Journal of Cellular Biochemistry 83:281–290 (2001)

Expression of Different NF-κB Pathway Genes in Dendritic Cells (DCs) or Macrophages Assessed by Gene Expression Profiling

Ioannis Baltathakis,[1,2] Orlando Alcantara,[1] and David H. Boldt[1,2]*

[1]Medicine/Hematology, University of Texas Health Science Center at San Antonio, San Antonio, Texas
[2]South Texas Veterans Health Care System, Audie L. Murphy Division, San Antonio, Texas

Abstract NF-κB/Rel transcription factors have been implicated in the differentiation of monocytes to either dendritic cells (DCs) or macrophages, as well as in the maturation of DCs from antigen-processing to antigen-presenting cells. Recent studies of the expression pattern of Rel proteins and their inhibitors (IκBs) suggest that their regulation during this differentiation process is transcriptional. To investigate differential gene expression between macrophages and DCs, we used commercially available gene microarrays (GEArray KIT), which included four of the NF-κB/Rel family genes (p50/p105, p52/p100, RelB, and c-rel) and 32 additional genes either in the NF-κB signal transduction pathway or under transcriptional control of NF-κB/Rel factors. To generate macrophages and DCs, human adherent peripheral blood monocytes were cultured with M-CSF or GM-CSF + IL-4 respectively for up to 8 days. DCs (and in some experiments, macrophages) were treated with lipopolysaccharide (LPS) for the last 48 h of culture to induce maturation. Cells were harvested after 7 days, cDNA was prepared and radiolabeled with α-^{32}P-dCTP, then hybridized to gene arrays containing specific gene probes. β-actin and GAPDH or PUC18 oligonucleotides served as positive or negative controls, respectively. The expression of all four NF-κB/Rel family genes examined was significantly upregulated in maturing DCs compared to macrophages. The strongest difference was observed for c-rel. RT-PCR determinations of c-rel, RelB, and p105 mRNAs confirmed these observations. Among the 32 NF-κB/Rel pathway genes, 14 were upregulated in mature DCs compared to macrophages. These genes were IκBα, IKK-β, NIK, ICAM-1, P-selectin, E-selectin, TNF-α, TNFR2, TNFAIP3, IL-1α, IL-1R1, IL-1R2, IRAK, and TANK. By contrast, only mcp-1 (monocyte chemotactic protein 1) was upregulated in macrophages compared to DCs. NF-κB pathway genes upregulated in DCs compared to macrophages were constitutively expressed in monocytes then selectively downregulated during macrophage but not DC differentiation. LPS did not induce expression of most of these genes in macrophages but LPS did induce upregulation of IL-8 in mature macrophages. We conclude that NF-κB/Rel family genes, especially c-rel, are selectively expressed during differentiation of monocytes towards DCs. Moreover, this differential expression is associated both with activation of different NF-κB signal transduction pathways in DCs and macrophages and with expression of a unique subset of genes in DCs that are transcriptionally targeted by NF-κB/Rel factors. The results illustrate the ability of the NF-κB pathway to respond to differentiation stimuli by activating in a cell-specific manner unique signalling pathways and subsets of NF-κB target genes. J. Cell. Biochem. 83: 281–290, 2001.

Key words: NF-κB; dendritic cells; macrophages; cDNA arrays

Abbreviations used: DCs, dendritic cells; ELAM-1, endothelial leukocyte adhesion molecule-1; GAPDH, glyceraldehyde 3 phosphate dehydrogenase; IκB, inhibitor of κ-B; ICAM-1, intercellular adhesion molecule-1; IKK, inhibitor kappa B kinase; IL-IR, interleukin-1 receptor; IRAK, IL-1 receptor-associated kinase; IRF-1, interferon regulatory factor-1; LPS, lipopolysaccharide; mcp-1, monocyte chemotactic protein-1; NIK, NF-κB-inducing kinase; TANK, TRAF family member-associated NF-κB activator; TNFAIP3, tumor necrosis factor alpha induced protein-3; TNFR, tumor necrosis factor receptor; TRADD, tumor necrosis factor receptor-associated death domain; TRAF, tumor necrosis factor receptor-associated factor; VCAM-1, vascular cell adhesion molecule-1.

DOI 10.1002/jcb.1231

Grant sponsor: NIH; Grant number: RO1 DK5042.

Ioannis Baltathakis's present address is Department of Immunology and Histocompatibility, Evangelismos General Hospital, Athens, Greece.

*Correspondence to: David H. Boldt, Medicine/Hematology, Mail Code 7880, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900.
E-mail: boldt@uthscsa.edu

Received 2 May 2001; Accepted 30 May 2001

Both dendritic cells (DCs) and macrophages are derived from the same CD34+ hematopoietic progenitor cell [Peters et al., 1997; Reid, 1997; Palucka et al., 1998; Traver et al., 2000]. Whereas controversy has existed regarding DC subtypes and their origins, it is clear that peripheral monocytes can give rise to cells with phenotypic and functional characteristics of both DCs and macrophages [Sallusto and Lanzavecchia, 1994; Chapuis et al., 1997; Palucka et al., 1998]. Recently investigators have begun to examine DC and macrophage differentiation from monocytes at the molecular level using serial analysis of gene expression (SAGE) [Hashimoto et al., 1999a,b, 2000]. These studies indicated that the NF-κB family gene, p105/50 was more highly expressed in lipopolysaccharide (LPS)-stimulated monocytes than in their unstimulated counterparts and also was differentially expressed between mature and immature DCs, being more highly expressed in the former [Hashimoto, 2000]. Other investigators also have reported differential expression of Rel/NF-κB family genes during human monocyte differentiation to macrophages and DCs [Rescigno et al., 1998; Ammon et al., 2000; Neumann et al., 2000]. In addition, RelB-deficient mice have substantially reduced numbers of myeloid-derived DCs in the thymus [Burkly et al., 1995; Weih et al., 1995; Wu et al., 1998], an observation further implicating the Rel/NF-κB family of transcription factors in DC development in a whole animal model.

NF-κB regulates expression of multiple genes important in immunological and inflammatory responses [May and Ghosh, 1997; Mercurio and Manning, 1999]. Functionally active NF-κB is a dimer composed of combinations of five mammalian proteins characterized by the Rel homology domain. These proteins are p65/RelA, c-rel, RelB, p100/p52/NF-κB2, and p105/p50/NF-κB1. The Rel homology domain is necessary for DNA binding, nuclear translocation, and protein–protein interactions. The consensus κB DNA-binding sequence is 5′-GGGRNNYYCC-3′ where R is purine and Y is pyrimidine [May and Ghosh, 1997]. NF-κB activity is regulated by a family of cytoplasmic inhibitory proteins, inhibitor of κ-B (IκBs), which interact with NF-κB and prevent its nuclear translocation [May and Ghosh, 1997; Mercurio and Manning, 1999]. NF-κB-mediated signaling is induced by a multiplicity of external stimuli (cytokines/growth factors, bacterial or viral products, pre-apoptotic or necrotic stimuli, UV-irradiation, reactive oxygen species, and others) acting through different signal transduction pathways and causing phosphorylation of IκBs, their subsequent proteolytic degradation, and release of active NF-κB for nuclear translocation [May and Ghosh, 1997; Mercurio and Manning, 1999]. Among the genes regulated by NF-κB are MHC molecules, cytokines and growth factors and their receptors, cell adhesion molecules, transcription factors, and the Rel/NF-κB and IκB proteins themselves [May and Ghosh, 1997].

Because of the documented association of NF-κB expression with DC differentiation we have used gene arrays to examine expression of NF-κB/Rel family genes and 32 additional κB pathway genes during DC and macrophage differentiation from human peripheral blood monocyte precursors.

MATERIALS AND METHODS

Monocyte Isolation

Peripheral blood was obtained from normal volunteer donors after informed consent. Blood was anticoagulated with EDTA, and the platelet fraction was decreased by centrifugation of whole blood at 1.5G for 10 min and removing the plasma/platelet layer. Mononuclear cells were then isolated via the ficoll method. Monocytes were selected by plating the mononuclear cells into Costar flasks in serum free medium for 2 h at 37°C in an atmosphere of 5% CO_2-95% air, and then removing non-adherent cells. Complete medium (RPMI supplemented with 1% L-glutamine, 10% heat-inactivated FCS, 1% penicillin/streptomycin, and 50 μM 2-ME) was then added to ready the cells for culture.

Cell Cultures

Monocytes were cultured in the presence of IL-4 (250 U/ml) (R&D Systems, Minneapolis, MN) and GM-CSF (5 ng/ml) (R&D Systems, Minneapolis, MN) for 6 days at 37°C in an atmosphere of 5% CO_2-95% air to induce dendritic cell differentiation. LPS (100 ng/ml) (Sigma, St. Louis, MO) was added at Day 6 to induce maturation, and the cultures were continued for an additional 48 h to produce mature DCs. Alternatively, monocytes were cultured with M-CSF (50 U/ml) (R&D Systems, Minneapolis, MN) to induce macrophage differentiation. Cultures were fed every 2 days

by removing 1/3 of supernatant and adding back fresh media with growth factors. Mature DCs or macrophages were harvested at 7 days for microarray experiments or FACS immunophenotyping. Viability of cells in cultures exceeded 90%. Recovery of cells from cultures averaged from 60 to 80% for DCs and 50% for macrophages relative to numbers of monocytes plated. The immunophenotypes of mature DCs and macrophages generated in our laboratory using these protocols were CD14-CD64-CD40+ CD1a+CD80+CD83+CD86+HLA-DR+ or CD14+CD64+CD40+CD1a-CD80-CD83-CD86-HLA-DR+, respectively (Fig. 1). DCs stimulated proliferation of naïve allogeneic lymphocytes and 90% of macrophages phagocytosed latex beads [Kramer et al., 1999]. Therefore, these differentiated cells had immunophenotypic and functional characteristics of mature DCs or macrophages.

Microarray Experiments

For these experiments we purchased GEArray gene expression array systems (SuperArray, Bethesda, MD). Each array consists of 56 coordinates containing specific cDNA fragments spotted in duplicate as well as control sequences (PUC18 as negative control; β-actin and glyceraldehyde 3 phosphate dehydrogenase (GAPDH) for loading). Using different arrays we compared expression of 36 genes in peripheral blood monocytes, mature DCs, and macrophages. A list of genes examined is given in Table I. For these experiments total cellular RNA was isolated by a modification of the guanidinium isothiocyanate technique. Integrity of RNA was assessed by visualization of ethidium bromide-stained gels. The microarrays were employed according to the manufacturer's instructions. In brief, using reagents provided cDNA was prepared from total RNA by reverse transcription with MMLV reverse transcriptase, radiolabeled using [α-^{32}P] dCTP (3,000 Ci/mM), then hybridized under precisely specified conditions to a positively charged nylon membrane containing the arrayed DNA. After washing, the arrays were visualized by autoradiography. Loading was adjusted based on intensity of hybridization signals to the housekeeping genes, β-actin and GAPDH, then gene expression was quantitated by scanning densitometry. Each experiment was performed at least twice to ensure reproducibility of results.

RT-PCR

RT-PCR was performed to confirm results of selected expression array experiments. After extraction of total cellular RNA, reverse transcription was initiated using the GeneAmp© RNA PCR kit (Perkin Elmer, Branchbury, NJ). Reverse transcribed cDNA was amplified in the linear range of amplification in presence of specific primer sets using the following conditions: denaturation at 94°C (60 s); [92°C (30 s), 60°C (30 s), and 75°C (90 s)] 30 times using an MJ Research PTC-100 thermocycler. PCR products were separated on 1.6% agarose gels stained with ethidium bromide to view the amplified bands.

RESULTS

NF-κB/Rel Family Genes are Differentially Expressed by Macrophages and DCs

Figure 2 shows array results presented to highlight expression of four NF-κB/Rel family genes in monocytes, macrophages and DCs. The four genes are c-rel, RelB, p105/p50, and p100/p52. In Figure 2, expression is normalized for GAPDH. Compared to macrophages expression of all four NF-κB/Rel family genes is substantially increased in DCs. The greatest difference is seen with c-rel. However, all four genes also are expressed by undifferentiated peripheral monocytes. Confirmation of array data by RT-PCR for three genes, p105/p50, relB, and c-rel, is shown in the lower portion of Figure 2. Increased expression of c-rel and p105/p50 in DCs compared to macrophages was seen consistently in 7 of 7 experiments and that of Rel B and p100/p52 was observed in 3 of 5 separate experiments.

These data indicate that DCs and macrophages differ in expression of NF-κB/Rel family genes. The data also suggest that during differentiation from monocytes to macrophages expression of these genes is actively down-regulated whereas expression is maintained during DC differentiation.

Differential Expression of NF-κB/Rel Family Pathway Genes in Macrophages and DCs

The observation that four NF-κB/Rel family genes were differentially expressed between DCs and macrophages implied that differential expression of NF-κB/Rel family pathway genes may contribute to the different functional

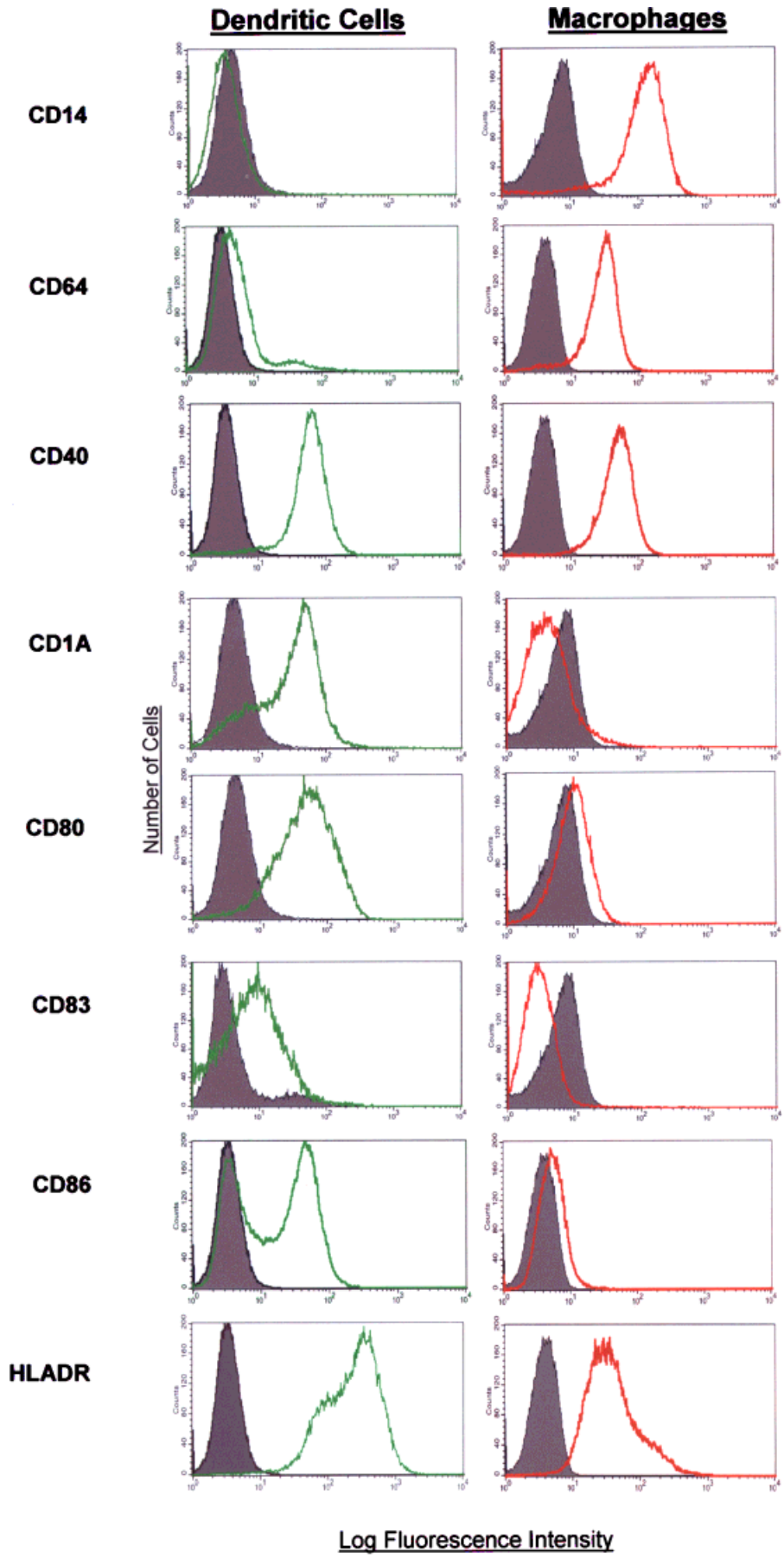

Dendritic Cells
Macrophages
CD14
CD64
CD40
CD1A
CD80
CD83
CD86
HLADR
Number of Cells
Log Fluorescence Intensity

TABLE I. NK-κB-Pathway Genes Examined

Gene class	Genes
Cytokine/growth factors (n = 10)	IL-1α, IL-1β
	IL-2
	IL-6
	IL-8
	TNF-α, TNF-β
	G-CSF
	GM-CSF
	mcp-1
Transcription/growth regulatory factors (n=17)	c-myc
	IL-1R1, IL-1R2*
	TNFR1, TNFR2*
	TRAF2, TRAF6*
	IKK-α, β, γ*
	IκBα (mad 3)
	IRF-1
	TANK (I-TRAF)*
	TRADD*
	TNFAIP3*
	IRAK*
	NIK*
Adhesion molecules (n = 4)	ICAM-1
	ELAM-1
	VCAM-1
	P-Selectin
NF-κB-components (n = 4)	c-rel, RelB, p100/p52, p105/−50
Other (n = 1)	iNOS

*Genes marked with an asterisk are NF-κB signal transduction pathway components. Other genes are NF-κB target genes.

phenotypes of these two cell types. Therefore, we used cDNA arrays to examine expression of 32 NF-κB/Rel family pathway genes in monocytes, macrophages and DCs (Fig. 3). The genes examined are listed in Table I. Results are summarized in Table II. Twelve of 32 NF-κB/Rel pathway genes consistently were upregulated in mature DCs compared to macrophages. These genes were IκBα, NF-κB-inducing kinase (NIK), intercellular adhesion molecule-1 (ICAM-1), P-selectin, E-selectin (ELAM-1), TNFR2, tumor necrosis factor alpha induced protein-3 (TNFAIP3), IL-1α, IL-1R1, IL-1R2, IRAK, and TANK. Two other genes, TNFα and inhibitor kappa B kinase (IKK-β), also were upregulated selectively in DCs in a majority of experiments. All of these genes also were more highly expressed in peripheral monocytes than in macrophages indicating that macrophage differentiation was associated with downregulation of a set of NF-κB pathway genes. In DCs these genes were not newly expressed but remained turned on during the differentiation from monocytes to DCs.

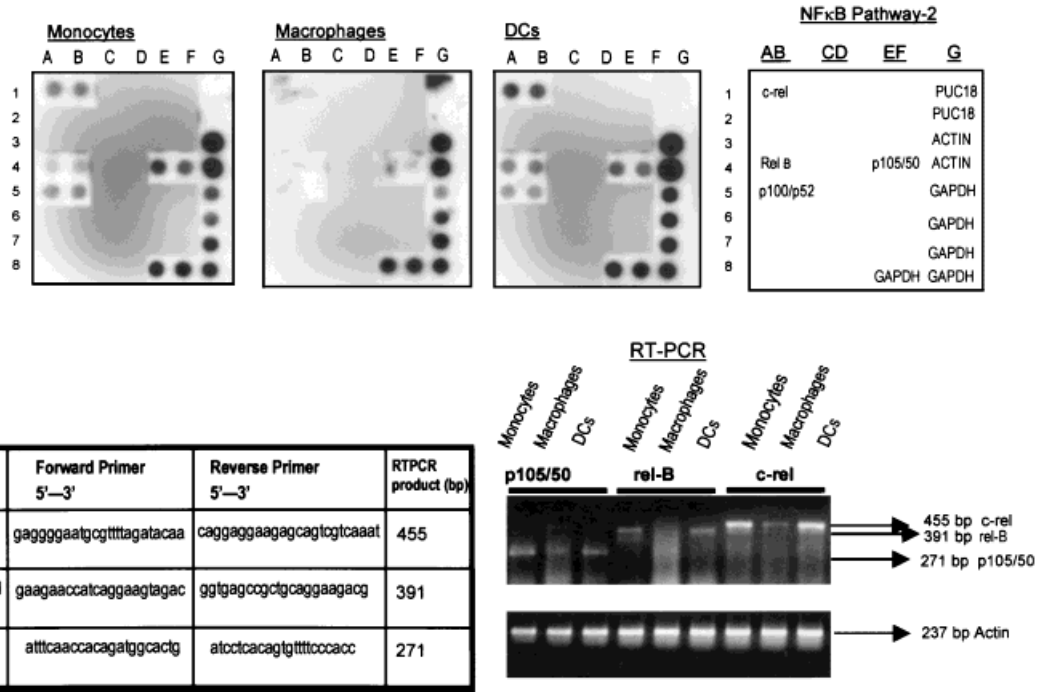
Paradoxically, one NF-κB/Rel target gene, monocyte chemotactic protein-1 (mcp-1), was more highly expressed in macrophages relative to DCs or monocytes indicating that it was upregulated during differentiation. Because other NF-κB/Rel pathway genes were downregulated during macrophage differentiation, mcp-1 upregulation may be mediated by an independent signaling pathway under these conditions. In this regard, mcp-1 expression has been reported to be regulated by the AP-1 pathway in endothelial cells [Wang et al., 1999] and by retinoids in monocytes [Zhu et al., 1999].

Monocytes are characterized by high IL-8 expression (Fig. 3). This expression is downregulated in both macrophages and DCs. A similar observation was made by Hashimoto et al. who used the SAGE technique to analyze gene expression [Hashimoto et al., 1999b]. Because IL-8 is an NF-κB target gene this observation suggests that IL-8 expression may be inhibited predominantly by non-NF-κB/Rel family genes during both macrophage and DC differentiation.

Effect of LPS Treatment on NF-κB/Rel Pathway Gene Expression by Macrophages

Generation of DCs but not macrophages involved maturation by exposure to LPS for 48 h [Palucka et al., 1998]. LPS itself may induce expression of many genes including cytokines, chemokines, and transcription factors [Yang et al., 1998]. This effect of LPS is mediated by signaling through the toll-like receptor-2 and activation of NF-κB [Kirschning et al., 1998; Yang et al., 1998, 1999]. Therefore, we examined the effect of LPS on expression of NF-κB/Rel family pathway genes in macrophages. In these experiments peripheral monocytes were treated with M-CSF for 6 days, then LPS, 100 ng/ml, was added for an additional 48 h. Results of a representative array experiment, comparing macrophages±LPS are shown in Figure 4. The major effect of LPS was induction of IL-8 expression whereas minor affects were observed on ICAM-1 and TNFAIP3. Upregulation of monocyte/macrophage IL-8 expression by LPS has been reported previously [Strieter et al., 1990; Lieber et al., 1994; Agrawal et al., 1995; Bauermeister et al., 1998]. Importantly,

Fig. 1. Immunophenotype analyses of DCs and macrophages from standard cultures. DCs and macrophages were generated with GM-CSF/IL-4 or M-CSF, respectively, as described in Materials and Methods. Cells were harvested then incubated at 4°C with fluorescent tagged monoclonal antibodies as indicated. Gated analyses of at least 10,000 events were performed using a Becton Dickinson FACStar Plus instrument. Shaded histograms indicate isotype controls. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]



Gene Name	Forward Primer 5'—3'	Reverse Primer 5'—3'	RTPCR product (bp)
C-rel	gaggggaatgcgttttagatacaa	caggaggaagagcagtcgtcaaat	455
Rel B/I-rel	gaagaaccatcaggaagtagac	ggtgagccgctgcaggaagacg	391
p105/50	atttcaaccacagatggcactg	atcctcacagtgttttcccacc	271

Fig. 2. NF-κB/Rel family genes are differentially expressed by mature macrophages and DCs. Peripheral monocytes were isolated and macrophages or DCs generated with M-CSF or GM-CSF/IL-4/LPS, respectively, as described in Materials and Methods. Radiolabeled cDNAs were prepared from the cells and hybridized to the gene arrays according to the manufacturer's directions. The data presentation in the upper panel has been manipulated to illustrate expression of only the four NF-κB/Rel family genes and positive (actin; GAPDH) and negative (PUC18) controls. A key to gene coordinates is shown at the right. Lower panel, RT-PCR detection of mRNA for p105/p50, rel-B, and c-rel in cells used for the array experiments above. Primers are shown at the left.

we now have observed this effect of LPS on IL-8 expression only in macrophages and not in DCs (compare Figs. 3 and 4). Essentially no effect of LPS was observed on expression of other NF-κB/Rel family pathway genes by macrophages. These data indicate that the differential gene expression noted between DCs and macrophages is not due primarily to LPS-mediated transcriptional activation but rather is most dependent on the specific cytokine milieu, be it either IL-4/GM-CSF or M-CSF.

DISCUSSION

Based on previous studies in mice and humans implicating NF-κB/Rel family genes in DC and macrophage differentiation [Burkly et al., 1995; Weih et al., 1995; Rescigno et al., 1998; Wu et al., 1998; Ammon et al., 2000; Neumann et al., 2000] we have examined expression of four NF-κB/Rel family genes, c-rel, RelB, p105/p50, and p100/p52, and 32 NF-κB pathway genes (Table I) by peripheral blood monocytes, and differentiated macrophages and DCs. Our results indicate that these four NF-κB/Rel family genes and 15 of 32 pathway genes were differentially expressed between macrophages and DCs (Fig. 2, Table II). For the NF-κB/Rel family genes and 14 of the pathway genes, expression was greater in DCs than macrophages, whereas for the mcp-1 gene the reverse was found to be true. Furthermore, all of these genes with the exception of mcp-1 were constitutively expressed by precursor monocytes. This observation indicates that macrophage differentiation from monocytes under our specific in vitro conditions was associated with active downregulation of multiple genes whereas DC differentiation was associated with persistent expression of these same genes, not new induction. By contrast, expression of mcp-1 in macrophages did appear to represent new induction. Differential gene expression between DCs and macrophages was not due primarily to LPS as LPS treatment of macrophages did not upregulate expression of most of the NF-κB pathway genes more highly expressed by DCs. Furthermore, Day 5 or 6 immature DCs (before LPS) already expressed these genes more highly than Day 5 or 6 macrophages (data not

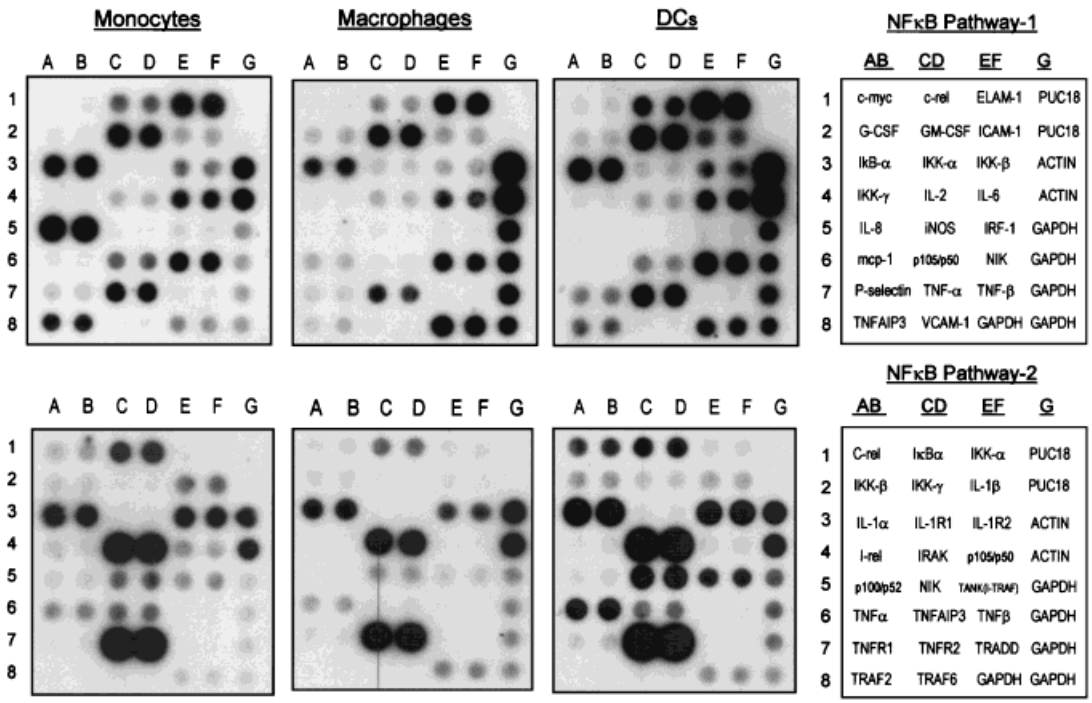


Fig. 3. cDNA array analyses of NF-κB pathway gene expresion by monocytes, macrophages, and DCs. Peripheral monocytes, macrophages, or DCs were prepared as described in Materials and Methods. Radiolabeled cDNAs were prepared and hybridized to two different arrays according to the manfacturer's directions. Keys to gene coordinates for each array are shown at the right.

shown). Taken together, these observations point to the cytokine milieu provided by IL-4 and GM-CSF as being permissive for continued expression (associated with only modest upregulation) during DC differentiation of a group of NF-κB pathway genes already turned on in peripheral monocytes. LPS treatment of mature macrophages caused striking upregulation of IL-8 (Fig. 4), an effect not observed with LPS treatment of immature DCs (Fig. 3). Because IL-8 is an NF-κB-dependent gene, this observation demonstrates the capacity of the NF-κB pathway to activate, in a cell- and stimulus-specific manner, only a subset of the repertoire of κB target genes.

Other investigators have reported differential expression of NF-κB family genes by monocyte-derived DCs and macrophages but their results using different culture conditions differed in various ways from our findings. Ammon et al. [2000] analyzed expression and/or DNA binding activities of 4 NF-κB family members [p50, p52, p65(RelA), and RelB] in human monocytes, macrophages, and DCs. In their studies DCs were generated with IL-4/GM-CSF and LPS and macrophages by culturing monocytes on hydrophobic Teflon foils in culture medium containing human serum but without added M-CSF. They observed constitutive expression of RelB and p65 mRNAs as well as p50, p65, and RelB DNA binding activities in unstimulated monocytes. No change in p65 expression was observed in either DC or macrophage cultures but addition of LPS to either macrophage or immature DC cultures induced p65 mRNA. Similar to our findings, RelB mRNA decreased more in macrophage than in DC cultures. However, RelB was substantially upregulated by LPS in DCs, a difference from our observations where the effect of LPS was more modest. These investigators did not comment on expression of p50 mRNA. NF-κB DNA binding patterns were complex and distinctive for different differentiation stages [Ammon et al., 2000].

Neumann et al. [2000] prepared DCs with GM-CSF/IL-4 or macrophages with GM-CSF/G-CSF, and then used monocyte conditioned medium or a cytokine cocktail (IL-1α, IL-6, TNF-α, IL-1β, and PGE_2) to induce maturation, eschewing use of LPS. In their experiments cytokine-induced maturation of DCs was associated with an increase in nuclear RelB, p50, p52, and c-rel as judged by immunoblotting of

TABLE II. κB-Dependent Genes Differentially Expressed by DCs and Macrophages in Figure 2[a]

Gene	Coordinates	Reproducibility[b]
NF-κB pathway 1 array (upper panel)		
E-selectin (ELAM-1)	1EF	7/7
ICAM-1	2EF	6/7
IκB-α	3AB	7/7
IKK-β	3EF	5/7
IL-8[c]	5AB	4/4
mcp-1[d]	6AB	6/7
NIK	6EF	7/7
P-selectin	7AB	7/7
TNF-α	7CD	4/7
TNFAIP3	8AB	6/7
NF-κB pathway 2 array (lower panel)		
IκB-α	1CD	7/7
IKK-β	2AB	5/7
IL-1α	3AB	5/5
IL-1R1	3CD	4/5
IL-1R2	3EF	5/5
IRAK	4CD	5/5
NIK	5CD	7/7
TANK	5EF	5/5
TNF-α	6AB	4/7
TNFA1P3	6CD	6/7
TNFR2	7CD	5/5

[a]All genes listed except mcp-1 and IL-8 were expressed more highly in DCs than macrophages.
[b]Reproducibility = number of experiments with differential gene expression/total experiments.
[c]IL-8 expression was down regulated in both macrophages and DCs relative to monocytes.
[d]mcp-1 expression was increased in macrophages relative to DCs.

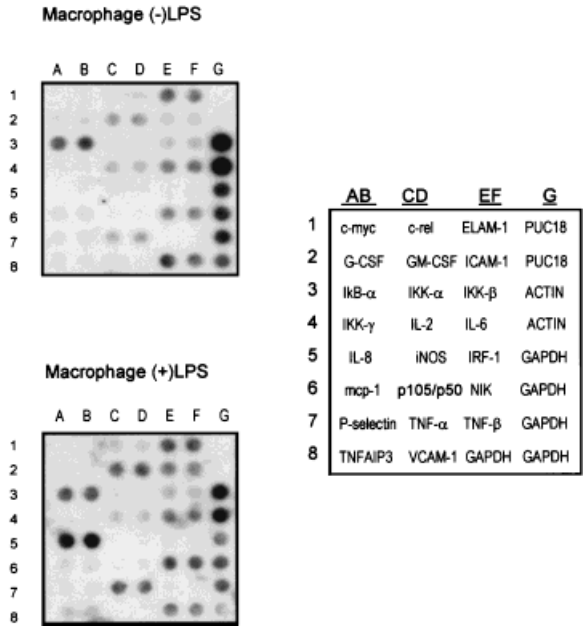


Fig. 4. Effect of LPS stimulation on NF-κB family gene expression by macrophages. Macrophages were generated by treatment of peripheral monocytes with M-CSF for 6 days. Cultures were carried out for an additional 48 h ± LPS, 100 ng/ml. Radiolabeled cDNAs were analyzed by hybridization to arrays as described in Materials and Methods. A key to array coordinates is provided at the right.

nuclear extracts. As in our experiments, c-rel expression gave the greatest increment. Also as in our experiments, peripheral monocytes constitutively expressed all four genes. Compared to monocytes there was increased expression of c-rel, RelB, and p50 proteins in nuclear extracts from mature macrophages, but nuclear levels of these proteins, especially c-rel, were substantially more increased in mature DCs [Neumann et al., 2000]. These differences between DCs and macrophages in the experiments of Neumann et al. [2000] appeared to represent different responses of each cell type to treatment with monocyte conditioned medium. It is difficult to compare these observations with our findings as these investigators did not examine mRNA levels directly.

Neumann et al. [2000] also examined effects of different maturation stimuli on nuclear expression of NF-κB family genes. Different patterns of protein expression in nuclear extracts were observed depending on whether immature DCs were treated with monocyte conditioned medium, TNF-α, or cytokine cocktail to induce terminal maturation. These data indicate that caution must be used in extrapolating from one in vitro culture system to another especially when different factors are used to induce DC maturation. Our data from experiments with LPS, therefore, may not be directly comparable to other systems in which LPS was not employed.

In our experiments, LPS added to immature DC cultures to induce terminal differentiation produced only modest upregulation of NF-κB pathway genes all of which were already expressed by peripheral monocytes and GM-CSF/IL-4-treated cells (immature DCs). In addition, LPS did not induce expression of any of these genes in macrophages. These observations indicate that the maturation-inducing effect of LPS for DCs in this system must be due to induction of genes other than those examined in our experiments. Using different cDNA arrays we have observed other genes that are consistently upregulated in mature DCs relative to macrophages including the transcription factor, E_2F_4 [Pierce et al., 1998], the cyclin dependent kinase inhibitor, p21 (WAF1/CIP1) [El-Deiry et al., 1993], gadd 45 (growth arrest and DNA damage inducible gene) [Smith et al., 1994], and pig 7 (p53 inducible gene-7;

LPS-induced TNF-α factor) [Polyak et al., 1997; Myokai et al., 1999] (not shown). We have shown previously that expression of p21 (WAF1/CIP1) is necessary for full maturation of both DCs and macrophages, despite its lower expression in the latter [Kramer et al., 1999]. However, the story is very complex and it is apparent that no single gene will likely be found solely responsible for generating the complex cellular phenotypes which characterize mature DCs and macrophages.

To date, expression of NF-κB target genes has not been extensively compared in DCs and macrophages. Although we have not surveyed the entire repertoire of NF-κB responsive genes, our survey of 32 pathway genes includes 18 target genes (Table I) and is the most comprehensive to date. Neumann et al. [2000] showed that the κB target genes, IκBα, IκBϵ, and Bcl-3, were upregulated in cytoplasmic extracts of mature DCs relative to monocytes and that both Bcl-3 and IκBα were more highly expressed in DCs than macrophages. We, too, found increased expression of IκB-α in DCs but did not examine other IκBs or Bcl-3. Other NF-κB target genes newly recognized to be expressed differentially by DCs and macrophages in our experiments included important cytokines (TNF-α, IL-1α, and mcp-1), adhesion molecules (ELAM-1, ICAM-1, and P-selectin), and the regulatory factor, IKK-β. Furthermore, we found differential expression of NF-κB signal transduction genes (TNFR2, TNFAIP3, TANK, NIK, IRAK, IL-1R1, and R2) between macrophages and DCs reflecting engagement of different signalling pathways during DC or macrophage differentiation. These differences have not previously been reported. It is likely that differential expression of specific NF-κB pathway genes by mature DCs and macrophages contributes significantly to the complex functional phenotypes of these cells, but additional experiments will be required to establish this point directly and to examine the relative importance of each of these genes. Furthermore, it is important to note that we have examined only a subset of NF-κB target genes and that other NF-κB responsive genes may be equally or more important to the differentiation process.

ACKNOWLEDGMENTS

The authors thank Cheryl Muzzi Adams for excellent secretarial assistance. IB was supported by a special fellowship from the South Texas Veterans Health Care System, Audie L. Murphy Division.

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