

Analysis of the Gene Expression Profiles of Immature versus Mature Bone Marrow-Derived Dendritic Cells Using DNA Arrays

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Received November 16, 2001

Dendritic cells (DCs) are professional antigen-presenting cells of the immune system and can be generated *in vitro* from bone-marrow cells. In this study, we systematically investigated by DNA array analysis the expression profiles of 514 immunologically relevant genes in two populations of mouse bone marrow-derived DC, immature (DC_{IMAT}), and lipopolysaccharide (LPS)-stimulated mature (DC_{MAT}) DCs. Our data showed that DC_{IMAT} expressed transcripts for 69 (13.42% of the 514) of these genes and that, upon maturation, 32 (6.23%) of these were up-regulated and 40 (7.78%) down-regulated. Maturation-dependent up-regulation, defined by a differential expression (DE) ratio of >2, was observed among five cytokine (Flt-3L, TNF- α , IL-1 α and -1 β , and IL-6), three chemokine (RANTES, MIP-2 and GRO α) and three other (iNOS, MMP-13, and STRAP) genes. Reciprocally, maturation-dependent down-regulation occurred with one cytokine (IGF-1), two chemokine receptor (CCR2 and CCR5), and three other (RP105, Ax1, and UCP2) genes. Lower level, but nevertheless significantly enhanced expression of the chemokine receptor CCR7 and of NF- κ B was also observed upon DC maturation. This DC maturation profile confirms previous findings from other lab, but it also substantially broadens our view of these cells by documenting expression changes among genes (e.g., IGF-1, MMP-13, STRAP) not reported previously in these cells. © 2002 Elsevier Science

Key Words: cytokine profile; DNA array; dendritic cell maturation.

Dendritic cells (DCs) are one of the most potent antigen presenting cells (APCs). They are distributed widely throughout the body, where they are

variously represented by cells such as interdigitating reticular cells (lymphoid organs; ref. 1), blood monocytes (2), and Langerhans cells (epidermis; ref. 3). They are heterogenous cells that display multiple differences in morphology, phenotype and function (4, 5). In general, immature DCs, which have a great proclivity for endocytosis, migrate via the vasculature from the bone marrow into various organs, where they reside in an inactive state. Immature DCs do not effectively induce primary immune responses because they express neither the requisite costimulatory molecules nor stable complexes of antigenic peptides with major histocompatibility complex (MHC) molecules (6). In the presence of inflammatory signals they rapidly take up foreign antigens and undergo a differentiation/maturation process that downregulates further antigen-processing capacity, but enhances their expression of MHC, costimulatory, and other molecules important for successful antigen presentation. They then migrate to the lymphoid organs where they interact with, and activate, naive T cells (7). Because of the critical roles DCs have in the generation of primary immune responses, an important avenue of investigation is their potential for modulating functions such as the induction of immune tolerance or tumor immunity.

The maturational processes of DCs are efficiently regulated, such that these cells can achieve different states of activation/maturation and thereby different functional properties, depending on the precise nature of the signals they receive from their microenvironment. Maturation can be triggered by multiple stimuli, including contact allergens, bacteria and viruses, proinflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor- α (TNF- α), lipopolysaccharide (LPS), immunostimulatory unmethylated CpG oligonucleotides, and poly (I:C) (8–12), and signaling molecules (CD40 ligand) (13). However, the molecular biology of the differentiation and maturation

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of DCs, especially an understanding of DC maturation based on a global analysis of gene expression, has been less studied. The gene expression profiles of human monocyte-derived DC, as determined by serial analysis of gene expression (SAGE) or DNA array, have been documented (14–16). However, similar studies that target mouse bone marrow (BM)-derived DCs have not yet been reported. In the present study, we systematically analyzed the gene expression profiles of mouse BM-derived DCs by flow cytometry and DNA array approaches.

MATERIALS AND METHODS

Cell lines, antibodies, cytokine, peptides, and animals. Monoclonal rat anti-mouse H-2K^b, Ia^b, CD11b, CD11c, CD40, CD54, CD80, and CD86 antibodies were purchased from Pharmingen (San Diego, CA). The fluorescein isothiocyanate (FITC)-conjugated goat anti-rat IgG antibodies were purchased from Bio/Can Scientific (Mississauga, ON, Canada). Recombinant mouse interleukin (IL)-4, and granulocyte/monocyte-colony stimulation factor (GM-CSF) were purchased from Endogen (Woburn, MA). Female C57BL/6 (H-2K^b) mice were obtained from Charles River Laboratories (St. Laurent, PQ, Canada) and housed in the animal facility of the Saskatoon Cancer Center.

Generation of dendritic cells. Two procedures were used for generation of bone-marrow (BM)-derived dendritic cells (DCs) of differing maturational stages, the immature DCs (DC_{IMAT}) (17) and the lipopolysaccharide (LPS)-stimulated mature DCs (DC_{MAT}) (18). Briefly, BM cells prepared from femorae and tibiae of normal C57BL/6 mice were depleted of red blood cells with 0.84% ammonium chloride on day 1 and plated in Dulbecco's modified Eagle medium (DMEM; GIBCO, Gaithersburg, MD) plus 10% fetal calf serum (FCS) and low dose (2 ng/ml) GM-CSF alone for generation of DC_{IMAT} and in high dose (10 ng/ml) GM-CSF plus IL-4 (10 ng/ml) for generation of DC_{MAT}. On day 3, the nonadherent granulocytes, and T and B cells were gently removed and the respective fresh media were added, and two days later the loosely adherent proliferating DC aggregates were dislodged and re-plated. On day 7, the released nonadherent DC_{IMAT} was harvested. To stimulate DC maturation, lipopolysaccharide (LPS; 1 µg/ml) was added to the day7 GM-CSF/IL-4 DCs, and one day later the released nonadherent DC_{MAT} were harvested. All DCs were then subjected to phenotypic analysis by flow cytometry and DNA array.

Immunophenotypic analysis. For phenotypic analyses by flow cytometry, DCs were stained for 30 min on ice with antibodies specific for H-2K^b, Ia^b, CD11b, CD11c, CD40, CD54, CD80, and CD86 (each, 5 µg/ml), washed three times in phosphate-buffered saline (PBS), and then incubated for an additional 30 min on ice with FITC-conjugated goat anti-rat IgG antibody (1:60). After three more washes with PBS, the cells were analyzed by flow cytometry. Isotype-matched monoclonal antibodies were used as controls.

DNA array analysis. The DNA array analysis was conducted by using the mouse Cytokine DNA Array kit (R&D Systems, Minneapolis, MN). The kit comprised cDNAs of target genes (i.e., 514 characterized and 14 housekeeping genes; described at www.rndsystems.com) spotted in duplicate on a positively charged nylon membrane (8 × 12 cm). Prior to hybridization, the membranes were washed in 2 × SSPE at room temperature for 5 min, then prehybridized at 65°C for 2–3 h with hybridization solution supplemented with 100 µg/ml heat-denatured salmon sperm DNA (R&D Systems).

To generate the membrane probes for the analyses, total RNA was isolated from the DCs using a commercial kit (Qiagen Inc., Mississauga, ON) and digested with DNase I (GIBCO) for 15 min at room temperature. The digestion was stopped by addition of EDTA, then

the RNA was purified using RNase mini spin columns (Qiagen Inc.) and its concentration and quality determined spectrophotometrically (OD₂₆₀) and by denaturing agarose gel analysis, respectively. The generation of labeled cDNAs was performed according to the manufacturer's protocol (R&D Systems). Briefly, mouse specific cytokine gene primers (4 µl) were annealed to 2 µg of each RNA sample (final volume, 15 µl) at 42°C for 2 min. The primed RNA was then incubated at 42°C for 2 h with 333 µM dATP, dGTP, and dTTP, 1.7 mM dCTP, 0.6 U/µl RNasin, and 6.6 pM (³²P]-dCTP (3000 Ci/mmol; Amersham Canada Ltd.) in a final volume of 30 µl reverse transcriptase buffer. The labeled cDNA was purified on Sephadex G-25 spin columns (R&D Systems), denatured at 90–95°C for 10 min, then incubated with the array membranes for 16 h at 65°C in roller bottles. The probed arrays were washed in 0.5 × SSPE/1%SDS twice for 3 min at room temperature and twice for 20 min at 65°C, and then for 20 min in 0.1 × SSPE/1%SDS at 65°C, and finally exposed in a phosphorimaging cassette for different periods of time before scanning with a Molecular Imager FX (Bio-Rad Laboratories, Mississauga, ON).

The numerical data corresponding to the integrated radioactive intensity of each DNA array spot were quantified using *Quantity One* software (Bio-Rad Laboratories). The expression level (EL) value for each target mRNA represents the normalized signal-to-background (S/B) ratio of the DNA array spot, while the differential expression (DE) value represents the relative difference in gene expression between the two populations of cells. The assigned cut-off for significance of gene expression is defined as a S/B ratio ≥3.0 (R&D Systems). Since a DE value of ≥2.0 is more likely to reflect a real change in gene expression (19), gene expression DE values of <2.0 were considered to represent equivalent gene expression in the two groups of cells. The positive and negative DE values represent the up- and down-regulation of gene expression in the mature DC_{MAT} in relative to the immature DC_{IMAT}, respectively. DE values of 2 to 6, 6 to 12, and >12 were characterized as mild, moderate, and dramatic increases, respectively, in gene expression.

RESULTS AND DISCUSSION

The Cell Surface Phenotype of DC_{IMAT} and DC_{MAT}

The dendritic cells (DCs) used in this study were derived from mouse BM cells cultivated in the complete medium containing GM-CSF (2 ng/ml) alone, or GM-CSF/IL-4 (each, 10 ng/ml) plus LPS (1 µg/ml), for generation of DC_{IMAT} or DC_{MAT}, respectively. Both populations of cells demonstrated a typical DC morphology (data not shown). The immature DC_{IMAT} displayed high expression of MHC class I (H-2K^b), CD11b, CD11c (data not shown), and adhesion molecule CD54 (ICAM-1; Fig. 1). In addition, they exhibited very low expression of cell-surface MHC class II (Ia^b), CD80 and CD86, and no discernable expression of CD40. On the contrary, the LPS-stimulated mature DC_{MAT} showed dramatically up-regulated expression of cell-surface MHC class II (Ia^b), CD40, CD54, CD80, and CD86 (Fig. 1), each of which play critical roles in the activation of T cells (7). The expression of MHC class I, CD11b, and CD11c in these DCs remained unchanged (data not shown). The observed phenotypes of our DC_{IMAT} and DC_{MAT} were thus consistent with previous descriptions of immature and mature DCs (12, 13).

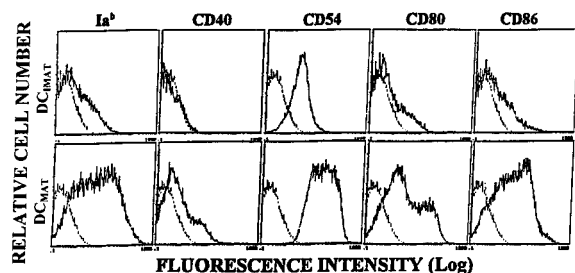


FIG. 1. Comparison of the phenotypic changes induced in mouse bone marrow-derived DCs by maturational signals. Mouse bone marrow-derived DCs were generated in either low dose (2 ng/ml) GM-CSF alone (DC_{IMAT}), or high dose (10 ng/ml) GM-CSF/IL-4 followed by LPS (1 g/ml) stimulation (DC_{MAT}) for the last two days of culture. The cells were then stained for analysis of MHC class II (Ia^b), CD40, CD54, CD80, and CD86 expression by flow cytometry using FITC-labeled antibodies (solid lines). Isotype-matched monoclonal irrelevant specificity antibodies were used as controls (dotted lines). One representative experiment of three is shown.

Global Characterization of Gene Expression in Bone Marrow-Derived DCs

A technology that identifies differential gene expression provides an important tool to cell biologists, and

several such methods have been available (e.g., Northern blotting, RT-PCR, differential display, and RNase protection assays; refs. 20–22). However, a new method, DNA array analysis that can simultaneously probe hundreds of genes in a sample has recently been developed (23). The advantage of these DNA arrays is that the genes to be assessed are meticulously selected for their biological relevance. We used an array of 514 genes that play key roles in many different biological processes relevant to DC maturation. We found that, of these, DC_{IMAT} expressed 69 (i.e., 13.42% of the 514) transcripts with an expression level (EL) value of ≥ 3 , while the DC_{MAT} similarly expressed 65 (12.65% of the 514) such transcripts. The expression levels of 20 (3.89% of the 514) of the shared identified transcripts were equivalent in the two populations of DCs (Table 1). On the other hand, some 72 genes were differentially expressed by DC_{IMAT} and DC_{MAT} (Tables 2 and 3); 32 (6.23%) of these were up-regulated (Table 2), and 40 (7.78%) down-regulated (Table 3), in association with the maturation process. The list of differentially regulated genes included numerous cytokines and cytokine receptors, chemokines and their receptors, alternate surface proteins (e.g., B7, adhesion molecules), cyto-

TABLE 1
List of Genes That Were Expressed at Equivalent Levels in the Immature and Mature DCs

Genes	GenBank Accession No.	DC _{IMAT} (EL)	DC _{MAT} (EL)	DE
Cytokines/Cytokine Receptors:				
Interleukine-3 Receptor β (IL-3 R β)	NM_007781	14.9	21.5	<2.0
Interleukine-1 receptor α (IL-1 R α)	M57525	10.7	17.9	<2.0
Interleukine-2 receptor γ (IL-2 R γ)	L20048	11.7	11.3	<2.0
Interleukine-7 receptor α (IL-7 R α)	NM_008372	5.4	5.2	<2.0
Interferon- γ receptor 2 (IFN- γ R2)	NM_008338	3.6	3.2	<2.0
Chemokines/Chemokine Receptors:				
C10	NM_009139	9.0	8.2	<2.0
Macrophage inflammatory protein 1 γ (MIP-1 γ)	NM_011338	22.4	25.5	<2.0
Thymus and activation-related chemokine (TARC)	AJ242587	7.5	10.8	<2.0
Macrophage-derived chemokine (MDC)	NM_009137	55.2	39.7	<2.0
Cell Surface Proteins:				
Vascular endothelial growth factor-A (VEGF-A)	NM_009505	6.5	8.0	<2.0
Signal Transduction Molecules:				
RhoA	NM_016862	9.4	8.5	<2.0
Other Molecules:				
A1	L16462	26.6	26.6	<2.0
Matrix metalloproteinase-14 (MMP-14)	NM_008608	4.2	4.8	<2.0
Bag-1	NM_009736	3.0	3.6	<2.0
Matrix metalloproteinase-12 (MMP-12)	NM_008605	60.2	101.7	<2.0
Notch-1	NM_008714	5.9	4.9	<2.0
Protein inhibitor of NOS1 (PIN)	AA041883	8.7	5.9	<2.0
Notch-2	D32210	8.0	5.5	<2.0
Matrix metalloproteinase-8 (MMP-8)	NM_008611	28.3	17.4	<2.0
Transforming growth factor- β (TGF- β)	NM_011577	18.6	11.9	<2.0

Note. The expression level (EL) value, representing the normalized signal-to-background (S/B) ratio of each DNA array signal. The differential expression (DE) value represents the fold change of gene expression between two groups of DCs. DC_{MAT}:DC_{IMAT} DE values of <2 are considered to represent equivalent gene expression in the two populations of DCs.

TABLE 2

List of Genes for Which the Observed Expression Was Up-Regulated in the Mature DC Population

Genes	GenBank Accession No.	DC _{IMAT} (EL)	DC _{MAT} (EL)	DE
Cytokines/Cytokine Receptors:				
Interleukin-12p35 (IL-12P35)	M86672	1.0	3.8	3.8
Granulocyte colony stimulating factor (G-CSF)	NM_009971	1.1	5.5	5.0
Fms-like tyrosine kinase-3 ligand (Flt-3L)	U04607	1.3	9.7	7.5
Tumor necrosis factor- α (TNF- α)	M13049	1.0	13.0	13.0
Interleukin-1 α (IL-1 α)	NM_010554	6.0	106.7	17.8
Interleukin-6 (IL-6)	X54542	1.3	36.0	27.7
Interleukin-1 β (IL-1 β)	NM_008361	1.3	89.1	68.5
Interferon- α receptor 2 (IFN- α R2)	NM_010509	5.2	10.4	2.0
Interleukin 2-receptor α (IL-2 R α)	NM_008367	1.7	5.9	3.5
Chemokines/Chemokine Receptors:				
Macrophage inflammatory protein-1 α (MIP-1 α)	NM_011337	10.2	34.4	3.4
Monocyte chemotactic protein-1 (MCP-1)	NM_011333	4.3	19.4	4.5
Macrophage inflammatory protein-1 β (MIP-1 β)	M35590	3.0	14.5	4.8
Regulated upon activation normal T cell express sequence (RANTES)	M77747	13.2	86.9	6.6
Macrophage inflammatory protein-2 (MIP-2)	NM_009140	3.5	36.1	10.3
Growth related oncogene α (GRO α)	G201043	1.1	19.5	17.7
CC-chemokine receptor-7 (CCR-7)	NM_007719	1.2	4.0	3.3
Cell Surface Proteins:				
4-1BB	NM_011612	12.6	26.2	2.1
Intercellular adhesion molecule-1 (ICAM-1)	X52264	2.8	9.6	3.4
CD80	X60958	1.5	8.4	5.6
Glucocorticoid induced TNF receptor family related gene ligand (GITR)	NM_009400	1.1	7.1	6.5
CD86	L25606	1.2	9.6	8.0
Signal Transduction Molecules:				
Cytokine-inducible SH2-containing protein 1 (CIS 1)	NM_009895	2.2	5.6	2.5
Cytokine-inducible SH2-containing protein 3 (CIS 3)	NM_007707	1.7	5.7	3.4
Cytokine-inducible SH2-containing protein 2 (CIS 2)	NM_007706	1.9	8.7	4.6
Nuclear factor kappa Bp65 (NF- κ Bp65)	NM_010589	1.3	6.8	5.2
Serine-threonine kinase receptor-associated protein (STRAP)	NM_011499	2.2	24.7	11.2
Other Molecules:				
Extracellular matrix metalloproteinase inducer (EMMPRIN)	Y16256	5.8	11.7	2.0
Notch-3	NM_006716	1.7	4.7	2.8
Tissue inhibitors of metalloproteinases 1 (TIMP-1)	NM_011593	1.2	3.7	3.1
Cyclooxygenase-2 (Cox-2)	NM_011198	1.7	8.2	4.8
Matrix metalloproteinase-13 (MMP-13)	NM_008607	1.5	12.0	8.0
Inducible nitric oxide synthase (iNOS)	NM_010927	2.0	18.0	9.0

Note. Up-regulated gene expression was based on the DE value for the mature DC_{MAT} relative to the immature DC_{IMAT} being ≥ 2 .

plasmic signal transduction intermediates (e.g., JAK, STAT), and an array of alternate immunologically relevant factors (e.g., iNOS).

Impact of DC Maturation on the Cellular Expression of Cytokines, Chemokines and Their Respective Receptors

Among the cytokines and their receptors expressed by our DC, cellular maturation led to a mild-to-moderate up-regulation of G-CSF, IL-12, Flt-3L, IFN α R2, and IL-2R α , and a dramatically increased expression of the immunoinflammatory mediators IL-1 α and -1 β , and TNF α , as well as IL-6. Simultaneously, DC_{MAT} experienced a down-regulated expression of several cytokine receptors (TNF α RII, IFN- γ R1,

IL-10R β , and M-CSFR), as well as a few cytokines themselves; IGF-1 mRNA levels were dramatically reduced, while OPN and Mer expression were more mildly affected. In concert with this, the cells down-regulated their receptors for an array of inflammatory chemokines (CCR1, CCR2, CCR5, IL-8R, and CXCR4). However, they mildly increased their expression of chemokines MCP-1, MIP-1 α , and -1 β , moderately increased MIP-2 and RANTES expression, and dramatically increased their GRO α expression. The steady-state levels of mRNA for the important lymphoid tissue-homing chemokines receptor, CCR7, were also upregulated in the DC_{MAT}.

The significantly up-regulated expression of some of these cytokines and chemokines and their respective

TABLE 3

List of Genes for Which the Observed Expression Was Down-Regulated in Mature DC Population

Genes	GenBank Accession No.	DC _{IMAT} (EL)	DC _{MAT} (EL)	DE
Cytokines/Cytokine Receptors:				
Osteopontin (OPN)	NM_009263	46.6	20.6	-2.3
Mer	U21301	3.4	1.0	-3.4
Insulin-like growth factor-I (IGF-I)	NM_010512	22.5	1.0	-22.5
Tumor necrosis factor receptor II (TNF RII)	NM_011610	22.2	11.0	-2.0
Interferon- γ receptor 1 (IFN- γ R1)	NM_010511	6.9	2.3	-3.0
Interleukin-10 receptor β (IL-10R β)	NM_008349	11.2	3.6	-3.1
Macrophage colony stimulating factor receptor (M-CSFR)	NM_007779	19.1	3.9	-4.9
Chemokines/Chemokine Receptors:				
Cytokine-responsive gene-2 (CGR-2)	M86829	4.2	1.9	-2.2
CC-chemokine receptor-1 (CCR-1)	NM_009912	4.3	1.0	-4.3
Interleukin-8 receptor (IL-8R)	NM_009909	4.2	0.8	-5.3
CXC-chemokine receptor-4 (CXCR-4)	NM_009911	16.0	2.7	-5.9
CC-chemokine receptor-5 (CCR-5)	NM_009917	10.7	1.4	-7.6
CC-chemokine receptor-2 (CCR-2)	NM_009915	14.3	1.1	-13.0
Cell Surface Proteins:				
Fas	NM_010177	4.1	2.0	-2.1
LPS binding protein (LPS BP)	NM_011029	45.5	17.3	-2.6
Integrin β 5A	NM_010580	9.2	3.3	-2.8
CD45	NM_011210	10.0	3.4	-2.9
Integrin β 2	NM_008404	44.9	14.1	-3.2
Latent transforming growth factor- β binding protein-3 (LTBP3)	NM_008520	6.5	1.8	-3.6
C5a receptor (C5aR)	NM_007577	8.5	2.3	-3.7
L-Selectin	NM_011346	5.2	1.0	-5.2
Radioprotective 105 kD (RP 105)	NM_006533	5.1	0.9	-5.7
MD-1	NM_010745	12.9	1.6	-8.1
Signal Transduction Molecules:				
Signal transducers and activators of transcription 1 (STAT1)	NM_009283	15.1	7.7	-2.0
Signal transducers and activators of transcription 6 (STAT6)	NM_009284	3.2	1.5	-2.1
Small conductance Ca (2+)-activated potassium (SK) channels 1 (SK 1)	U14173	3.7	1.8	-2.1
TRAF family member-associated NFKB Activator (TANK)	NM_011529	5.9	2.8	-2.1
14-3-3 eta	NM_011738	16.9	7.8	-2.2
Ski-related novel gene non Alu-containing (SnoN)	NM_011386	6.8	3.0	-2.3
Janus family of tyrosine kinases-1 (JAK-1)	S63728	12.9	3.7	-3.5
Janus family of tyrosine kinases-2 (JAK-2)	NM_008413	7.3	1.6	-4.6
Other Molecules:				
Bcl-x	NM_009743	3.1	1.5	-2.1
Caspase-3	NM_009810	3.0	1.4	-2.1
Bcl-2 antagonist/killer (BAK)	NM_007523	5.7	2.4	-2.4
Leptin	NM_008493	3.2	1.1	-2.9
MCH	BB175332	3.4	1.1	-3.1
A disintegrin and metalloproteinase-10 (ADAM-10)	NM_007399	4.8	1.5	-3.2
Uncoupling protein 3 (UCP3)	NM_009464	4.7	0.8	-5.9
Tyrosine kinase receptor (Axl)	NM_009465	11.2	1.0	-11.2
Uncoupling protein 2 (UCP2)	NM_011671	49.4	3.2	-15.4

Note. Down-regulated gene expression was based on the DE value for the mature DC_{MAT} relative to the immature DC_{IMAT} being ≥ -2 .

receptors in the DC_{MAT} would be expected to have important immunoregulatory effects. For example, RANTES, MIP-1 α and -1 β , MCP-1, and MIP-2 are each chemotactic for both T cells and macrophages (24), two of the major cell populations involved in immune responses (7). In addition, TNF- α has the ability to activate both T cells (25) and DCs (26, 27), and the IL-12 expressed by DC_{MAT} can effect Th1-skewing of the immune repertoire (28). Down-regulation on the maturing DC themselves of the TNF α , IL-10, and IL-8 recep-

tors is consistent with their new role of emigration from the inflammatory focus into the lymphatics. Another prelude to such emigration would be a reduction in responsiveness to the inflammation-induced chemokines (e.g., MCP-1 and MIP3 α), through the observed down-regulation of their cellular receptors CCR1, CCR2, and CCR5. Similarly, an up-regulation of responsiveness to the lymphoid organ-homing chemokines MIP3 β and secondary lymphoid tissue chemokine (SLC) is requisite for this DC migratory function,

which is mediated through the CCR7 (29, 30). The FLT-3L plays a role in the regulation of hematopoiesis, possessing a growth-stimulatory effect on DCs (31). Its expression by maturing DC, whose departure from the tissues would be imminent, could perhaps be designed to foster subsequent recruitment of new, immature DC into the resolving lesions.

DC Maturation Affects the Expression of Numerous Alternate Cell Surface Molecules and Intracellular Signal Transduction Intermediates

Our FACS data for augmented expression of the antigen-presentation-associated molecules ICAM-1 (CD54), CD80, and CD86 were fully supported by the DNA array mRNA data. The significance of these molecules in DC biology was noted above. However, maturation was also associated with increased steady state levels of mRNA for 4-1BB and the glucocorticoid-induced TNF receptor family-related gene ligand (GITR), but reduced expression of the binding proteins/receptors for lipopolysaccharide (LPS-BP) and C5a (C5aR), two other biological response modifiers expressed within inflammatory lesions, as well as that for latent transforming growth factor- β (LTBP3). Also down-regulated were expression of integrins- β 2 and - β 5a, and L-selectin, the apoptosis-related receptor Fas, CD45, MD-1, and RP1-5. Of these, the maturational process dramatically affected only GITR, CD86, and MD-1. Cell surface receptors normally effect their functions via cytoplasmic signal transduction intermediates, and we found that multiple members of the JAK/STAT family of mediators were more strongly expressed in DC_{IMAT}, as were SKI, TANK, 14-3-3-eta, and SnoN. On the other hand, several CIS proteins, NF κ B and STRAP were differentially up-regulated in the DC_{MAT}, with the latter two being very substantially affected by the LPS-driven maturation.

It is known that LPS binds to DCs via their Toll-like receptor (TLR)-4 in the mouse (32), but the mechanism(s) of LPS-induced intracellular signaling are less well-studied. Previous studies have shown that during DC differentiation there is a substantial translocation of NF κ B-p65 from the cytoplasm to the nucleus (33–35), and our DNA array data confirmed also that there are significantly (5.2-fold) enhanced levels of NF- κ B mRNA in LPS-stimulated DC. Our cumulative data are thus consistent with reports which demonstrated that NF- κ B/Rel transcription factors also regulate cytokine and chemokine gene expression in LPS-stimulated monocytes (36, 37).

The last grouping of mediators we examined incorporated apoptosis- and weight regulation-related products, as well as metalloproteinase-associated molecules, COX-3, Notch-3, and type 2 nitric oxide synthase (iNOS). Of these, only MMP-13 (collagenase 3) and iNOS were significantly up-regulated during the DC

maturation process. The connective tissue matrix metalloproteinases (MMPs) that possess elastolytic activity are members of a family of at least 15 Zn⁺⁺-dependent endopeptidases that function extracellularly (38). The MMP family of enzymes contributes to both normal and pathological tissue remodeling and plays a key role in the cell migration through the body (14). In this study, we observed an eightfold increase in MMP-13 expression in the mature DC_{MAT} relative to the DC_{IMAT}, and this is consistent with a recent report by Lapteva *et al.* (16). This enhanced expression of MMP-13 by DC_{MAT} could promote their CCR7-dependent migration from the tissue site(s) of inflammation to the regional lymph nodes, and thereby indirectly contribute to the initiation of immune responses *in vivo*.

Inducible nitric oxide synthase was originally described as an enzyme that is expressed in activated macrophages, where it generates nitric oxide (NO) from amino acid L-arginine, and thereby contributes to the control of replication or killing of intracellular microbial pathogens (39, 40). Recently, it has been shown that iNOS can also control the function of natural killer (NK) cells and the expression of cytokines such as IFN- γ or transforming growth factor- β , through activation of Tyk2 kinase, and thus IL-12 and IFN- α/β signaling in NK cells (41). In this study, we observed a 9.0-fold increase in expression of iNOS in the mature DC_{MAT} compared with the immature DC_{IMAT}, indicating that the LPS-stimulated mature DCs become more efficient in regulation of NK response.

In conclusion, we assessed the expression within maturing mouse bone marrow-derived DC of some 514 genes that play critical roles at various stages of normal immunoregulation. These cells are well known for their immunoregulatory functions (6), and this represents a first application of this powerful DNA array method with such cells. Most of genes observed to be up-regulated in our mature DCs are associated with functions such as cell migration, chemoattraction and activation of other immune cells, and our results largely agree with those of previous studies that targeted gene expression in human monocyte-derived DCs (14–16). Taken together, our data both validate finding in other systems and contribute further to our understanding of the biological processes that occur during BM-derived DC maturation and to the biological functions of DCs in the host immunity.

ACKNOWLEDGMENT

This study was supported by a research grant (ROP-15151) from the Canadian Institutes of Health Research.

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