account the normalized diameter and background noise. Spots were considered for analysis if their response was significantly higher than the background noise plus three standard deviations. Data analysis was

performed using proprietary software (ApiAnalyser™). The signals from genes of interest were normalized per well using the signal corresponding to the chosen house-keeping gene, and then the inter-well ratios were calculated independently for each gene. The limit of detection of the OLISA technology is 3 femtomoles which corresponds to $3 \cdot 10^3$ attomoles. The method is capable of detecting any kind of transcripts, provided that their RNA inputs are not less than $3 \cdot 10^3$ attomoles.

Statistical analysis of the microarray data

OLISA™ data were analyzed with analysis of variance (ANOVA) for comparison between vehicle- and chemical-treated iDC. OLISA results were obtained from 3 to 7 donors and only genes tested at least on 3 donors were further considered for statistical analysis. Level of significance was set at $p \leq 0.05$. In our experimental conditions, and according to Pichon et al., variations in gene expression were considered as significant when the ratio exceeded 1.25 or was below 0.75 for up-regulated or down-regulated genes, respectively.

Principal Component Analysis (PCA)

Results from analysis of the microarray data were subjected to Principal Component Analysis, a multivariate statistical method, to obtain a general view of how treatments interact with the genes studied. For this principal component analysis, data obtained with microarray technology were expressed as the mean gene expression level for each gene and each treatment after GAPDH and control (referred to as vehicle-treated iDC) normalization. From a set of genes, the objective was to analyze the relationships between treatments. For this purpose, a correlation circle was created, and correlations between treatments calculated.

RESULTS

Standardization of cultures of immature DC

In order to standardize the DC used for the genomic assays, myeloid DC were derived in vitro from monocytes isolated from healthy human donors and analyzed phenotypically by flow cytometry (Figure 1 and [Table 2](#)). After 6 days of culture, monocyte-derived DC had a phenotype characteristic of immature DC (iDC), i.e. CD1a high, HLA-DR intermediate, CD80 intermediate, CD86 low and CD83 negative ([Figure 1, one representative donor](#)). Of note, some variations in the level of expression of surface antigens were noted between donors. DC cultures that did not meet the specific expression criteria were excluded from further analysis.

Incubation of iDC with Eug, HC and SDS for 24 hrs did not modify the iDC phenotype, as compared to iDC incubated for 24 hrs with medium alone. Incubation of iDC with Isoeug only slightly up-regulated CD83 as compared to DNBS, while it increased the expression of the CD86 marker. In contrast, incubation of iDC with LPS for 24 hrs induced iDC maturation, as shown by up-regulation of CD83, CD86 and CD80 markers ([Table 2](#)).

These data indicate that our culture conditions were appropriate for the development of iDC and that haptens were unable to induce full DC maturation after a short time exposure, i.e. 24 hrs. This is in agreement with recent studies showing that a prolonged time of incubation with the chemicals is necessary for the induction of DC maturation (Staquet et al., 2004).

Selection of candidate genes

The gene profiling studies tested the expression of 165 genes comprising 155 genes involved in DC biology (immunological functions, transcription, signal transduction, trafficking), 5 miscellaneous genes, 4 house-keeping genes (HPRT, GAPDH, S26 ribosomal protein, S40 ribosomal protein) and one negative control (S18 ribosomal subunit) ([Table 1](#)). We have previously optimized the parameters for reproducible analysis of gene expression in iDC using the OLISA™ technology (Bonnet-Duquennoy et al., 2006). Namely, GAPDH was selected as a suitable house-keeping gene for data normalization and use in OLISA and qRT-PCR analysis ([Cluzel-Tailhardat et al., 2007](#)). Preliminary experiments have shown that the intra-experiment variability was low, but revealed variable results obtained with iDC derived from different donors. Therefore, each experimental condition was tested using iDC from at least 3 different donors to assess for reproducibility of the data.

Gene expression profile on chemical-treated iDC

A total of 23 iDC cultures from 23 different donors were tested. Vehicle-treated iDC expressed transcripts for a mean of 119/165 genes tested, i.e. 72% [range :101/165 to 125/165]. Donor to donor variability was low (<20%).

Following 24 hrs incubation with chemicals, the number of genes detected were 127/165 (77%) in DNBS-treated iDC (including 90.5% of common genes expressed by vehicle-treated iDC).. Eug-treated iDC and Isoeug-treated iDC expressed 124/165 (75%) and 106/165 (66%) transcripts, respectively, with 83 % of genes common to vehicle-treated iDC. Finally, HC- and SDS-treated iDC expressed 100/165 (61%) and 81/165 (49%) gene products with 77% and 87% of common vehicle-treated iDC genes, respectively.

Hapten-treatment of iDC induced changes in only 21/165 genes as compared to control iDC cultured with the vehicle alone. Table 3 shows the 21 genes that, after allergen treatment, showed statistically significant up or down-regulation relative to vehicle-treated control iDC. The results were expressed in two different ways to analyze either the significance or the reproducibility of the data. In the first set of results, a gene was scored positive by statistical analysis of the whole microarray data bank, regardless of the number of donors scoring positive (Table 3). For example, although TLR2 was up-regulated in HC-treated iDC in only 25% of donors, it was scored positive. As shown in Table 3, no gene was affected by all the chemicals tested, suggesting that each hapten is able to induce a preferential pattern of iDC activation. This is confirmed by the observation that a given chemical induced the modulation of a limited but chemical-specific set of genes. Although DNBS and Eug modulated similar numbers of genes (8 for DNBS and 10 for Eug), only one gene (Glycam 1) was modulated by both haptens. The same observation holds true for all chemicals. Eug and Isoeug modulated 10 and 2 genes, respectively, with no gene commonly affected by both haptens. Three genes were modulated by HC. Of note, the irritant SDS regulated 8 genes that were not affected by any of the haptens tested here, except TLR2 and CCR5, which were modulated by DNBS, Isoeug, HC and by DNBS, Eug, respectively. For clarity, Table 3 gives the number of times that a particular gene was tested and registered positive for a chemical.

Alternatively, in the second way of expressing the results, a given gene was scored if it was modulated significantly by a given chemical in at least 66% of the experiments, i.e. in iDC recovered from 2 out of 3 donors. As shown in Table 4 a limited number of genes are

reproducibly up or down-regulated by each of the 5 chemicals and therefore can be considered as markers of this chemical. CCL19 and CCR5 were modulated by DNBS. CCL27 and TLR2 were modulated by Isoeug. CCL2, CCR5 and β 1 integrin were regulated by Eug. CCR7 and CD80 were regulated by HC. Finally, SDS significantly modulated only CXCL10.

Principal component analysis (PCA) of the microarray data

The results obtained with analysis of variance as shown in Table 3 considered each chemical treatment and each gene separately. In order to have a more general view of the changes in gene expression obtained by the different treatments, we performed a principal component analysis (PCA) of the microarray data. PCA is a statistical method analyzing data generated for different genes upon several treatments thus allowing a direct correlation between treatments and genes. In this study, PCA was conducted on the 21 genes significantly modulated in iDC by the treatment with the 5 chemicals (Table 3). The correlation circle shown in Figure 2 represents the relationships between treatments along 2 axes. We showed that Eug and HC appear strongly related since they are found in identical directions. DNBS and Isoeug treatments, which are organized in opposite direction, are therefore not related. The position of SDS near the center of this circle indicates that this chemical is not related to any hapten tested.

DISCUSSION

Our data show that gene expression profiling analysis of DC is a sensitive and efficient approach allowing the identification of chemicals endowed with skin sensitizing properties. Moreover, gene microarray using a limited set of genes is sufficient to provide reproducible detection of DC genes relevant to the sensitizing properties of weak haptens, to which normal individuals are frequently exposed.

We demonstrate that different types of chemicals with strong, moderate or low sensitizing properties can induce activation of DC, resulting in the modification of gene expression levels that could be detected by hybridization to OLISA™ probes. All the haptens tested induced DC gene expression changes that could be detected in our assay. The pattern of gene expression profiles in chemical-induced DC activation was hapten-specific and manifested by up-and/or down regulation of a limited set of genes in DC derived from different healthy human donors. As previously reported (Pichowski et al., 2001), the high variability in the response obtained with DC derived from different healthy human donors emphasizes the need for testing DC from at least three different donors, before drawing conclusions on a given chemical.

Our data confirm the hypothesis that gene profiling of hapten-treated iDC is an efficient *in vitro* assay suitable for the characterization of the sensitizing properties of haptens, especially of weak contact sensitizers (Gildea et al., 2006). In this respect, gene profiling studies appear more sensitive than phenotypic and functional *in vitro* studies which, so far, could only reproducibly detect haptens endowed with strong/moderate activities. Moreover, we demonstrated that a low-density transcriptomic array testing for the expression of only 165 genes is sufficient for the routine analysis of the sensitizing properties of various types of chemicals. This technology has a number of advantages over the pan-genomic analysis, including the possibility of testing multiple experimental conditions, screening of a large number of chemicals and of the iDC sensitivity from different human donors.

Remarkably, all types of haptens, ranging from moderate/strong to weak sensitizers, were able to induce gene expression changes in iDC. This suggests that iDC represent a target highly sensitive to the activating properties of haptens, that could be linked either to their adjuvant or antigenic properties. The moderate/strong hapten DNBS induced profound changes in DC with a total of 7 genes modulated in DNBS-treated DC cultures from 7 different donors. Two of these 7 genes (CCL19, CCR5) were regulated in > 66% of the donors and one gene (CCR5) was

modulated in all donors. This high activation property of DNBS was expected and has been previously reported using gene profiling as well as phenotypic studies of DC (Ryan et al., 2004a, Ryan et al., 2004b). Indeed, Ryan et al., reported that DNBS could modulate several thousand genes in DC using pan-genomic analysis (Ryan et al., 2004a).

More importantly, activation of iDC has been observed with weak haptens, such as Eug and HC, which exhibit low pro-inflammatory properties, weak toxicity and are unable to sensitize naive normal mice (Vocanson et al., 2006). From the 3 genes up-regulated by HC in all DC cultures, two of them (CCR7 and CD80) were modulated in 3 out of 4 donors. Eug modulated as many as 10 genes, a number similar to that modulated by DNBS, among which genes encoding CCL2, CCR5 and β 1 integrin were modulated in > 66% of donors. Isoeug, a moderate contact sensitizer, only affected two genes (CCL27 and TLR2) but this was observed in a majority (2/3) of the donors. Finally, the irritant SDS was also responsible for DC activation with modulation of 8 genes, one of which (CXCL10) was consistently up-regulated in 2/3 donors.

Importantly, no single iDC gene out of the 21 different genes modulated by the chemicals tested was affected by all the 5 chemicals. Thus, each chemical induced a specific pattern of modulated genes (Table 3). PCA of expression of the 21 genes in the whole array databank revealed four different profiles discriminating between the 5 chemicals tested (Figure 2). More precisely, each of the five chemicals could be defined by a specific set of one to three genes, which were reproducibly modulated in at least 2 out of 3 donors and therefore could be considered as compound specific (Table 4). That hapten-induced iDC changes are specific implies that chemicals may activate DC through different pathways. However, future studies testing a bigger set of test chemicals and a higher number of donors are required to confirm these observations and to conclude to the usefulness of low-density microarray for the screening of weak haptens.

Although it would have been logical to assume that a common set of genes could identify all skin sensitizing chemicals (and therefore define a “dermatitis gene profile”), our study did not confirm this hypothesis as much as distinct chemicals were able to activate limited but different groups of genes. Does this mean that each individual chemical can activate a unique set of genes and results in a “chemical specific” gene profile ? We cannot answer this question because we have not tested enough chemicals endowed with similar sensitizing properties. Alternatively, our data raise the possibility that qualitative rather than quantitative changes in gene expression make

the differences between moderate/strong and weak haptens. Indeed, our results may be interpreted as a demonstration, at the gene level, of the different biological consequences of skin exposure to haptens endowed with different pro-inflammatory properties. It is somehow expected that the activation pattern of iDC induced by strong/moderate haptens (e.g.DNBS) could differ from that obtained with weak haptens (e.g. eugenol). In this respect, recent studies have shown that nickel, DNFB, DNCB can activate distinct intracellular signalling pathways involving MAP kinases and NF-kB, respectively and resulting in the up-regulation of unique cell surface markers and production of different sets of cytokines (Cruz et al., 2002; Boislevé et al., 2005). Therefore, we postulate that a more extensive analysis may confirm the results of present study and only define a few groups of genes characteristics of the physico-chemical properties of haptens and which could represent a “sensitization potency” gene profile.

CONFLICT OF INTEREST STATEMENT

The authors state no conflict of interest.

REFERENCES

- Aeby, P., Wyss, C., Beck, H., Griem, P., Scheffler, H., and Goebel, C., 2004. Characterization of the sensitizing potential of chemicals by in vitro analysis of dendritic cell activation and skin penetration. *J Invest Dermatol.* 122, 1154-1164.
- Aiba, S., Terunuma, A., Manome, H., and 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.
- Arrighi, J.F., Rebsamen, M., Rousset, F., Kindler, V., and Hauser, C., 2001. A critical role for p38 mitogen-activated protein kinase in the maturation of human blood-derived dendritic cells induced by lipopolysaccharide, TNF-alpha, and contact sensitizers. *J Immunol.* 166, 3837-3845.
- Boisleve, F., Kerdine-Romer, S., and Pallardy, M., 2005. Implication of the MAPK pathways in the maturation of human dendritic cells induced by nickel and TNF-alpha. *Toxicology.* 206, 233-244.
- Boisleve, F., Kerdine-Romer, S., Rougier-Larzat, N., and Pallardy, M., 2004. Nickel and DNCB induce CCR7 expression on human dendritic cells through different signalling pathways: role of TNF-alpha and MAPK. *J Invest Dermatol.* 123, 494-502.
- Bonnet-Duquennoy, M., Abaibou, H., Tailhardat, M., Lazou, K., Bosset, S., Le Varlet, B., Cleuziat, P., and Kurfurst, R., 2006. Study of housekeeping gene expression in human keratinocytes using OLISATrade mark, a long-oligonucleotide microarray and q RT-PCR. *Eur J Dermatol.* 16, 136-140.
- Caux, C., Massacrier, C., Vanbervliet, B., Dubois, B., Durand, I., Cella, M., Lanzavecchia, A., and Banchereau, J., 1997. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to granulocyte-macrophage colony-stimulating factor plus tumor necrosis factor alpha: II. Functional analysis. *Blood* 90, 1458-1470.
- Cluzel-Tailhardat, M., Bonnet-Duquennoy, M., Abaibou, H., Pelle de Queral, D., Vocanson, M., Lazou, K., Kurfurst, R., Courtellemont, P., and Le Varlet, B., 2007. Which house-keeping genes for dendritic cells transcriptomic studies. *Eur J Dermatol.* 17, in press.
- Cruz, M.T., Duarte, C.B., Gonçalo, M., Figueiredo, A., Carvalho, A.P., and Lopes, M.C., 2002. Differential activation of nuclear factor kappa B subunits in a skin dendritic cell line in response to the strong sensitizer 2,4-dinitrofluorobenzene. *Arch Dermatol Res* 294, 419-425.
- Degwert, J., Steckel, F., Hoppe, U., and Kligman, L.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. *Toxicol.in vitro* 11, 613-618.

Gerberick, G. F., Ryan, C. A., Kern, P. S., Schlatter, H., Dearman, R. J., Kimber, I., Patlewicz, G. Y., and Basketter, D. A., 2005. Compilation of historical local lymph node data for evaluation of skin sensitization alternative methods. *Dermatitis* 16, 157-202.

Gildea, L.A., Ryan, C.A., Foertsch, L.M., Kennedy, J.M., Dearman, R.J., Kimber, I., and Gerberick, G.F., 2006. Identification of Gene Expression Changes Induced by Chemical Allergens in Dendritic Cells: Opportunities for Skin Sensitization Testing. *J Invest Dermatol.* 126, 1813-1822.

Granucci, F., Vizzardelli, C., Virzi, E., Rescigno, M., and Ricciardi-Castagnoli, P., 2001. Transcriptional reprogramming of dendritic cells by differentiation stimuli. *Eur J Immunol.* 31, 2539-2546.

Griffin, M.D., Xing, N., and Kumar, R., 2004. Gene expression profiles in dendritic cells conditioned by 1alpha,25-dihydroxyvitamin D(3) analog. *J Steroid Biochem Mol Biol.* 89-90, 443-448.

Guironnet, G., Dalbiez-Gauthier, C., Rousset, F., Schmitt, D., and Peguet-Navarro, J., 2000. In vitro human T cell sensitization to haptens by monocyte-derived dendritic cells. *Toxic. In Vitro* 14, 517-522.

Holliday, M.R., Dearman, R.J., Corsini, E., Basketter, D.A., and Kimber, I., 1996. Selective stimulation of cutaneous interleukin 6 expression by skin allergens. *J. Applied Toxicol.* 16, 65-70.

Kuhn, U., Brand, P., Willemsen, J., Jonuleit, H., Enk, A.H., van Brandwijk-Petershans, R., Saloga, J., Knop, J., and Becker, D., 1999. Induction of tyrosine phosphorylation in human MHC class II-positive antigen-presenting cells by stimulation with contact sensitizers. *J Immunol.* 160, 667-673.

Lore, K., Sonnerborg, A., Spetz, A.L., Andersson, U., and Andersson, J., 1998. Erratum to "Immunocytochemical detection of cytokines and chemokines in Langerhans cells and in vitro derived dendritic cells". *J Immunol Methods.* 218, 173-187.

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

Pichon, J.S., 2006. Multiplex degenerate PCR coupled with an oligo sorbent array for human endogenous retrovirus expression profiling. *Nucleic Acids Res.* 34 (6), e46.

Pichowski, J.S., Cumberbatch, M., Dearman, R.J., Basketter, D.A., and 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 (2), 115-121.

Pichowski, J.S., Cumberbatch, M.D., Basketter, D.A., and Kimber, I., 2002. Investigation of induced changes in interleukin 1beta mRNA expression by cultured human dendritic cells as an in vitro approach to skin sensitization testing. *Toxicol In Vitro* 14 (4), 351-360.

- Reutter, K., Jager, D., Degwert, J., and Hoppe, U., 1997. In vitro model for contact sensitization : II. Induction of IL-1 β mRNA in human blood-derived dendritic cells by contact sensitizers. *Toxicol.in vitro* 11, 619-626.
- Rougier, N., Redziniak, G., Mougin, D., Schmitt, D., and Vincent, C., 2000. In vitro evaluation of the sensitization potential of weak contact allergens using langerhans-like dendritic cells and autologous T cells. *Toxicology* 145, 73-82.
- Ryan, C.A., Gildea, L.A., Hulette, B.C., Dearman, R.J., Kimber, I., and Gerberick, G.F., 2004a. Gene expression changes in peripheral blood-derived dendritic cells following exposure to a contact allergen. *Toxicol Lett.* 150, 301-316.
- Ryan, C.A., Hulette, B.A., Gildea, L.A., Dearman, R.J., Kimber, I., and Gerberick, G.F., 2004b. Examination of phenotypic changes in peripheral blood derived dendritic cells following exposure to a contact allergen : cell surface marker and gene expression. *Cutaneous and ocular toxicology* 23, 91-104.
- Ryan, C. A., Cruse, L. W., Skinner, R. A., Dearman, R. J., Kimber, I., and Gerberick, G. F., 2002. Examination of a vehicle for use with water soluble materials in the murine local lymph node assay. *Food chem Toxicol* 40, 1719-1725.
- Saint-Mezard, P., Rosieres, A., Krasteva, M., Berard, F., Dubois, B., Kaiserlian, D., and Nicolas JF, 2004. Allergic contact dermatitis. *Eur J Dermatol.* 14, 284-295.
- Schoeters, E., Verheyen, G.R., Van Den Heuvel, R., Nelissen, I., Witters, H., Van Tendeloo, V.F., Schoeters, G.E., and Berneman, Z.N., 2005. Expression analysis of immune-related genes in CD34(+) progenitor-derived dendritic cells after exposure to the chemical contact allergen DNCB. *Toxicol In Vitro.* 19, 909-913.
- Schoeters, E., Nuijten, J. M., Van Den Heuvel, R. L., Nelissen, I., Witters, H., Schoeters, G. E., Van Tendeloo, V. F., Berneman, Z. N., and Verheyen, G. R., 2006. Gene expression signatures in CD34+-progenitor-derived dendritic cells exposed to the chemical contact allergen nickel sulfate. *Toxicol.Appl.Pharmacol.*
- Staquet, M.J., Sportouch, M., Jacquet, C., Schmitt, D., Guesnet, J., and Peguet-Navarro, J., 2004. Moderate skin sensitizers can induce phenotypic changes on in vitro generated dendritic cells. *Toxicol In Vitro.* 18, 493-500.
- Suciu-Foca Cortesini, N., Piazza, F., Ho, E., Ciubotariu, R., LeMaoult, J., Dalla-Favera, R., and Cortesini, R., 2001. Distinct mRNA microarray profiles of tolerogenic dendritic cells. *Hum Immunol.* 62, 1065-1072.
- Tang, Z. and Saltzman, A., 2004. Understanding human dendritic cell biology through gene profiling. *Inflamm Res.* 53, 424-441.
- Tuschl, H. and Kovac, R., 2001. Langerhans cells and immature dendritic cells as model systems for screening of skin sensitizers. *Toxicol In Vitro* 15 (4-5), 327-331.

Tuschl, H., Kovac, R., and Weber, E., 2000. The expression of surface markers on dendritic cells as indicators for the sensitizing potential of chemicals. *Toxicol In Vitro* 14 (6), 541-549.

Vocanson, M., Hennino, A., Cluzel-Tailhardat, M., Saint-Mezard, P., Benetiere, J., Chavagnac, C., Berard, F., Kaiserlian, D., and Nicolas, J.F., 2006. CD8+ T cells are effector cells of contact dermatitis to common skin allergens in mice. *J Invest Dermatol.* 126, 815-820.

Figures legend

Figure 1. Phenotypic analysis of iDC. Cell surface marker expression in un-treated immature DC (iDC) and hapten- or LPS-treated iDC were studied by flow cytometry. Cytometry overlay histograms of isotype control (hollow) and untreated or treated iDC (shaded) cells for each of the surface markers examined. X-axis represents the fluorescence intensity over a four decade log scale; Y-axis represents the number of cells.

Figure 2. Principal component analysis (PCA) of the microarray derived data. 21 genes significantly regulated in microarray data were subjected to PCA. The figure represents the projection of the different treatments on the correlation circle showing relationships between each of them. The position of treatment on the scatter plot determine correlations between each treatment in relation with their gene expression levels for the set of 21 genes studied. Treatments positioned in the same direction are correlated and those in opposite or divergent directions are unrelated.

Figure 1.

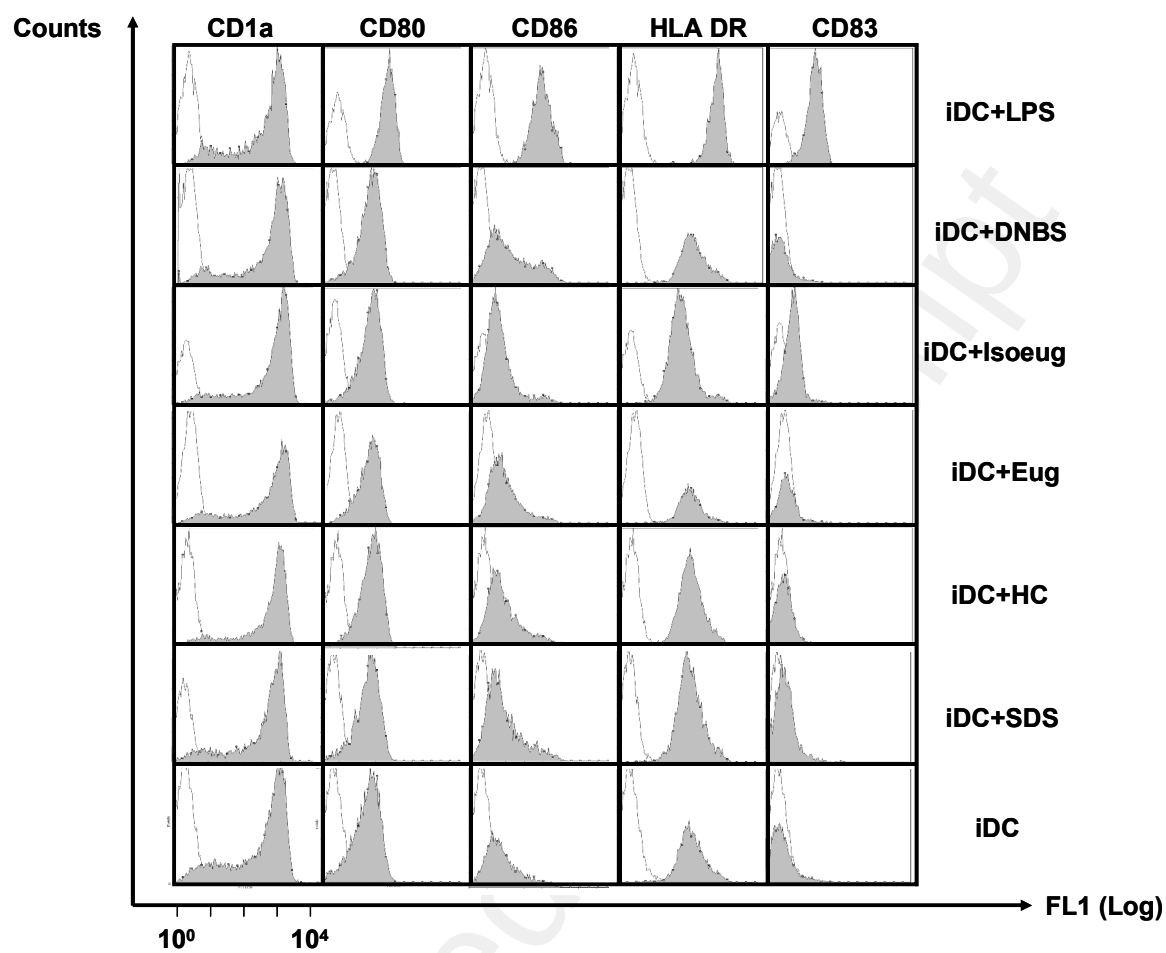


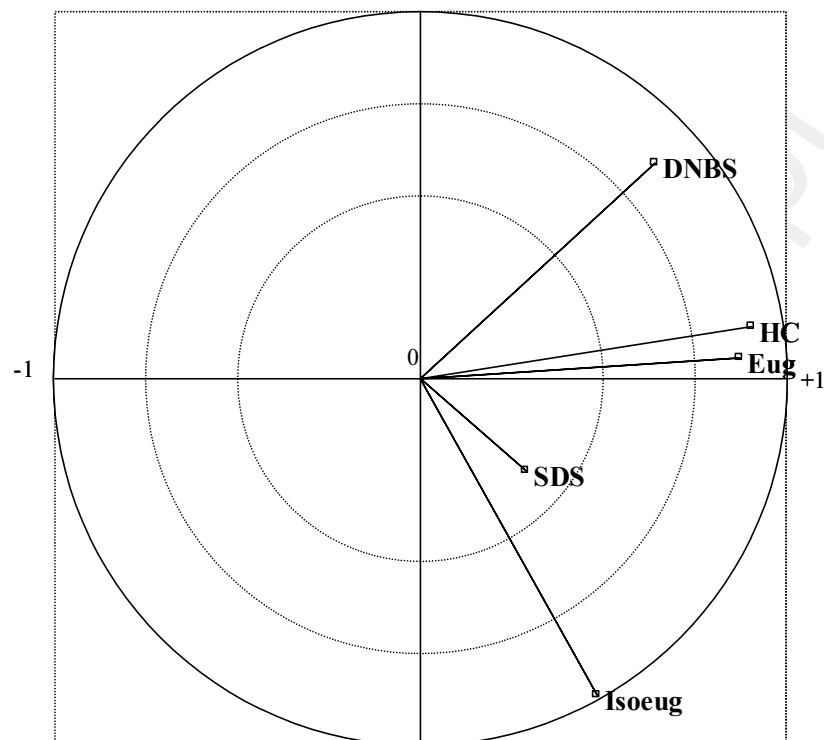
Figure 2.

Table 1. Composition of the low density microarray

Accession number (NCBI source)	Gene Symbol	Common names	Accession number (NCBI source)	Gene Symbol	Common names
House-keeping genes			Stress and apoptosis		
M31642	HPRT		X15480	GSTP1	
X69654	26S		U34683	GSS	GSH synthetase
M33197	GAPDH	G3PD, GAPD	X64177	MT1	
NM_001013	40S		X97260	MT2	
X03205	18S		J04026	TXN	
Proliferation			AF270920	TXN2	
M14694	P53		AF183420	PMSR	
BC039815	P63	P73-like	AF308602	Notch 1	
Y11416	P73	P53-related protein	S90469	CYP P450	
X65550	KI-67		AY028632	Catalase	
NM_002592	PCNA		U37518	TRAIL	TNFSF10
AF015950	hTRT	Telomerase associated protein	L22473	BAX	Bcl2 associated X protein
L27211	P16	Cyclin dependent inhibitor	U58334	Bcl2	
S67388	P21	CIP1, WAF1, CDKN1A	Z23115	Bcl-xL	
Z12020	mdm2	P53-binding protein mdm2	Z23116	Bcl-xS	
Chemokines and receptors			M67454	FasL	
NM_002982	CCL2	MCP1, SCYA2	X89101	Fas LR	
NM_002984	CCL4	MIP1 beta, SCYA4	AF376061	CASP12	
M21121	CCL5	RANTES, SCYA5	AF100928	AIF1	
U59808	CCL13	MCP4, SCYA4	AF149794	APAF1	
NM_002987	CCL17	TARC, SCYA17	D29011	PSMB5	
NM_002988	CCL18	PARC, MIP4, SCYA18	D00759	PSMA1	
U77180	CCL19	MIP 3 b, SCYA19	NM_015089	PARC	
NM006274	CCL20	MIP 3a, SCYA20, LARC	AB020027	Hsp27	
NM_002990	CCL22	MDC, SCYA22, STCP-1	X06985	Hsp32	Heme oxygenase 1
AJ243542	CCL27	CTACK, SCYA27	AF087870	Hsp40	DNAJ
BC013615	CXCL8	IL8, SCYB8	X61598	Hsp47	Serpin H2
NM_001565	CXCL10	IP10, SCYB10	M34664	Hsp60	GROEL
U28694	CCR3	Eosinophil cation receptor	AF352832	Hsp70	
U57840	CCR5	CD195	NM_016292	Hsp75	TRAP1 (TNF receptor associated protein 1)
NM_004367	CCR6	LARC receptor	X15183	Hsp90A	
NM_001838	CCR7	CDW197, EB11	M16660	Hsp90B	
NM_001504	CXCR3	CD183, IP10-R, Mig-R	L09210	iNOS	NOS
AF348491	CXCR4	CD184, NPY3R, fusin,	X02317	SOD1	
NM_032966	CXCR5	MDR15	M36693	SOD2	
M28983	IL1a	Hémanopoiétine 1	NM_001909	CPSD	
M63099	IL1 RA	IL1RN	D21243	HMOX2	
U25676	IL2	TCGF (T cell growth factor)	Y00433	GPX 1	
NM_002183	IL3RA	CD123	X15722	GSR 1	
M13982	IL4	BSF1 (B cell stimulating factor)	M25627	GST A1	
X04688	IL5	EDF (eosinophil differentiation factor)	U90313	GSTH	
M54894	IL6	IFN b2	BC024005	GSTM1	
AJ029171	IL10	CSIF (cytokin synthase inhibitor factor)	Cytokines and hormones		
Translation and cell signaling			AF180563	IL12P40	
NM_002228	AP-1	c-jun	L06801	IL13	
NM_005354	Jun D		AY 044641	IL18	IFNg inducing factor
NM_002229	Jun B		M54886	IFN a	
NM_005252	c-fos		M25460	IFN b	
L16785	c-myc	transcription factor p64	X02812	TGF b1	TGF b
NM_002502	NFKB p100		Structure		
M62399	NFKB p65	RelA	NM_004925	AQP3	
NM_003998	NFKB p50	NFKB, NFKB P105	NM_001170	AQP7	
NM_004364	CEBPA		NM_181501.1	ITGA1	CD49a, VLA1
NM_005194	CEBPB		NM_002203	ITGA2	CD49b, VLA2
U48866	CEBPE		M59911	ITGA3	CD49c, VLA3
NM_001806	CEBPG		L12002	ITGA4	CD49d, VLA4
NM_138473.2	SP1	TSFP1, Sp1	X53586	ITGA6	CD49f, VLA6
M97190	SP2	Sp2	NM_002209	ITGAL	CD11a, LFA1
NM_006238	PPARD	PPARB (peroxisome proliferative activated receptor)	NM_00632	ITGAM	CD11b
D83233	PPARG2		NM_002208	ITGAE	CD103
L35253	P38 MAPK	MAPK14, SAPK2A	X07979	ITGB1	CD29, VLAB
XM_498102	Ras		BC005861	ITGB2	CD18
X60188	ERK1	MAPK3, P41ERK1	NM_000212	ITGB3	CD61
M84489	ERK2	MAPK1, P42MAPK	NM_000887	ITGAX	CD11c
U17743	JNK1	MAPK8, SAPK1	AF011333	DEC205	CD205, LY75
X03484	RAF	RAF1	AJ489592	GLYCAM1	
NM_002738	PKCB	PRKCB1	X05232	MMP3	stromelysin 1, progelatinase
L07032	PKC theta		NM_002423	MMP7	matrilysin
U08015	NFATC1	NFAT2 (nuclear factor of activated T cell)	J05070	MMP9	gelatinase B
AF290985	SLAP	SLAP1	Antigen presentation		
AY040554	SLAM	CD150, SLAMF1, CDw150	NM_001766	CD14	
X14454	IRF1		NM_001250	CD40	
U88540	TLR1	CD281	J04536	CD43	sialophorin, gpL115
U88878	TLR2	CD282	NM_002162	CD50	ICAM3, CDw50
U88879	TLR3	CD283	J03132	CD54	ICAM1
U88880	TLR4	CD284	Y00636	CD58	LFA3
AB060695	TLR5		NM_005191	CD80	B7-1
AB020807	TLR6		NM_004233	CD83	
AF245702	TLR7		M60334	HLADRA	
AF245703	TLR8		M20503	HLADRB1	
AF245704	TLR9		AF290886	DC-SIGN	CD209
AF296673	TLR10		Z22555	CLA1	CD361.1, SCARB1 (scavenger receptor B1)
Miscellaneous			Z13009	CDH1	
Z71389	DEFB4	SAP1	AB021288	B2M	
X06234	MRP8	S100A8			
X06233	MRP14	S100A9			
Z18538	SKALP	PI3 (protease inhibitor 3), elafin			
AF016535	MDR1	CD243, ABCB1			

Table 2. Variation of expression of iDC surface markers by chemical treatment

	CD1a	CD80	CD86	HLA-DR	CD83
iDC+LPS	0,7	3,3	5,7	3,4	25,8
iDC+DNBS	0,9	1,2	1,8	1,1	0,8
iDC+Isoeug	1,0	1,2	0,8	0,8	2,2
iDC+Eug	1,1	1,0	0,8	0,9	1,1
iDC+HC	1,1	1,2	0,9	0,9	0,6
iDC+SDS	1,0	1,0	1,0	0,9	1,1

Results are expressed as the ratio of the MFI in chemical-treated iDC to the MFI in untreated iDC. Data are from one representative donor. The differences were considered significant when the ratio exceeded 1.25 or was below 0.75 for up-regulated or down-regulated markers respectively.

Table 3. iDC genes significantly regulated by chemicals

	Number of positive donor response / Number of donors tested					
Gene symbol	NCBI Accession Number n°	DNBS	Isocug	Eug	HC	SDS
PROLIFERATION						
P63	BC039815			(b) - (3/7)		
hTRT	AF015950					- (2/7)
CHEMOKINES						
CCL2	NM_002982			- (5/7)		
CCL19	U77180	(a) + (5/7)		+ (2/6)		
CCL27	AJ243542		+ (2/3)			
CCL13	U59808	+ (2/7)		+ (2/7)		
CCR5	U57840	+ (4/4)		+ (3/4)		- (3/6)
CCR7	NM_001838	+ (3/7)			+ (3/4)	
CXCL8	BC013615					- (2/4)
CXCL10	NM_001565					+ (4/6)
ANTIGEN PRESENTATION						
CD80	NM_005191				+ (3/4)	
CD40	NM_001250					- (2/7)
Beta 2 microglobulin	AB021288			+ (2/7)		
CELL ADHESION						
E-Cadherin	Z13009	+ (2/4)				
Beta 1 Integrin	X07979			+ (3/3)		
Alpha 6 Integrin	X53586			+ (2/7)		
Glycam 1	AJ489592	+ (1/4)		+ (2/5)		
SIGNALING						
TLR4	U88880			+ (2/7)		
TLR2	U88878	+ (4/7)	+ (4/6)		+ (1/4)	+ (2/7)
MISCELLANEOUS						
MRP 14	X06233					- (2/4)
SKALP	Z18538					- (1/4)

(a) + : up-regulation

(b) - : down-regulation

Table 4. iDC genes reproducibly regulated by chemicals

CHEMICAL	DNBS	Isoeug	Eug	HC	SDS
CCL2	(a) ↑ (71%) ^(b)		↓ (71%)		
CCL19		↑ (66%)			
CCL27	↑ (100%)		↑ (75%)		
CCR5				↑ (75%)	
CCR7					↑ (66%)
CXCL10				↑ (75%)	
CD80			↑ (100%)		
Beta 1 Integrin		↑ (66%)			
TLR2					

(a) : up- (↑) or down- (↓) regulation

(b) : percent of positive donor response

The list gives the names of genes regulated by chemicals in > 66% of donors.