Dendritic cells (DCs) are antigen-presenting cells that play a major role in initiating primary immune responses. We have utilized two independent approaches, DNA microarrays and proteomics, to analyze the expression profile of human CD14+ blood monocytes and their derived DCs. Analysis of gene expression changes at the RNA level using oligonucleotide microarrays complemented by 6300 human genes that were expressed in DCs. A total of 255 genes (4%) were found to be regulated during DC differentiation or maturation. Most of these genes were not previously associated with DCs and included genes encoding secreted proteins as well as genes involved in cell adhesion, signaling, and lipid metabolism. Protein analysis of the same cell populations was done using two-dimensional gel electrophoresis. A total of 900 distinct protein spots were included, and 4% of them exhibited quantitative changes during DC differentiation and maturation. Differentially expressed proteins were identified by mass spectrometry and found to represent proteins with Ca++ binding, fatty acid binding, or chaperone activities as well as proteins involved in cell motility. In addition, proteomic analysis provided an assessment of post-translational modifications. The chaperone protein, calreticulin, was found to undergo cleavage, yielding a novel form. The combined oligonucleotide microarray and proteomic approaches have uncovered novel genes associated with DC differentiation and maturation and has allowed analysis of post-translational modifications of specific proteins as part of these processes.

Dendritic cells (DCs) are highly specialized antigen-presenting cells that have an essential role in the initiation and control of the cytotoxic T cell response. As "professional" antigen-presenting cells, they are specialized to take up, process, and present soluble antigens in complexes with either class I or class II MHC molecules (1, 2). They are present in most tissues in a relatively immature state, but in the presence of inflammatory signals, they rapidly take up foreign antigens and undergo maturation into potent antigen-presenting cells that migrate to lymphoid organs where they initiate an immune response. Their phenotypic and functional characteristics are intimately linked to their stage of maturation. However, the specific genes whose expression mediates differentiation of pluripotent progenitors to DCs are largely undefined. The generation of large numbers of DCs has become feasible through the in vitro cultivation of progenitors using exogenous hematopoietic cytokines to support their growth, differentiation, and maturation (3, 4). Human myeloid DCs can be generated from various sources, including blood, bone marrow, and CD34+ stem cells. Monocytes from peripheral blood have served as a ready source for generating myeloid DCs in vitro following incubation with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) for use in immunotherapy (4–6). Thus, DCs have become accessible for detailed molecular and cell biological analysis and for clinical applications. Microarrays and proteomics technologies for identifying the mRNA and protein constituents of living organisms and determining their pattern of expression are emerging (7–9). Few studies have been undertaken that simultaneously analyzed cell populations at both RNA and protein levels. Potential sources of discordance between RNA and protein levels include translational control and altered protein stability. Additionally, proteomic analysis may uncover post-translational modifications that are not predictable at the RNA level. Here we used in vitro cultures of circulating CD14+ monocytes treated with GM-CSF and IL-4 followed by treatment with TNF-α, in order to analyze systematically gene expression during DC differentiation and maturation, using both oligonucleotide microarrays and proteomics.

**Experimental Procedures**

**Generation of DCs from Peripheral Blood—**Generation of DCs was performed as described previously (10). Peripheral blood mononuclear cells (PBMCs) were obtained from leukapheresis specimens of normal donors after Ficoll-Paque density gradient separation. PBMCs were washed twice in Hank’s balanced salt solution (Life Technologies, Inc.) and were resuspended in XVIVO-15 medium (BioWhittaker, Walkersville, MD). PBMCs were incubated with anti-CD14 monoclonal antibody coated with microbeads (Miltenyi Biotec, Auburn, CA), and CD14+ monocytes were isolated by passing the PBMCs through a magnetic cell separation system (Miltenyi Biotec). CD14+ monocytes (2 × 10^7) were cultured in XVIVO-15 medium containing GM-CSF (100 ng/ml) and IL-4 (50 ng/ml) for 7 days of culture, fresh XVIVO-15 medium containing GM-CSF (100 ng/ml) and IL-4 (50 ng/ml) plus TNF-α (10 ng/ml) was added to the cells for 7 additional days. All cytokines were purchased from PeproTech (Rocky Hill, NJ).

**Cell Surface Antigen Analysis—**The analysis of cell surface antigens...
was performed by direct immunofluorescence (FACScan, Becton Dickinson, Mountain View, CA). Cells were washed twice with culture cell medium and incubated for 30 min on ice with each test monoclonal antibody diluted to the optimal concentration for immunostaining. Labeled cells were then washed, fixed in 1% paraformaldehyde, and analyzed for fluorescence. Data analysis was based on examination of 10,000 cells/sample. Staining was performed with the following FITC-and phycoerythrin (PE)-labeled monoclonal antibodies: FITC-CD1a, FITC-CD14, FITC-HLA-DR, FITC-CD83, FITC-mouse IgG1, FITC-mouse IgG2 (all from PharMingen, San Diego, CA); PE-CD86 (Coulter/Immunotech, Miami, FL); and PE-mouse IgG1 (Becton Dickinson). Primary antibodies were compared with the appropriate isotype-matched controls.

Preparation of cDNA and Gene Chip Hybridization—Total RNA was isolated using Trizol reagent (Life Technologies, Inc.) and used to generate cRNA probes. Preparation of cRNA, hybridization, and scanning of the HuGeneFL arrays were performed according to the manufacturer’s protocol (Affymetrix, Santa Clara, CA). Briefly, 5 μg of the RNA was converted into double-stranded cDNA by reverse transcription using a cDNA synthesis kit (SuperScript Choice, Life Technologies, Inc.) with an oligo(dT)$_{18}$ primer containing a T7 RNA polymerase promoter site added 3’ of the poly(T) (Genet, La Jolla, CA). After second-strand synthesis, labeled cDNA was generated from the cDNA sample by an in vitro transcription reaction supplemented with biotin-11-CTP and biotin-16-UTP (Enzo, Farmingdale, NY). The labeled cRNA was purified by using RNeasy spin columns (Qiagen, Valencia, CA). Fifteen micrograms of each cRNA was fragmented at 94 °C for 35 min in fragmentation buffer (40 mM Tris acetate, pH 8.1, 100 mM potassium acetate, 30 mM magnesium acetate) and then used to prepare 300 μl of hybridization mixture (100 mM MES, 0.1 mg/ml herring sperm DNA (Promega), 1 mM sodium chloride, 10 mM Tris, pH 7.6, 0.0005% Triton X-100) containing a mixture of control cRNAs for comparison of hybridization efficiency between arrays and for relative quantitation of measured transcript levels. Before hybridization, the cRNA samples were heated at 94 °C for 5 min, equilibrated at 45 °C for 5 min, and clarified by centrifugation (14,000 × g) at room temperature for 5 min. Aliquots of each sample (10 μg of cRNA in 200 μl of the master mix) were hybridized to HuGeneFL Arrays at 45 °C for 16 h in a rotisserie oven set at 60 rpm then washed with non stringent wash buffer (6 × saline/sodium phosphate/EDTA) at 25 °C, followed by stringent wash buffer (100 mM MES (pH 6.7), 0.1 M NaCl, 0.01% Tween 20) at 50 °C, stained with streptavidin-phycocerythrin (Molecular Probes), washed again with 6 × saline/sodium phosphate/EDTA, stained with biotinylated anti-streptavidin IgG, followed by a second staining with streptavidin-phycocerythrin, and a third washing with 6 × saline/sodium phosphate/EDTA. The arrays were scanned using the GeneArray scanner (Affymetrix). Data analysis was performed using GeneChip 4.0 software. The software includes algorithms that determine whether a gene is absent or present and whether the expression level of a gene in an experimental sample is significantly increased or decreased relative to a control sample. To assess differences in gene expression, we selected genes based on a sort score value equal or greater than 2. The sort score is calculated by Affymetrix software by using a combination of actual values of the average differences.

Two-dimensional Polyacrylamide Gel Electrophoresis—The procedure followed was as described previously (11). Cells were solubilized in 200 μl of lysis buffer containing 9.5 μM urea (Bio-Rad), 2% Nonidet P-40, 2% carrier ampholytes pH 4–8 (Gallard/Schlessinger, Carle Place, NY), 2% β-mercaptoethanol, and 10 mM phenylmethylsulfonyl fluoride. Aliquots containing ~5 × 10$^6$ cells were applied onto isofocusing gels. Isoelectric focusing was conducted using pH 4–5 carrier ampholytes at 700 V for 16 h, followed by 1000 V for an additional 2 h. The first-dimension gel was loaded onto the second-dimension gel, after equilibration in 125 mM Tris, pH 6.8, 10% glycerol, 2% SDS, 1% dithiothreitol, and bromophenol blue. For the second-dimension separation, a gradient of 11–14% of acrylamide (Serva, Crescent Chemical, Haupauge, NY) was used. Proteins were transferred to an Immobilon-P polyvinylidene difluoride membrane (Millipore, Bedford, MA) or visualized by silver staining of the gels (11, 12). Phosphoproteins were visualized by phosphorimaging technology.

Radioactive Labeling and Heat Shock Treatment—[$^{32}$P]Orthophosphate labeling was performed by preincubating the cells for 2 h with 200 μCi/ml [32P]orthophosphate (Amersham Pharmacia Biotech) in phosphate-free culture medium (Life Technologies, Inc.). Heat shock treatment was then performed by incubating the cells for 1 h at 42–44 °C as described previously (12, 13).
### Table I

**Up-regulated mRNAs in monocyte-derived DCs**

RNA from CD14+ monocytes (D1), immature (D7), and mature (D14) DCs were hybridized onto Affymetrix oligonucleotide arrays and quantified as indicated under “Experimental Procedures.” For each gene, the fold change was calculated by Affymetrix software; NC indicates no change; the -> indicates fold change calculation for which the smaller value is replaced by an estimate of the minimum value for detectable transcripts. PPA-γ is peroxisome proliferator-activated receptor-γ.

| Description                                      | GenBank™ accession no. | Fold change | D7/D1 | D14/D7 |
|--------------------------------------------------|------------------------|-------------|-------|--------|
| **Cell surface proteins**                         |                        |             |       |        |
| CD1a                                             | M28825                 | ~17.5       | -5.4  |        |
| CD1b                                             | M28826                 | 16.8        | -3.6  |        |
| CD1c                                             | M28827                 | 16          | -3.2  |        |
| Fc-ε RII/CD23A                                   | M15059                 | 12.2        | -2.3  |        |
| Macrophage mannose receptor (MRC1)               | M93221                 | 11.3        | NC    |        |
| CD36/FAT                                         | Z22555                 | 9.3         | NC    |        |
| MHC class II, HLA-DQA                            | M54996                 | 8.8         | NC    |        |
| MHC class II, HLA-DP light chain                 | M57466                 | 8.3         | NC    |        |
| MHC/HLA-DQ protein 41                            | M28590                 | 6.6         | NC    |        |
| CCR7                                             | L31584                 | NC          | 6.5   |        |
| E-cadherin                                       | Z35402                 | 5.7         | 2     |        |
| TIG1                                             | U27185                 | -5.4        | 2.8   |        |
| PKD2                                             | U50928                 | 5.1         | NC    |        |
| C3b/C4b receptor (CR1)                           | X05309                 | ~5.0        | NC    |        |
| CD26/DPP4                                        | X60708                 | NC          | ~5.0  |        |
| CD59                                             | M84349                 | 4.6         | 1.9   |        |
| Inteegrin β, subunit                             | X53002                 | ~4.3        | NC    |        |
| Transcript associated with monocyte to macrophage differentiation | X85750     | 4.3 | NC | |
| Fc-γ RIβ3/CD3                                    | M19333                 | 4.1         | NC    |        |
| MHC class II, HLA-DR3,3                          | K02405                 | 3.8         | NC    |        |
| MHC class II, HLA-SB alpha                       | X03100                 | 3.3         | NC    |        |
| CD71                                             | M11507                 | 2.9         | NC    |        |
| CD86                                             | U04343                 | 2.8         | NC    |        |
| MHC class II, HLA-DQw1.1 β chain                 | X03560                 | 2.4         | NC    |        |
| Class II invariant γ chain/CD74                  | X65561                 | 2.3         | NC    |        |
| CD83                                             | Z11697                 | 1.6         | 4.7   |        |
| **Secreted proteins**                            |                        |             |       |        |
| Macrophage metalloelastase (HME)                 | L23808                 | ~80.6       | NC    |        |
| TARC (CCR4 ligand)                               | D43767                 | 26.5        | 3     |        |
| α₁-macroglobulin                                 | M11313                 | 25.1        | NC    | ~21.4  |
| Mac-2-binding protein                            | L13210                 | NC          | ~21.4 |        |
| MCP-4                                            | U46767                 | ~18.6       | 2.5   |        |
| Factor XIIIa                                     | M14539                 | ~16.1       | ~3.9  |        |
| Growth arrest-specific Gas6                      | L13720                 | ~14.7       | NC    |        |
| Cathepsin C                                      | X87212                 | 13.1        | NC    |        |
| Macrophage-derived chemokine (MDC)               | U83171                 | 10.1        | NC    |        |
| Bikum                                            | U78085                 | 8.6         | NC    |        |
| Complement C1q B chain                           | K03430                 | 6.9         | NC    |        |
| Bone-derived growth factor (BPFG-1)              | L42379                 | 5.6         | NC    |        |
| TNF-inducible protein 6 (TSG-6)                   | M31165                 | NC          | ~5.4  |        |
| TGF-β                                            | AB000584               | 5           | NC    |        |
| Extracellular matrix protein 1 (ECM1)            | U65932                 | 4.9         | NC    |        |
| Semaphorin E                                     | AB000220               | 4.6         | NC    |        |
| CSF-1                                            | M37435                 | 4.4         | NC    |        |
| Osteopontin                                      | U20758                 | 4.2         | NC    |        |
| Activin β, subunit                               | X57579                 | 3.5         | NC    |        |
| Autotaxin-t                                      | L46750                 | ~3.4        | NC    |        |
| TGF-α                                            | X70340                 | NC          | 3     |        |
| **Nuclear proteins**                             |                        |             |       |        |
| DHP2                                             | U56814                 | ~14.5       | 4.5   |        |
| MINOR                                            | U12767                 | 10.4        | NC    |        |
| PPAR-γ                                           | L40904                 | 9           | NC    |        |
| LXR-α                                            | U22662                 | 8.5         | NC    |        |
| Interferon regulatory factor 4 (IRF4)             | U52682                 | ~8.2        | 3.4   |        |
| C/EBPα                                           | U34070                 | 5.1         | ~10   |        |
| SOX-4                                            | X70683                 | NC          | ~3.9  |        |
| AES-1                                            | X73558                 | 3.5         | NC    |        |
| TRIP7                                            | L40357                 | 3.2         | 2.1   |        |
| SmRNP                                            | J04615                 | 3.1         | NC    |        |
| Mrg1                                             | U65093                 | 3           | NC    |        |
| **Signal transduction/growth control**            |                        |             |       |        |
| RIL                                              | X93510                 | ~24.2       | NC    |        |
| GTGase-activating protein (Rap1GAP)               | M64788                 | ~18.4       | ~16   |        |
| Cyclin D2                                        | D13639                 | 9.5         | ~2.2  |        |
| Inhibitor of apoptosis MIHC                      | U37546                 | NC          | 8     |        |
| Dual-specificity phosphatase 5                   | U15932                 | ~7.6        | NC    |        |
| Cyclophilin C                                    | S71018                 | ~5.7        | 1.6   |        |
| Stac                                             | D86640                 | ~5.3        | NC    |        |
| cAMP-dependent protein kinase catalytic subunit, Cβ | M34181   | ~5.1        | NC    |        |
| Putative serine/threonine kinase GS3955          | D87119                 | ~4.8        | NC    |        |
Western Blotting—Following transfer, membranes were incubated for 2 h in blocking buffer containing 5% milk in 10 mM Tris-HCl, pH 7.5, 2.5 mM EDTA, pH 8, 50 mM NaCl. The membranes were incubated for 2 h with antibodies against calreticulin, SPA-600 (StressGen, Victoria, Canada), T-19 (Santa Cruz Biotechnology, Santa Cruz, CA), vimentin, V9 (Santa Cruz Biotechnology), or FABP5 (kindly provided by Professor Celis, University of Aarhus, Denmark) at a dilution 1:1,000,000, 1:10,000, 1:100,000, and 1:1000, respectively. The membranes were then incubated for 1 h with horseradish peroxidase-conjugated anti-rabbit (Amersham Pharmacia Biotech) or anti-goat (Sigma) IgG antibodies, at a dilution 1:1000. Immunodetection was accomplished by enhanced chemiluminescence (ECL) (Amersham Pharmacia Biotech) followed by autoradiography on hyperfilm MP (Amersham Pharmacia Biotech).

In-gel Enzymatic Digestion—The two-dimensional gels were silver-stained by successive incubations in 0.02% sodium thiosulfate for 2 min, 0.1% silver nitrate for 40 min, and 0.014% formaldehyde plus 2% sodium carbonate. The proteins of interest were excised from the two-dimensional gels and destained for 5 min in 15 mM potassium ferricyanide and 50 mM sodium thiosulfate as described (14). Following 3 washes with water, the gel pieces were dehydrated in 100% acetonitrile for 5 min and dried for 30 min in a vacuum centrifuge. Digestion was performed by addition of 100 ng of trypsin (Promega, Madison, WI) in 200 mM ammonium bicarbonate or by the addition of 100 ng of the endoproteinase Glu-C (Promega, Madison, WI) in 100 mM ammonium bicarbonate. The Lys-C digestion was performed with 500 ng of the endoproteinase Lys-C (Roche Molecular Biochemicals) in 100 mM Tris-HCl, pH 9. Following enzymatic digestion overnight at 37 °C, the peptides were extracted twice with 50 ml of 60% acetonitrile, 1% trifluoroacetic acid. After removal of acetonitrile by centrifugation in a vacuum centrifuge, the peptides were concentrated by using pipette tips C18 (Millipore, Bedford, MA).

Mass Spectrometry—Analyses were performed primarily using a Perseptive Biosystem matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) Voyager-DE mass spectrometer (Framingham, MA), operated in delayed extraction mode. Peptide mixtures were analyzed using a saturated solution of α-cyano-4-hydroxycinnamic acid (Sigma) in acetone containing 1% trifluoroacetic acid (Sigma). Peptides were selected in the mass range of 800–4000 Da. Spectra were calibrated using calibration mixture 2 of the Sequazyme peptide mass
RNA from CD14+ monocytes (D1), immature (D7), and mature (D14) DCs were hybridized onto Affymetrix oligonucleotide arrays and quantified as indicated under “Experimental Procedures.” For each gene, the fold change was calculated by Affymetrix software; NC indicates no change; the ~ indicates fold change calculation for which the smaller value is replaced by an estimate of the minimum value for detectable transcripts.

| Description | GenBank™ accession no. | D7/D1 | D14/D7 |
|-------------|------------------------|-------|--------|
| **Cell surface proteins** | | | |
| CD14 | X13334 | ~92.1 | ~5.6 |
| Ficolin-1 | D83920 | ~51.3 | NC |
| Galectin-2 | M87860 | ~37.7 | NC |
| Platelet-activating factor receptor | D10202 | ~22.4 | NC |
| LST1/C splice variant | AF000424 | NC | ~18.2 |
| CCR5 | U95626 | 12.7 | NC |
| Epithelial membrane protein 1 | Y07909 | NC | ~8.3 |
| CRX2 | U02905 | ~8 | ~2.1 |
| CD163 | Z22968 | ~11.2 | NC |
| C3a receptor | U95626 | 12.7 | NC |
| CCR2 | U03905 | 8 | 2.1 |
| CD11a (LFA-1α) | Y00796 | 7.3 | NC |
| C5a anaphylatoxin receptor/CD88 | M62505 | 22.4 | NC |
| CSF-1 receptor/CD115 | M62505 | 22.4 | NC |
| RP105 | D83597 | ~6.2 | NC |
| Receptor gp250 (SORL-1) | D10202 | ~4.8 | ~2.9 |
| HLA-B-associated transcript 2 | D10202 | 7.3 | NC |
| Activation-induced C-type lectin (AIICL) | D10202 | 7.3 | NC |
| Fc-εRIIA | X68090 | 5.4 | ~3.4 |
| Ninjurin-1 | U72661 | 5.8 | NC |
| LDL receptor | D89050 | 4.8 | 6.3 |
| EV12 | M53267 | 4.8 | NC |
| CD157 | D21878 | 4.8 | NC |
| Uroplakin II | AF000562 | NC | ~4.7 |
| MacMARC5 | J05070 | ~32.1 | NC |
| TNF receptor | J05070 | ~32.1 | NC |
| MHC class I-related protein | J05070 | ~32.1 | NC |
| IL-2 receptor γ chain | J05070 | ~32.1 | NC |
| Toll-like receptor 1 | J05070 | ~32.1 | NC |
| Type II interleukin-1 receptor | J05070 | ~32.1 | NC |
| uPAR/CD87 | X68090 | 8.4 | NC |
| Syndecan 2 | U28488 | 5.4 | ~3.4 |
| CD13/N-aminopeptidase | U28488 | 5.4 | ~3.4 |
| Disintegrin-metalloprotease MADM (ADAM10) | U28488 | 5.4 | ~3.4 |
| Presenilin 1 | U28488 | 5.4 | ~3.4 |
| CD44E | U28488 | 5.4 | ~3.4 |
| IL-8 | U28488 | 5.4 | ~3.4 |
| Cathepsin O/cathepsin K precursor | U28488 | 5.4 | ~3.4 |
| Neuraminidase B (NMB) | U28488 | 5.4 | ~3.4 |
| PBEF | U28488 | 5.4 | ~3.4 |
| Cathepsin O/cathepsin K precursor | U28488 | 5.4 | ~3.4 |
| MIP-2α | U28488 | 5.4 | ~3.4 |
| a1-antitrypsin | U28488 | 5.4 | ~3.4 |
| Platelet factor 4 (PF4) | U28488 | 5.4 | ~3.4 |
| ENA-38 | U28488 | 5.4 | ~3.4 |
| TNF-α | U28488 | 5.4 | ~3.4 |
| GP-39 | U28488 | 5.4 | ~3.4 |
| Fibrinogen-like 2 | U28488 | 5.4 | ~3.4 |
| Nuclear proteins | U28488 | 5.4 | ~3.4 |
| TAL2 | D50645 | 2.4 | NC |
| Histone H2B | D50645 | 2.4 | NC |
| Nuclearosome assembly protein 2 (NAP-2) | D50645 | 2.4 | NC |
| CBFB | D50645 | 2.4 | NC |
| CBP | D50645 | 2.4 | NC |
| Interferon regulatory factor 7A (IRF 7A) | D50645 | 2.4 | NC |
| PSCDBP | D50645 | 2.4 | NC |
| Sterol regulatory element binding protein 2 | D50645 | 2.4 | NC |
The search program MS-Fit, developed by the University of California at San Francisco, was used for searches in the database NCBI. Search parameters were as follows: maximum allowed peptide mass error of 400 ppm, consideration of one incomplete cleavage per peptide, and pH range between 4 and 8. MALDI-TOF mass spectrometry was also used for molecular weight determination as described (15). In some cases, the amino acid sequence of some peptides of interest was determined by electrospray ionization-mass spectrometry analysis.

**RESULTS**

**Surface Phenotype of CD14⁺ Monocytes-derived DCs—Differentiation of CD14⁺ blood monocytes into mature dendritic cells**

**TABLE II—continued**

| Description | GenBank™ accession no. | Fold change |
|-------------|------------------------|-------------|
| Spliceosomal protein SAP 49 | L35013 | ~4.7 ~4.9 |
| Enigma (LIM domain protein) | L35240 | NC ~4.6 |
| Siah-binding protein 1 (siahBP1) | U51586 | NC ~3.8 |
| EGF-response factor 2 | X78992 | –3.5 ~9.3 |
| Ribonuclease protein SS-A/Ro | M25077 | –3.4 ~1.9 |
| H4 histone | S81914 | –3.4 –7 |
| Nucleoporin Nup214 | D14689 | –3.3 NC |
| RFG/ARA70/EL1 | X77548 | –3.2 NC |
| RING3 | D43040 | –3.2 NC |
| SRC/Hingenase HRH1 | D59487 | –3.1 NC |
| CCAAT transcription binding factor-γ | Z47972 | –2.7 NC |
| TSC-22 | U53048 | –2.7 NC |
| Tax interaction protein 1 | U90913 | NC –2.6 |
| Net transcription factor | Z36715 | ~2.3 NC |

**Signal transduction/growth control**

| Description | GenBank™ accession no. | Fold change |
|-------------|------------------------|-------------|
| MAP kinase phosphatase 3 (MKP-3) | X93920 | ~10.2 NC |
| Fyn-binding protein (Fyb) | U93049 | –9.6 NC |
| Anti-death protein/IEF1 | S81914 | NC ~7 |
| GAP-binding protein p62 | U70987 | NC ~5.8 |
| MAP kinase activated protein kinase 2 | U12779 | NC ~5.2 |
| Guanine nucleotide exchange factor 2 (GEF2) | L13858 | NC ~4.7 |
| Protein tyrosine kinase 2β (PTK2B) | U43522 | ~4.3 NC |
| Protein phosphatase 2A (PP2A) | L7602 | ~4.2 ~4.6 |
| S100A12 | D83657 | ~4.1 ~2.2 |
| Rab6 GTPase-activating protein | D31886 | ~3.5 NC |
| Ras p21 protein activator | X59399 | NC ~2.7 |
| ISG15 | M13755 | NC ~2.6 |
| Leukocyte elastin | M69043 | ~2.6 NC |
| Inositol 1,4,5-trisphosphate receptor type 1 | U23850 | ~2.5 NC |
| Cyclophilin F | M80254 | ~2.4 NC |
| BTG2 | U72649 | ~2.1 NC |

**Metabolism/enzymes**

| Description | GenBank™ accession no. | Fold change |
|-------------|------------------------|-------------|
| MRP8 | M21005 | ~31.6 NC |
| Cysteine protease | D55696 | ~14.2 ~20.8 |
| Neutrophil cytosol factor 1 (NCF1) | M55067 | ~12.3 NC |
| MRP14 | M8311 | ~11.2 NC |
| Short chain alcohol dehydrogenase | U73514 | ~8.2 ~8.3 |
| Nrap1 | L38593 | ~7.8 ~6.3 |
| ATPase | Z69881 | NC ~5.9 |
| Cytochrome b245 | X04011 | ~5.6 ~3.6 |
| Superoxide dimutase SOD2 | X07854 | ~5.4 ~2.7 |
| Malic enzyme 1 | U43944 | ~5.2 NC |
| Not56-like protein | Y90922 | NC ~4.7 |
| Erythrocyte-specific AMP deaminase | D12775 | ~4.3 2.4 |
| l-Kynurenine hydrolase | U57721 | ~4.2 NC |
| Dihydroriboside dehydrogenase | U05681 | ~3.7 3.6 |
| Leukotriene A-4 hydrolase | J03459 | ~3.6 NC |
| Glyceraldehyde-3-phosphate dehydrogenase | L31943 | ~3.5 NC |
| Carboxylesterase | L07765 | ~3.3 NC |
| Bcat1 | U23515 | ~2.9 NC |
| Glutathione reductase | X15722 | ~2.9 NC |
| Glutamate transporter | D26443 | ~2.6 NC |
| Fructose-biphosphatase 1 | U21931 | NC ~2.4 |
| α,β-Galactosidase A | X14448 | ~2.3 NC |
| Iduorotate-2-sulfatase | L46586 | ~2.8 NC |
| S100 calcium-binding protein | M89563 | NC ~7.5 |

**Not classified**

| Description | GenBank™ accession no. | Fold change |
|-------------|------------------------|-------------|
| KIAA0064 | D31764 | NC ~13.2 |
| Clone S19, CpG-enriched DNA | L33999 | NC ~13 |
| KIAA0246 | D87433 | ~9.7 NC |
| Neuroendocrine-specific protein A | L10333 | ~7.4 NC |
| KIAA0206/LHFPL2 | D69681 | ~6.6 NC |
| Tubulin | X06956 | ~4.5 NC |
| Corcin | X89109 | ~4.4 ~17.1 |
| HHCPA78 | S73391 | ~3 NC |
| Keratin 10 type 1 intermediate filament | J04029 | ~2.9 NC |
can be induced in vitro by treatment with a combination of GM-CSF, IL-4, and TNF-α (10, 16). We isolated CD14+ cells from PBMCs obtained from leukapheresis specimens of healthy donors. Adherent CD14+ monocytes were cultured for 7 days in the presence of GM-CSF (100 ng/ml) and IL-4 (50 ng/ml) followed by 7 additional days in the presence of GM-CSF (100 ng/ml), IL-4 (50 ng/ml), and TNF-α (10 ng/ml). The differentiation stage of the cells was determined by two criteria, morphology and cell surface expression of specific markers. At day 7, the DCs displayed phenotypic and morphologic characteristics of immature DCs. The cells expressed CD1a, the costimulatory molecule CD86 (50 and 52% positive cells, respectively), high levels of MHC class II antigens, while being negative for the monocyte marker CD14, as determined by fluorescence-activated cell sorter analysis. Following further culture in the presence of TNF-α for 7 days, almost all the cells exhibited high levels of HLA-DR, CD86, and CD83, which represent markers of mature DC (Fig. 1) (16–19). Development of the dendrite/neurite morphology was progressively more prevalent and pronounced after addition of TNF-α (data not shown). Furthermore, up-regulation of CD83 expression presented the same temporal kinetics as the morphologic changes.

Analysis of Overall Gene Expression in CD14+ Monocytes and Their Derived DCs by Oligonucleotide Arrays—Three independent differentiation experiments were performed, and RNA transcript levels for different genes were determined at day 1 (CD14+ monocytes) and after 7 days of GM-CSF/IL-4 treatment (immature DCs) and 14 days of GM-CSF/IL-4 plus TNF-α treatment (mature DCs), using oligonucleotide arrays. Transcripts for ~40% of the 6,300 unique genes assessed were detected in all the cell populations tested. We identified a subset of genes that differed in their expression levels during DC differentiation and maturation, by 2.5-fold or greater, in all three experiments. The 255 genes identified are presented in Tables I and II, for up-regulated and down-regulated genes, respectively. The number of genes whose expression decreased upon DC differentiation and/or maturation was as large as the number of genes whose expression increased. In addition, comparison of overall gene expression between immature and mature DCs showed only few differences. Genes known to be
differentially expressed during DC differentiation changed their expression accordingly in our analysis. This group included the monocytic marker CD14, CD163, and C5a anaphylatoxin receptor (CD88), which were strongly down-regulated, and the cell surface proteins CD1a, CD1b, CD1c, CD36, CD59, CD83, CD86, and CCR7, which were up-regulated with DC differentiation and maturation. Up-regulation of Fc-eRII and Fc-gRII, of several genes encoding for MHC class II, and of genes encoding for the secreted proteins TARC (CCR4 ligand), MCP-4, and the macrophage-derived chemokine was also observed.

Most of the 255 genes we have uncovered were not previously known to be expressed differentially in DCs. Novel changes included differential expression of many cell surface molecules related to cell adhesion, such as E-cadherin, galectin 2, CD11a/LFA-1α, nijurin-1, macmarcks, syndecan 2, CD44E, and presenilin 1. Transcript levels for genes encoding for several secreted proteins increased as the cells differentiated. These genes include the growth factor BPGF1, TGF-β, CSF-1, sema- phorin E, activin β subunit, and the macrophage chemoattractant osteopontin. In contrast, expression of the chemokines belonging to the IL-8 superfamily (IL-8, CTAPIII, MIP-2α, MIP-2β, platelet factor 4 (PF4), and ENA-78) was decreased. We also observed a decrease of neuromedin B, PEDF, and PBEF mRNAs. Expression of mRNAs encoding for proteins localized in the nuclear compartment or involved in signaling has been poorly described in DCs. Our results demonstrate that expression of the interferon regulatory factor 4, C/EBPa, FIG. 3. Identification of four vimentin isoforms. Close-up sections of silver-stained two-dimensional gel of DCs (left panel) and of a Western blot using a specific monoclonal antibody V9 against vimentin (right panel).

| Spot # | Identification | NCBI accession no. | Matching peptides | Estimated MW/pI | Protein covered % |
|--------|----------------|--------------------|------------------|-----------------|------------------|
| Up-regulated |
| 111 | hsp73 | 5729877 | 17/48 (35%) | 54.9 /5.5 | 30 |
| 278 | Vimentin* | 2119204 | 13/31 (41%) | 41.1 /4.7 | 34 |
| 279 | Vimentin* | 2119204 | 22/55 (62%) | 42.0 /4.8 | 45 |
| 348 | Macrophage capping protein | 4502561 | 8/37 (21%) | 40.8 /6.0 | 19 |
| 538 | Guanylate kinase | 4504221 | 3/8 (37%) | 29.7 /5.3 | 19 |
| 574 | hsp27* | 123571 | 5/7 (18%) | 28.5 /5.9 | 33 |
| 701 | Ferritin light chain | 120523 | 3/8 (37%) | 21.9 /5.4 | 22 |
| 802 | FABP5* | 4557581 | 8/10 (80%) | 11.0 /6.1 | 67 |
| 827 | FABP4 | 4557579 | 8/14 (57%) | 15.8 /5.9 | 76 |
| 867 | ACBP | 118276 | 6/8 (75%) | 14.3 /5.5 | 50 |
| 870 | S100C | 5032057 | 4/7 (57%) | 14.8 /5.8 | 42 |
| Down-regulated |
| 138 | Calreticulin* | 4757900 | 6/13 (46%) | 49.3 /4.5 | 18 |
| 182 | Calreticulin* | 4757900 | 5/18 (27%) | 45.8 /4.4 | 17 |
| 535 | RNCC protein | 4337097 | 7/12 (58%) | 30.0 /5.1 | 40 |
| 824 | MRP14* | 4506773 | 9/16 (56%) | 14.8 /5.2 | 70 |
| 826 | MRP14* | 4506773 | 9/11 (81%) | 15.3 /6.4 | 72 |
| 834 | MRP8* | 115442 | 6/8 (75%) | 15.8 /6.7 | 43 |
| 873 | MRP8* | 115442 | 3/5 (60%) | 15.2 /6.2 | 32 |

* The identity is further confirmed by Western-blotting analysis or heat-shock treatment.

FIG. 4. hsp27 regulation during DC differentiation and heat shock. A, close-up sections of silver-stained two-dimensional gels from CD14+ monocytes and DCs showing an increase in the unphosphorylated form hsp27A and a minor increase in the phosphorylated form hsp27B during DC differentiation. B, CD14+ monocytes untreated or treated for 7 days in the presence of GM-CSF and IL-4 were labeled for 2 h with [32P]orthophosphate and subjected (HS) or not (C) to a heat shock treatment of 45 min at 42 °C. Two-dimensional electrophoresis and autoradiography showed that all the phosphorylated forms hsp27B, -C, and -D were induced in both cell types.
MRG1, peroxisome proliferator-activated receptor-γ, TRIP7, SLA, Rap1GAP, cAMP-dependent protein kinase, IP3 protein kinase B, cyclopilin C, and cyclins A1, D2, G2, and H genes, was increased. Expression of interferon regulatory factor 47A, TAL2, NAP-2, epidermal growth factor response factor 2, CTPB, IEX-1, SAP49, HRH1, IeBa, Fyb, Net, and cyclphilin F genes is decreased. Finally, the regulation of a large group of genes encoding for lipid-binding proteins or enzymes involved in lipid metabolism was observed. Levels of acyl-CoA thioester hydrolase, 15-lipoxygenase, lysosomal acid lipase, lipoprotein lipase, lysophospholipid homolog (HU-K5), FABP3, FABP4, FABP5, apolipoproteins C-I and E, and 3-oxoacyl-CoA thiolase mRNAs was observed. Myeloid-related proteins MRP14 and MRP8 down-regulation was progressive upon DC differentiation and maturation, leading to a 9- and 12-fold decrease in spot intensities, respectively. Again, the results obtained for these two genes were highly concordant (Table I). Concomitant with the up-regulation of FABP4 and FABP5, we observed a strong down-regulation of two members of the S100 family, the myeloid-related proteins MRP14 and MRP8. Interestingly, it has been recently shown that the heterodimer MRP8/MRP14, designated fatty acid p34 (FA-p34), exerts a fatty acid binding activity (20–22). MRP4 and MRP8 down-regulation was progressive upon DC differentiation and maturation, leading to a 9- and 12-fold decrease in spot intensities, respectively. Again, the results obtained for these two genes at the RNA and protein levels were highly concordant (Table II).

There were discrepancies between the protein and gene expression data for vimentin and hasp27 that were previously shown to be induced at the mRNA level during DC differentiation (25). Close analysis of the microarray hybridization data showed saturation level intensities for these genes resulting from their high level expression. Therefore, the discordance between mRNA and protein levels observed in our data for these genes most likely reflects their high expression level, reaching saturation at the RNA hybridization level using microarrays but not at the protein level using two-dimensional gels. Vimentin and hasp27 proteins can be resolved into several isoforms on two-dimensional gels. Therefore, we wished to analyze the expression of these isoforms in DCs, by Western blotting using specific antibodies. Four vimentin spots, including two (spots 278 and 279) previously identified by mass spectrometry and two additional spots (spots 237 and 327), were revealed by Western blotting using a specific antibody against vimentin (Fig. 3). All four spots increased in intensity with DC differentiation (see Fig. 2). There was no detectable differential expression of the four isoforms of vimentin during DC differentiation. Hsp27 was resolved by two-dimensional gel electrophoresis into four isoforms, a nonphosphorylated (hsp27A; pl = 6.6) and three phosphorylated forms (hsp27B, -C, and -D; pl = 6.2, 5.7 and 5.5, respectively), as
described previously (12, 13). Spot 574 was identified by mass spectrometry as corresponding to the unphosphorylated hsp27A isoform, and spot 570 was identified as the phosphorylated form of hsp27B. Expression of both hsp27A (spot 574) and hsp27B (spot 570) was increased during DC differentiation, whereas the hyperphosphorylated forms hsp27C and hsp27D remained undetectable (Fig. 4A). Heat shock treatment of either CD14+ monocytes, preincubated with [32P]orthophosphate, resulted in the induction of all phosphorylated forms hsp27C and hsp27D remained undetectable (Fig. 4A). A decrease in the unphosphorylated form hsp27A in response to heat shock treatment correlated with an increase in hsp27-phosphorylated forms, as determined by silver staining (data not shown). These results suggest that the increase in hsp27 expression observed during DC differentiation was not due to a stress response of the cells but was specific to their differentiation stage and that phosphorylation of hsp27 was not modulated during DC differentiation.

Identification of a Novel Calreticulin Isoform—The calreticulin protein was found to be down-regulated with DC differentiation in our two-dimensional gel analysis, whereas the corresponding transcript was unchanged at the RNA level by microarray analysis. Hybridization data for calreticulin transcript did not show any saturation. Interestingly, a protein (spot 412) with an estimated molecular mass of 32 kDa and pI of 4.1 was found to be induced in immature DCs (Fig. 2). After enzymatic digestions using trypsin or endoproteinase Lys-C and analysis of the resulting peptides by MALDI-TOF mass spectrometry, the peptide masses were consistent with those of peptides derived from calreticulin, a protein with a mass of 48 kDa and a pI of 4.3. Calreticulin is a chaperone protein localized in the endoplasmic reticulum (23, 24), and no forms of calreticulin with a mass of 32 kDa have been reported previously. Interestingly, the peptides obtained from the tryptic and Lys-C digestions matched only with the C-terminal portion of calreticulin (Table IV). Additional enzymatic digestions using the endoproteinase Glu-C were performed. Peptides that exhibited high intensities after Lys-C or Glu-C digestions were further analyzed by electrospray ionization-mass spectrometry in order to obtain the sequence of these peptides. Two peptides were identified as LIVRPDNTYEVK and LIVRPDNTYEVE. These two peptides, obtained after different digestions, contained the same N-terminal end and therefore correspond to the N-terminal end of the protein. The molecular mass and pI values of the novel calreticulin form were calculated as 28825.69 Da and 4.07, respectively, in agreement with the mass determined by MALDI-TOF (29 kDa) and with the mass/pI estimated based on migration in two-dimensional gels. Altogether, these results indicated that the protein in spot 412 is a cleavage product of calreticulin, corresponding to the C-terminal end (amino acids 157–400) (Fig. 5A). We designated this newly identified form of calreticulin as Crt32. Identification of spot 412 as the C-terminal portion of calreticulin was further confirmed by Western blotting using two specific antibodies against calreticulin, SPA-600 and T-19 antibodies, produced against a C-terminal and an N-terminal peptide, respectively. Spot 412 was revealed by SPA-600 antibody (Fig. 5B) but not by T-19 antibody (data not shown). Three additional spots (413, 414, and 415), present only in DCs, were also recognized by the antibody produced against the C-terminal region of calreticulin. These three isoforms remain to be characterized. Concomitantly with the increase in Crt32 levels, full-length calreticulin (spots 138 and 182) was decreased during DC differentiation and maturation. These data suggested that calreticulin is most likely cleaved during DC differentiation yielding Crt32. Therefore, whereas microarray analysis did not show any changes involving calreticulin, proteomic analysis allowed the detection of a post-translational modification of calreticulin occurring during DC differentiation.

**DISCUSSION**

DCs are professional antigen-presenting cells that are critically involved in the initiation of a primary immune response (1, 2). DCs acquire their function with differentiation that occurs through a programmed expression of specific proteins. In order to develop a better understanding of DC differentiation, we utilized two complementary approaches to identify specific genes regulated during DC differentiation and matu-
ration. One approach relies on the quantitative analysis of mRNAs by oligonucleotide microarrays. The other approach relies on quantitative analysis and identification of proteins by proteomics. Indeed, proteins represent the most functional compartment of a cell, and the information obtained at the protein level cannot simply be predicted from examining expression at the RNA level. The proteomics approach is also appropriate to identify post-translational modifications, which may regulate protein function. A systematic analysis of genes that are differentially expressed with monocyte-derived DC differentiation using SAGE has been reported recently (25). Most of the genes described as differentially expressed were also found to be differentially expressed in our study. In addition, we have uncovered a large number of additional genes. We identified close to 4% of the genes and proteins analyzed as regulated during DC differentiation. The regulated genes were in major part related to cell adhesion and motility, growth control, regulation of the immune response and antigen presentation, and lipid metabolism. These include all genes previously reported to be modulated during DC differentiation. A large number of additional genes not previously reported in DCs have been identified in this study.

Immature DCs traffic from the blood to tissues where they take up and process antigens. DCs subsequently migrate to the draining lymphoid organs where they are converted to mature DCs with up-regulation of co-stimulatory and HLA molecules, resulting in priming of naive T cells. Interestingly, we identified a large number of genes encoding for proteins involved in cell adhesion and motility that are regulated during DC differentiation. Expression of galectin 2, CD11a/LFA-1b, ninjurin 1, macmarcks, syndecan 2, CD44E, and presenilin 1 was down-regulated. Expression of secreted proteins involved in cell motility, autotaxin-t and semaphorin E, reported to play a role in axon guidance in the nervous system (26), was up-regulated. Up-regulation of the cytoskeleton-related proteins, the macrophage capping protein, and vimentin, all involved in cell motility (27, 28), were also observed during DC differentiation. Therefore, the concomitant decrease in expression of integrins and cell adhesion molecules, the increase in expression of genes involved in cell motility, and regulated expression of enzymes such as α,-antitrypsin and macrophage metalloelastase (HME) likely have an effect on the enhanced migration properties of DCs compared with their precursors. HME belongs to the family of related matrix-degrading enzymes that are important in tissue remodeling and repair during development and inflammation (29).

Differentiation of DCs was accompanied by differential expression of genes involved in the immune response. Noticeable was the up-regulation of genes encoding anti-inflammatory proteins such as cyclophilin C and TSG-6 (30, 31) with a concomitant decrease in the production of pro-inflammatory cytokines. Several genes encoding pro-inflammatory cytokines and their receptors, such as prionterleukin-1β, TNF-α, CD163, C5a anaphylatoxin receptor, IL-6 receptor, and TNF receptor, were down-regulated. A noticeable change was the down-regulation of a set of chemokines belonging to the IL-8 superfamily such as CTAPIII, MIP2-α, MIP2-β, ENA78, PF4, and IL-8. It has been reported that these chemokines are pro-inflammatory cytokines that act as potent neutrophil chemottractants and activators (32). Interestingly, these chemokines that were coordinately down-regulated have been co-localized to the same genomic region (33). Osteopontin, a key cytokine involved in T lymphocyte activation (34), was up-regulated. The maturation of DCs was accompanied by the up-regulation of Mac-2-binding protein. Mac-2-binding protein is an adhesion molecule with a potent immune stimulatory activity. Indeed, it has been demonstrated that Mac-2-binding protein stimulates host defense systems, such as NK and LAK cell activities and induces the secretion of IL-2 (35). Up-regulation of TGF-α was also observed during DC maturation.

An important function of DCs is antigen uptake, processing, and presentation. As expected, mRNAs for Fc-εRII and Fc-γRII as well as for several MHC class II genes were up-regulated. A marked increase in macrophage mannose receptor (MRC1) RNA was observed. MRC1 is involved in the capture of antigens by immature DCs and in their delivery to MHC class II compartments (36). Several proteins known for their chaperone activity including hsp73, hsp27, and calreticulin were also regulated during DC differentiation. An emerging hypothesis is that heat shock proteins participate in antigen processing and presentation and play a central role in the activation of T lymphocytes by DCs (37–39). hsp70 targets immature DC precursors to enhance antigen uptake (40). We observed an up-regulation of hsp73 protein, related to the hsp70 family (41), during DC differentiation. hsp73 has been recently reported to bind specifically to the cell surface of monocyctic and dendritic cell lines and to be internalized spontaneously by receptor-mediated endocytosis (38). In addition, the murine hsp73 has been recently reported to accumulate in exosomes from immature DCs (42). The role of hsp27 up-regulation during DC differentiation is less clear. It has been reported that an increase in cellular levels of hsp27 promotes a resistance of monocytes to apoptotic cell death (43, 44). Increased hsp27 expression in DCs may therefore have a protective role against cytotoxicity. In contrast to hsp27 and hsp73, the cognate chaperone protein calreticulin was down-regulated during DC differentiation due to post-translational modification. In addition, whereas the expression of hsp27 and hsp73 was maximal in immature DCs, calreticulin was mostly down-regulated during DC maturation. Calreticulin participates in the assembly of MHC class I with peptide and β2-microglobulin in the endoplasmic reticulum, a process required for the presentation of antigenic peptides to cytotoxic T lymphocytes at the cell surface (23, 24). In addition, it has been reported recently that calreticulin elicits tumor- and peptide-specific immunity (45). Calreticulin displays in vivo peptide binding activity and can elicit cytotoxic T lymphocyte responses against bound peptides (46). Proteomic analysis of DCs allowed us to identify a truncated form of calreticulin, present only in DCs. We designated this novel form of calreticulin as Crt32. This form contains the P-domain, a site of chaperone activity, the C-domain, which contains the endoplasmic reticulum retrieval sequence, but lacks the N-domain. A calreticulin fragment corresponding to the N-domain has been recently purified from the supernatant of an Epstein-Barr virus-immortalized cell line. This fragment, named vasostatin, is an angiogenesis inhibitor that exerts antitumor effects in vivo (47, 48). Therefore, even though the C-terminal end of vasostatin has not been characterized precisely, Crt32 most likely corresponds to the complementary part of vasostatin, following cleavage of calreticulin. A decrease in levels of the cognate form of calreticulin and an increase in Crt32 levels may be relevant to DC function, and the precise function(s) of Crt32 in mature DCs is currently under investigation.

This study suggests a role for genes involved in lipid metabolism in DC function. Several genes encoding enzymes or proteins involved in the production, uptake, transport, and solubilization of cholesterol and fatty acids were up-regulated in DCs. This group includes apolipoprotein E, apolipoprotein C-I, ABCG1, lysosomal acid lipase, and lipoprotein lipase. The fatty acids are translocated from the extracellular environment to the cytoplasm by the fatty-acid translocase (FAT/CD36) and
then solubilized and transported by FABPs to the site where they are metabolized (49). We observed marked up-regulation of CD36 as well as of the lipid-binding proteins FABP3, FABP4, FABP5, CRABPII, and acyl-CoA-binding protein. Up-regulation of FABPs was concomitant with a strong down-regulation of the S100 proteins, MRPs, and MRP14. Interestingly, MRPs and MRP14 are expressed by myeloid cells during inflammatory reactions, and it has been reported that MRP-8/MRP-14 heterodimer (FA-p34) has a fatty acid binding activity and specifically binds (poly)unsaturated fatty acids (49, 50). In this context, we observed an up-regulation of 15-lipoxygenase that promotes the formation of lipoxins that are modulators of lipoycete recruitment (51–53).

The oligonucleotide array and proteomics analyses undertaken in this study have uncovered novel genes and proteins with potential roles in DC function, differentiation and/or maturation. Microarray analysis has identified important changes in genes involved in cell adhesion and motility, immune response, growth control, as well as in lipid metabolism. Following the simultaneous analysis of several thousand genes at the mRNA level, the challenge is to utilize efficiently this information to develop a better understanding of DC function. This study also demonstrates that a proteomics approach may provide information that could not be obtained at the RNA level, due to poor correlation between mRNA and protein levels or due to post-translational modifications that may result in several isoforms generated from one mRNA, as in the case of calreticulin in our study. Genes and proteins identified to be expressed selectively in DCs may provide further understanding of the biological function of DCs in host defense system and of the mechanisms of antigen processing and presentation.

REFERENCES

1. Banchereau, J., and Steinman, R. M. (1998) Nature 392, 245–252
2. Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y. J., Pulendran, B., and Palucka, K. (2000) Ann. Rev. Immunol. 18, 767–811
3. Young, J. W. (1998) Ann. Rev. Med. 49, 35–43
4. Prieto, J., Subira, M. L., Castilla, A., Arroyo, J. L., and Serrano, M. (1998) J. Immunol. 160, 243–282
5. Zhou, M., Lambert, H., and Landry, J. (1993) J. Biol. Chem. 268, 35–43

Gene Expression in Dendritic Cells

17931