

Profiling of genes expressed in human monocytes and monocyte-derived dendritic cells using cDNA expression array

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Summary. Using a human cDNA expression array, we obtained expression profiles of 588 genes in CD14⁺ monocytes and monocyte-derived dendritic cells (DCs). Overall, 22 genes were upregulated, and nine genes were downregulated in DCs of both samples from two different individuals. Many of the genes that were upregulated in DCs encode proteins that are related to differentiation, cell structure, migration, termination of cell cycle as well as proliferation, e.g. tumour necrosis factor- α (TNF- α), tumour necrosis factor receptor II (TNFRII), thymosin β -10, epithelial discoidin domain receptor 1, replication factor C, putative transcription factor DB1, alpha catenin, transforming growth factor- β 1, prohibitin, p53-regulating protein and neu differentiation factor. Among the downregulated genes in DCs were genes that encode proteins of cell cycle

regulation: mitotic growth and transcription activator, platelet-derived growth factor receptor- β subunit, interleukin 2 receptor (IL-2R)- γ subunit, IL-7R- α subunit, leucocyte interferon- γ (IFN- γ) and granulocyte-macrophage colony-stimulating factor receptor (GM-CSFR). Semi-quantitative reverse transcription-polymerase chain reaction method confirmed the upregulated expression levels in DCs for TNFRII, TNF- α , alpha catenin and downregulation of IFN- γ , GM-CSFR on four different donor samples of DCs and monocytes. Moreover, our data show the presence of a 'switch-on' step for the TNF- α and TNFRII gene expression in immature DCs for further differentiation into mature DCs.

Keywords: dendritic cell, monocyte, differentiation, expression profiling.

Dendritic cells (DCs) are heterogeneous cell populations with distinctive morphology and are distributed ubiquitously in various tissues. DCs originate from the bone marrow, and their precursors migrate via the bloodstream to almost all organs of the body (Steinman, 1991). At present, known DC populations include interdigitating reticulum cells in lymphoid organs (Bjorck *et al.*, 1997), blood DCs (O'Doherty *et al.*, 1993; Thomas *et al.*, 1993), Langerhans cells in the epidermis of the skin (Rowden *et al.*, 1977; Romani *et al.*, 1989) and dermal DCs (Cerio *et al.*, 1989). They capture and process antigens (Ag) in non-lymphoid tissues and migrate to T-cell-dependent areas of secondary lymphoid organs through afferent lymph or the bloodstream to prime naive T cells and initiate the immune response. During this process, DCs lose their Ag-capturing/processing ability as they differentiate into mature, fully

stimulatory antigen-presenting cells (Austyn, 1996). Although there is considerable interest in DCs, their very low frequency in blood and tissues has made the isolation and further study of their biology difficult (Reid, 1998). A recent major forward step has been the generation of a large number of DCs *in vitro* either from CD34⁺ haemopoietic progenitor cells or from peripheral blood (PB) CD14⁺ monocytes using different combinations of cytokines (Peters *et al.*, 1993; Romani *et al.*, 1994; Sallusto & Lanzavecchia, 1994; Zhou & Tedder, 1996).

In spite of the rapid progress in the field of generating a large number of DCs, the molecular biology of differentiation and maturation of DCs remains unclear (Hart, 1997). In the few past years, several approaches have been used for monitoring the expression of genes in DCs, such as quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis (DeSaint-Vis *et al.*, 1998), differential display (DD), random sequencing of cDNA libraries (Marland *et al.*, 1997) and serial analysis of gene expression (SAGE) (Hashimoto *et al.*, 1999). All these techniques have

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their own advantages and disadvantages. The conventional RT-PCR is a popular, accurate and sensitive method of quantifying transcription levels of rare mRNA, but is inherently too slow and cumbersome to provide the parallel information about thousands of genes required to generate a global gene expression profile (Spinella *et al*, 1999). DD has been used widely because it can provide data on novel genes and is relatively fast, inexpensive, sensitive and simple to perform, although the major drawbacks of the method are the lack of quantitative precision and reproducibility (Baldwin *et al*, 1999). Moreover, the excised band from a DD gel usually represents several species of cDNA, which leads to a more complicated analysis (Spinella *et al*, 1999). A recently developed method called SAGE is a powerful tool for monitoring a large number of known and novel mRNA transcripts simultaneously. A major drawback of SAGE is its reliance on PCR amplification to generate di-tags, which compromises the quantitative aspects of the method. In SAGE, the frequency of tag isolation is influenced not only by the starting frequency of the mRNA templates, but also by the intrinsic PCR amplification efficiency of the individual tag sequences. Moreover, SAGE is sequencing intensive and, at present, quite a laborious procedure.

To begin to understand how the altered expression of a specific gene plays an integral role in a biological process of differentiation of monocytes into DCs, one can use the recently developed high-throughput technique called cDNA expression array analysis. Profiling of 588 known genes that have been reported to play key roles in many different biological processes yields highly informative results in a single hybridization and allows large-scale comparison of multiple nucleic acid sequences (Sehgal *et al*, 1998). Because of the wide spectrum of genes spotted on a nylon membrane (e.g. genes encoding cell cycle regulators, transcription factors, growth factors, interleukins, interferon receptors and cell surface antigens), the cDNA array technology is well suited to profiling general gene expression changes as cells undergo differentiation. In this study, we developed a non-radioactive approach for the visualization of an expression array and identified the complex genetic alteration involved in the differentiation of DCs from CD14⁺ PB monocytes in 7-d cultures in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4. Such an approach provides not only an insight into the underlying mechanism of differentiation but also the opportunity to identify specific genes that can be relevant to the development of certain human disorders in which DCs or monocytes are involved.

MATERIALS AND METHODS

Cell preparation. Human monocytes were isolated from normal peripheral blood mononuclear cells (PBMCs) by magnetic bead sorting with anti-CD14 monoclonal antibody (mAb) (MACS, Milteny Biotec, Germany). DCs were prepared from these monocytes by culturing for 7 d in AIM medium (Gibco BRL, Rockville, MD, USA) containing 10% fetal calf serum (FCS; Hyclone, Logan, UT, USA) in the presence of GM-CSF (400 U/ml; Kirin Brewery, Gunma, Japan) and IL-4

(400 U/ml; Becton Dickinson Labware, MA, USA). DCs used in this study strongly expressed major histocompatibility complex (MHC) class I, class II, CD80, CD1b, CD1c and CD1d, and significantly expressed CD86 and CD1a.

RNA isolation and DNase treatment. Twelve total RNA samples (from DCs and CD14⁺ PB monocytes from six donors) were extracted from cells by the isothiocyanate method using TRIzol reagent (Gibco BRL) according to the manufacturer's protocol. A total RNA sample (20 µg) was incubated for 15 min at 37°C with 1 unit of DNase I (Wako Nippon Gene, Tokyo, Japan) in a final volume of 50 µl containing 40 mmol/l Tris-HCl (pH 7.9), 10 mmol/l NaCl, 6 mmol/l MgCl₂, 10 mmol/l NaCl, 10 mmol/l CaCl₂, 2 mmol/l dithiothreitol (DTT) and 0.2 mg/ml bovine serum albumin (BSA).

Reverse transcription. A total RNA sample (2 µg) was mixed with 5 µmol/l Oligo dT₁₈ primer (Ambion, Austin, TX, USA) and heated at 70°C for 3 min, then cooled for 3 min on ice. The reaction mixture (total volume 20 µl) contained 4 µl of 5 × first-strand buffer (Gibco BRL), 2.5 µl of 0.1 mol/l DTT, 1 µl of a 10 mmol/l mixture of dNTPs (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and 400 units of reverse transcriptase SuperScript II (Gibco BRL). Reverse transcription was performed at 42°C for 1 h and terminated by heating at 92°C for 10 min.

Amplification of target DNAs. A cDNA mixture (3 µl) was amplified by PCR with 2 mmol/l MgCl₂, 200 µmol/l each dNTP, 0.02 µmol/l gene-specific primer mixture supplied with human Atlas cDNA expression array (Clontech, Palo Alto, CA, USA) and 1 unit of *Taq* Gold DNA polymerase (PE Biosystems, Foster City, CA, USA). Temperature conditions for PCR were as follows: heating to 95°C for 10 min, 40°C for 5 min, 72°C for 5 min; 94°C for 2 min, 40°C for 5 min, 72°C for 5 min; then 23 cycles at 94°C for 1 min, 50°C for 1 min and 72°C for 2 min and, finally, 72°C for 7 min. PCR products were phenol-chloroform purified and ethanol precipitated.

Labelling of target cDNAs, hybridization, detection and quantification analysis. Amplified target DNAs were labelled with alkaline phosphatase using a commercial kit (Amersham Pharmacia Biotech). Then, a cDNA expression array (human Atlas expression array; Clontech) was prehybridized for 1 h at 50°C with 10 ml of hybridization buffer (Amersham Pharmacia Biotech). The solution was then replaced with 15 ml of a fresh hybridization solution containing a denatured probe (20 ng/ml), and the mixture was incubated at 50°C overnight in an air incubator. After several washes (according to the manufacturer's recommendations), chemiluminescent signals were detected with CDP-Star reagent (Amersham Pharmacia Biotech). Membranes were exposed to X-ray films for 1 h. Then, the films were subjected to a scanner (Molecular Imager FX; Bio-Rad, Hercules, CA, USA). Subsequent quantification analysis was carried out using commercial software (Array Gauge; Fuji Film, Tokyo, Japan).

Semi-quantitative RT-PCR. In order to confirm the upregulation of selected genes, 1 µg of total RNA was mixed with 450 ng of random hexanucleotide primers, heated for 3 min to 70°C and chilled on ice. Then, 1 ×

first-strand buffer, 0.01 mol/l DTT, a 400 µmol/l mix of each dNTP and 200 units of reverse transcriptase SuperScript II (Gibco BRL) were added, and the mixture was incubated at 42°C for 1 h. Multiplex relative RT-PCR quantification was performed using a QuantumRNA 18S internal standards kit (Ambion); the gene-specific primer sequences are listed in Table I. cDNA was amplified by PCR containing 1 µl of the first-strand cDNA reaction mixture, 2 mmol/l MgCl₂, 200 µmol/l each dNTP, 0.5 µmol/l each oligonucleotide primer and 1 unit of *Taq* Gold DNA polymerase (PE Biosystems). The linear range of PCR product accumulation was determined for every pair (see Table I). Temperature conditions for the PCR were as follows: heating to 95°C for 10 min, then 22–28 (depending on the primer pair) cycles at 95°C for 1 min, 60°C for 1 min and 72°C for 1 min. A PCR mixture (4 µl) was analysed in 5% acrylamide gel, which was stained with Sybr Gold (Molecular Probes, Eugene, OR), and the intensity of the signals was quantified (Molecular Imager FX system; Bio-Rad).

RESULTS

Differential hybridization with a human cDNA expression array
To identify differences in gene expression between CD14⁺ PB monocytes and DCs, we compared the hybridization patterns of both cell types derived from two healthy donors and confirmed the hybridization signals by semi-quantitative RT-PCR in another four donors.

For each of four hybridizations (two for DCs and two for monocytes from two donors), we used a new cDNA array membrane. Figure 1 shows the hybridization patterns of DCs and CD14⁺ PB monocytes. On average, about 216 genes (37%) out of 588 genes spotted onto the membrane were detected. The remaining 372 genes did not show any signals.

Genes upregulated in DCs compared with CD14⁺ PB monocytes and genes downregulated in DCs after differentiation are listed in Table II. The differences in gene

expression levels were essentially the same between samples from different donors.

The expression profile showed that only part of the genes clearly changed their expression levels. We detected upregulation of 22 genes and downregulation of nine genes in differentiated DCs in both samples from two different individuals. The mRNA transcripts of highly expressed genes specifically detected in DCs and almost absent in monocytes include those that encode for the tumour necrosis factor-α (TNF-α), DNA repair protein RAD51 homologue, tyrosine kinase, X-ray repair protein complementing defective repair protein in Chinese hamster cells and adenosine A1 receptor.

In contrast, we observed two genes whose expression was clearly absent in DCs but present in CD14⁺ monocytes (downregulated in DCs): genes encoding JNK activating kinase 1 and IL-7R-α subunit.

The expression of housekeeping genes remained relatively constant: eight out of the nine genes spotted on the membrane were detected. Among these, the hybridization signals of six genes were of similar intensities between monocytes and DCs.

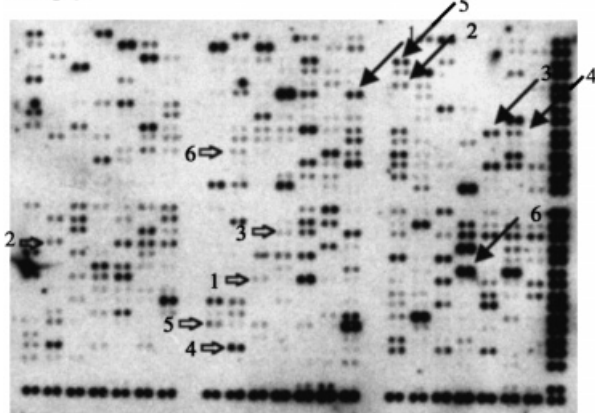
In addition, we checked the reproducibility of expression patterns of monocytes and DCs from two different donors. Among hybridization signals from the monocytes of the two different individuals, 15 out of 216 genes exhibiting positive signals (7%) showed intensity differences that were not similar. On the other hand, for the DCs exhibiting positive signals from two donors, the signal intensities of 32 out of 216 genes (15%) were not reproducible. The latter may be explained by the individual differences in genetic background and relative heterogeneity in the differentiating population of DCs. Reprobing hybridization with the same probe (as used for the DC sample) on the same stripped membrane showed that the intensities of most (95%) signals were reproducible.

To evaluate whether the variation between experiments was the result of differences between the probes or

Table I. Oligonucleotide primers used for relative RT-PCR analysis.

Gene name	Primer sequence	Length (nt)	PCR product size (bp)	PCR annealing temperature (°C)
Alpha catenin	5'-GATGGAGATGACAGACTTTACCCGAGG-3'	27	342	65
	5'-TAGGATGCCTTCACTGTCTGCACCAC-3'	26		
Human TNF gene	5'-TCTCGAACCCGAGTGACAA-3'	20	126	65
	5'-TATCTCTCAGCTCCACACCA-3'	20		
Human IFNγ	5'-AGTTATATCTTGGCTTTTCA-3'	20	355	55
	5'-ACCGAATAATTAGTCAGCTT-3'	20		
GM-CSFR alpha	5'-CCAGGACCTATCAGAAGCTGTCTGTACCTG-3'	29	305	65
	5'-GACGATGCCACAGACAAGGGTTCCACG-3'	28		
Human TNFR I	5'-ATTTGCTGTACCAAGTGCCACAAAGGAACC-3'	30	586	65
	5'-GTCGATTTCACACAAACATGGAGTAGAGC-3'	30		
Human TNFR II	5'-GAATACTATGACCAGACAGCTCAGATGTGC-3'	30	402	65
	5'-TATCCGTGGATGAAGTCGTGTTGGAGAACG-3'	30		
Zinc finger X-chromosomal protein	5'-CCCTGATGGACATCCTTTGACTGTCTAT-3'	28	364	65
	5'-GTGGCGATTCAATAACCTTGTTCAGCT-3'	28		

DCs



Monocytes

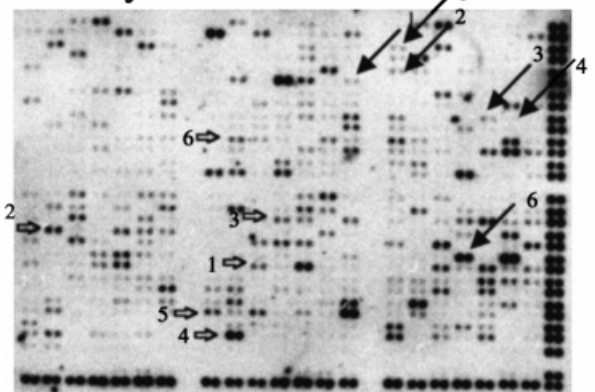


Fig 1. Expression pattern of genes in DCs and PB monocytes. Solid arrows indicate the six top upregulated genes in DCs; open arrows indicate the six top upregulated genes in monocytes. Numbers at the arrows are printed according to Table II.

differences between the membranes, we rehybridized the first donor's probe of DC sample to the stripped membrane (after hybridization of the second donor). The results showed that positive signal intensity was highly reproducible (94.7%), which was almost equal to hybridization of the same sample to the same membrane, and demonstrated the equality of two different membranes.

Semi-quantitative RT-PCR confirmation of expression profiling results

To confirm the differences observed in expression profiles obtained using cDNA arrays, we selected seven genes that showed different signal intensities in DCs and monocytes by the multiplex relative RT-PCR method. Based on the hybridization signal intensity, genes encoding alpha catenin (3.5 times upregulation), TNF- α (40 times) and human tumour necrosis factor receptor (TNFR I, II; 10.2 times) were upregulated in DCs. In contrast, genes encoding interferon- γ (IFN γ) (3.1 times upregulation) and granulocyte-macrophage colony-stimulating factor receptor (GM-CSFR; 2.3 times) were upregulated in monocytes. Zinc

finger X-chromosomal protein hybridization signals were similar in intensity in both cell types. As shown in Figure 2, six out of seven selected genes showed the same expression patterns obtained using RT-PCR and the cDNA arrays.

Interestingly, according to the results of hybridization with the cDNA arrays, the expression of TNFR I was upregulated in DCs. Consequent semi-quantitative RT-PCR showed that it was upregulated in four different donors samples of DCs and downregulated in the other two samples.

As endogenous standards for semi-quantitative RT-PCR, we used 18S rRNA transcript, because its expression does not vary during the cell cycle or between cell types. Although a commonly used endogenous standard, β -actin varies significantly between monocytes and DCs (Hashimoto *et al*, 1999).

DISCUSSION

In recent years, various molecular biological techniques have been applied to study the biological function of DCs. These methods include quantitative gene-specific RT-PCR (DeSaint-Vis *et al*, 1998), DD, random sequencing of cDNA libraries (Marland *et al*, 1997) and SAGE (Hashimoto *et al*, 1999).

We used another recently developed method of hybridization using nucleic acid arrays (Chalifour *et al*, 1994; DeRisi *et al*, 1996). The advantage of the cDNA expression profiling method is that the genes and probes included in the array are meticulously selected and have known biological functions. Therefore, our experiments can generate informative results for hundreds of genes simultaneously. We used a commercially available cDNA expression array with 588 genes that play key roles in many different biological processes.

In this study, we modified the manufacturer's original procedure of array analysis and applied a highly sensitive non-enzymatic chemiluminescent labelling technique for PCR-amplified cDNA targets, which were obtained from as little as 2 μ g of total RNA. The advantages of non-radioactive labelling and detection are short exposure time (minutes compared with hours or days using radioactively labelled probes), the stability of labelled probes (at least 1 year compared with several days with radioactively labelled probes) and elimination of inconvenience associated with working with radioactive materials (Ross *et al*, 1999).

According to immunophenotypic and functional analyses, immature DCs are derived *in vitro* from CD14⁺ PB monocytes cultured in the presence of GM-CSF and IL-4. Mature DCs are derived from CD14⁺ PB monocytes cultured in the presence TNF- α in addition to GM-CSF and IL-4 (Hart, 1997). In this study, we aimed to investigate the molecular biological changes during the early differentiation stages of monocytes into immature DCs.

It is important to note that, using a commercial cDNA array, we found several genes upregulated in DCs that have not been reported before. These genes include those encoding epithelial discoidin domain receptor 1, replication factor C, putative transcription factor DB1, prohibitin,

Table II. List of differentially expressed genes identified using human Atlas cDNA expression array.

Name	GenBank accession no.	Ratio
Genes upregulated in DCs		
TNF α	X01394	40
DNA repair protein RAD51 homologue	D13804	37.2
Transcription factor COUP 1	X12795	16
DNA polymerase alpha catalytic subunit	X06745	11.8
TNFR	M32315	10.2
Thymosin β -10	M92381	6
X-ray repair complementing defective repair protein in Chinese hamster cells 1	M36089	5.3
Epithelial discoidin domain receptor 1	X74979	5.2
Replication factor C, 37-kDa subunit	L07541	4.8
Transforming growth factor β receptor III precursor	L07594	4.7
Tyrosine kinase	U43408	4.7
Putative transcription factor DB1	D28118	4.3
Alpha catenin	D13866	3.5
Tyrosine-protein kinase lyn	M16038	3.2
Transforming growth factor β 1	X02812	3.2
Prohibitin	S85655	2.9
IL-1 beta precursor	K02770	2.9
P53-regulating protein	Z12020	2.8
Proto-oncogene RhoA	L25080	2.8
Neu differentiation factor	U02326	2.7
Adenosine A1 receptor	S56143	2.5
C-myc oncogene	V00568	2.4
Downregulated genes in DCs		
JNK activating kinase 1	L36870	1:55.5
IL-2R- γ subunit	D11086	1:17.2
Platelet-derived growth factor receptor β subunit	M21616	1:9.53
Mitotic growth and transcription activator	D26156	1:7.7
IL-7R- α subunit	M29696	1:5.8
LIM domain kinase 1	D26309	1:5.1
Nerve growth factor receptor-related	X60592	1:4.4
B-lymphocyte activation molecule		
IFN γ	X01992	1:3.1
GM-CSFR	X17648	1:2.3

proto-oncogene RhoA and neu differentiation factor. Epithelial discoidin domain receptor 1 or tyrosine protein kinase CAK is a collagen receptor with the potential to regulate cellular responses to the extracellular matrix. It has also been reported that activation of epithelial discoidin domain receptor 1 by collagen results in the upregulation of matrix metalloproteinase-1 expression. Our data support

recent findings obtained by SAGE regarding a change in the expression of metalloproteinase in DCs, which is probably involved in changing the cell structure and migration of DCs (Hashimoto *et al*, 1999).

In a previous study of DCs, upregulation of many genes encoding proteins associated with cell structure, such as alpha- and beta-actin, was detected (Hashimoto *et al*, 1999).

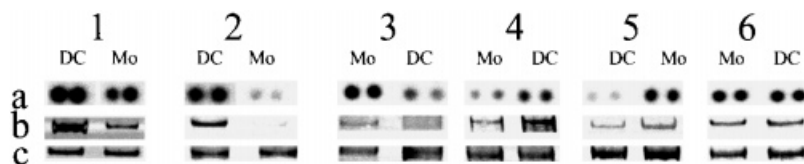


Fig 2. Confirmation of hybridization signals of six genes by relative RT-PCR. Results are constant within four DC samples and four monocyte (Mo) samples derived from four donors. Row a represents hybridization signals from the membrane; row b, gene-specific relative RT-PCR products; row c, amplified S18 rRNA internal control. Genes as follows: 1, alpha catenin; 2, human TNF- α ; 3, human IFN γ ; 4, human TNFR II; 5, GM-CSFR; 6, zinc finger X-chromosomal protein.

In our study, we found the upregulation of both proto-oncogene RhoA and putative transcription factor DB1 mRNA transcripts. RhoA is a member of the Ras superfamily of small GTP-binding proteins (Ridley, 1997). It was shown that RhoA interacts with DB1 and regulates various aspects of the actin cytoskeleton in various organisms (Lebowitz & Prendergast, 1998). Our findings demonstrate the involvement of RhoA and DB1 in the differentiation of DCs from PB monocytes.

Prohibitin is a 30-kDa protein located in the mitochondria and has an antiproliferative activity by inhibiting cell cycle and DNA synthesis (McClung *et al*, 1995; Dell'Orco *et al*, 1996). Upregulation of prohibitin in DCs may be evidence for the termination of proliferation in the differentiated form of monocytes.

Replication factor C is a multimeric primer recognition protein that is essential for the processive elongation of DNA chains catalysed by DNA polymerase delta or epsilon in human cells. Moreover, there is evidence that replication factor C could be involved in several other processes, such as transcription, S-phase checkpoint regulation, apoptosis and differentiation (Mossi & Hubscher, 1998). Therefore, the upregulation of the replication factor C gene may play a role in the differentiation of PB monocytes into DCs.

Among genes that were downregulated in DCs, we detected several genes regulating the cell cycle: JNK activating kinase 1, LIM domain kinase 1, mitotic growth and transcription activator, platelet-derived growth factor receptor β subunit. These proteins are involved in the regulation of proliferative and cell cycle processes (Dunaief *et al*, 1994; Derijard *et al*, 1995; Higuchi *et al*, 1997; Heldin *et al*, 1998).

Moreover, we observed higher expression levels of IL-2 and IL-7 receptors in monocytes. Our data are in agreement with those of recent studies, in terms of constitutive expression of IL-2 and IL-7 receptors, both on the cell surface and intracellularly in monocytes. Activated IL-2 and IL-7 receptors can induce intracellular signalling for cell proliferation (Pandrau-Garcia *et al*, 1994; Hodge *et al*, 2000). These results again confirm that the cell cycle in monocytes is much more active and, therefore, monocytes can differentiate into DCs.

It is noteworthy that we detected a higher level of IFN γ production in PB monocytes than in DCs. This finding was confirmed by our relative RT-PCR results and corresponds with those of previous studies (Zhou & Tedder, 1995).

Interestingly, we observed high expression levels of TNF α , TNFR I and II in DCs. Our results are in agreement with those of previous studies on TNF α , TNFR I and TNFR II mRNA expressions in DCs (McKenzie *et al*, 1995). In contrast to the results of a previous study using mature blood DCs (Hashimoto *et al*, 1999), we detected a higher level of TNF α expression in DCs than in monocytes. The differential intensity of hybridization signals for TNF α was confirmed by relative RT-PCR for four different samples of DCs and monocytes.

It was shown previously that DCs express both types of TNFR (McKenzie *et al*, 1995). In our study, the expression of TNFR II was confirmed by RT-PCR and shown to be

upregulated in DCs from six samples of monocytes and DCs (Fig 2). However, TNFR I expression was shown to be variable, as it was upregulated in four samples of DCs and downregulated in the other two samples. This variability could be caused by different genetic backgrounds of different donors. Moreover, our results show the 'switch on' of TNFR genes in immature DCs after their differentiation from PB monocytes. We presume that further culture in the presence of TNF α may provide the next differentiation step towards maturation of a DC population.

On the other hand, DCs actively expressed extracellular communication proteins, such as transforming growth factor-beta 1 (TGF- β 1), IL-1 and thymosin β -10. These results are consistent with those of previous studies (DeSaint-Vis *et al*, 1998; Hashimoto *et al*, 1999). Cytokine TGF- β is a pluripotent growth factor that regulates cell proliferation, extracellular matrix deposition, cell migration and differentiation. Cells that synthesize TGF- β may have specific receptors for this protein (Diamond, 1995). Thus, in DCs, we detected the upregulation of TGF- β 1 receptor type III together with TGF- β . Presumably, these proteins also play a role in the migration and differentiation of DCs.

In conclusion, the cDNA hybridization method was used for the first time for profiling the expression of a large number of genes during differentiation of DCs from CD14⁺ PB monocytes. Most of the upregulated genes in DCs were associated with differentiation, cell structure, migration and termination of proliferating activity. Our results agree with those of previous studies determining the up- and down-regulated genes during the differentiation of DCs from PB monocytes. Our report is the first description of the upregulation of several genes in DCs: epithelial discoidin domain receptor 1, RhoA, putative transcription factor DB1, prohibitin, replication factor C and neu differentiation factor. Moreover, our data show the presence of a switch-on step for TNFR II gene expression in immature DCs for further differentiation into mature DCs. Therefore, the data obtained should help towards understanding the biological processes that occur during DC differentiation as well as the functions of DCs. Moreover, our findings will probably be useful in studies on human disorders in which DCs or monocytes are involved.

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