Characterization of the Monocyte-Specific Esterase (MSE) Gene

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Carboxylic esterases are widely distributed in hematopoietic cells. Monocytes express the esterase isoenzyme (termed '*monocyte-specific esterase*', MSE) that can be inhibited by NaF in the α-naphthyl acetate cytochemical staining. We examined the expression of MSE in normal cells and primary and cultured leukemia–lymphoma cells. The MSE protein was demonstrated by isoelectric focusing (IEF); MSE mRNA expression was investigated by Northern blotting and reverse transcriptase-polymerase chain reaction (RT-PCR). The following samples were positive for MSE protein and Northern mRNA expression: 20/24 monocytic, 4/32 myeloid, and 1/20 erythroid-megakaryocytic leukemia cell lines, but none of the 112 lymphoid leukemia or lymphoma cell lines; of the normal purified cell populations only the monocytes were positive whereas, T, B cells, and granulocytes were negative; of primary acute (myelo) monocytic leukemia cells (CD14-positive, FAB M4/M5 morphology) 14/20 were Northern mRNA and 11/14 IEF protein positive. RT-PCR revealed MSE expression in 29/49 Northern-negative lymphoid leukemia–lymphoma cell lines. The RT-PCR signals in monocytic cell lines were on average 50-fold stronger than the mostly weak trace expression in lymphoid specimens. On treatment with various biomodulators, only all-*trans* retinoic acid significantly upregulated MSE message and protein levels but could not induce new MSE expression in several leukemia cell lines; lipopolysaccharide and interferon-γ increased MSE expression in normal monocytes. Analysis of DNA methylation with sensitive restriction enzymes showed no apparent regulation of gene expression by differential methylation; the MSE gene is evolutionarily conserved among mammalian species; the half-life of the human MSE transcripts was about 5–6 h. The extent of MSE expression varied greatly among different monocytic leukemia samples. However, the MSE overexpression in a significant number of specimens was not associated with gene amplification, gross structural rearrangements or point mutations within the cDNA region. Taken together, the results suggest that MSE expression is not absolutely specific for, but strongly associated with cells of the monocytic lineage; MSE is either not expressed at all or expressed at much lower levels in cells from other lineages. The biological significance, if any, of rare MSE messages in lymphoid cells detectable only by the hypersensitive RT-PCR remains unclear. Further studies on the regulation of this gene and on the physiological function of the enzyme will no doubt be informative with respect to its striking overexpression in some malignant cells and to a possible role in the pathobiology of monocytic leukemias.

INTRODUCTION

Esterases represent a diverse spectrum of enzymes with an ubiquitous tissue distribution that share certain features regarding substrate specificity (1). These esterases belong to the class of serine hydrolases that are defined as functionally related hydrolytic enzymes containing a serine residue in their active site (2). This enzyme class comprises the serine protease multigene family as well as various carboxyl-, cholin-, aryl-, acetyl- and acetylcholinesterases (3).

The carboxylesterases (EC 3.1.1.1) are a heterogeneous group of cellular enzymes capable of hydrolyzing a variety of aliphatic or aromatic esters under acidic or neutral conditions (4). In hematology these enzymes are known as *non-specific esterases,* acting most efficiently on short-chain (acetate and butyrate) esters. The enzymatic activity can be inhibited by sodium fluoride (NaF) in monocytic cells, but not in cells of the granulocytic series (5); however, it should be noted that NaF-resistance or sensitivity is here clearly a relative phenomenon (6).

Nevertheless, the unique substrate and inhibitor specificity of the esterase found in monocytes indicated early on that the high activity in monocytes might be due to enzyme variants that are not present in other leukocytes (7). This notion was strengthened by data from electrophoretic analyses of enzyme extracts, first by polyacrylamide gel electrophoresis and later by isoelectric focusing (IEF) (reviewed in (8)). These zymogram IEF studies of normal and malignant myeloid cells have consistently demonstrated the existence of two main groups of esterase bands. One group of IEF bands is common to all myeloid cells (termed *common esterase*, ComEst, by Scott *et al.* (9)) and one is additionally detected in cells of monocytic origin (termed *monocyte-specific esterase*, MSE). In the IEF analysis the ComEst group appears as a series of bands with isoeletric points (pI) ranging from 6.3–7.9 while MSE comprises 1–5 bands (depending on the IEF system used) with a narrow pI range 5.5–6.2 (3,9).

For a long time it was not known whether ComEst and MSE belong to a multigene family representing post-transcriptionally modified variants of the same enzyme or whether they are clearly distinct at the molecular and genetic level, being related only in their substrate specificity (9). Recent experimental evidence supports the second view, namely that ComEst and MSE are unrelated enzyme species: one appears to be a monomeric acetylesterase and the other a trimeric carboxylesterase, respectively (1,9).

We recently demonstrated the specificity of MSE as expression at the mRNA (examined by Northern blotting) and protein level (by IEF) were clearly restricted to cells committed to the monocyte–macrophage lineage (10). In order to substantiate these conclusions in a comprehensive survey and to further clarify the nature of MSE, we analyzed a greatly extended panel of primary and continuously cultured leukemia cells applying extremely sensitive detection methods, examined the gene expression under *in vivo* and manipulated *in vitro* conditions, and characterized the MSE gene in further detail.

Received February 15, 1994. Accepted April 22, 1994.
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LEUKEMIA

MATERIAL AND METHODS

Primary Normal and Malignant Cell Material

Fresh leukemia cells were taken from peripheral blood (PB) or bone marrow (BM) from patients with myeloid leukemia of the morphological M4 or M5 subtypes according to the French–American–British (FAB) classification. Samples were sent to the reference laboratory of author W.D.L. for immunophenotype analysis. PB or BM mononuclear cells were separated by standard Ficoll–Hypaque density gradient centrifugation (Lymphoprep, Nycomed, Oslo, Norway). All samples were examined with the following panel of surface markers using flow cytometry: CD2, CD3, CD4, CD7, CD10, CD13, CD14, CD15, CD19, CD33, CD34, CD41, CDw65, glycophorin A, HLA-DR. Cells were pelleted and frozen in liquid nitrogen.

Normal PB mononuclear cells taken directly by venupuncture of laboratory staff or from buffy coats (generously provided by the German Red Cross Blood Transfusion Center, Springe, Germany) were isolated by standard Ficoll–Hypaque density gradient centrifugation. The cells were separated from the vast majority of thrombocytes by repeated low-speed sedimentations (200 × *g* for 7 min). Mononuclear cells were adjusted to 2–20 × 10^6 cells/ml with macrophage-serum free medium (M-SFM; Gibco BRL, Eggenstein, Germany). Tissue culture dishes (Nunc, Wiesbaden, Germany) containing 10–15 ml of this cell suspension were incubated for 1 h at 37°C in a humidified incubator with 5% CO_2. Non-adherent cells were subsequently removed by washing the culture dishes repeatedly with warm phosphate-buffered saline (PBS) containing 0.5% M-SFM. For RNA preparation cells were washed from the dishes with guanidinium isothiocyanate. T cells were enriched by sheep red blood cell rosetting (ICN Flow, Meckenheim, Germany). Granulocytes were collected from the bottom of the Ficoll gradient and separated from erythrocytes by a dextran gradient (Dextran T-500; Pharmacia, Freiburg, Germany). Normal B cells were obtained from surgically removed tonsils after sheep red blood cell rosetting of the mononuclear cell preparation. The purities of the normal cell populations were verified by immunostaining and flow cytometric analysis (FACScan; Becton Dickinson, Heidelberg, Germany): T cells were enriched to 97% (CD3+), B cells to 97% (CD19+), monocytes to 86% (CD14+ and positive in the α-naphthyl acetate esterase cytochemical staining), and granulocytes to >95% (morphological analysis). In order to increase the quantity and the purity of available normal monocytes, monocytes were isolated from mononuclear cells (after Ficoll–Hypaque centrifugation) by counter-current elutriation (JGM-E Beckman centrifuge; Beckman, München, Germany) using a large-volume chamber (50 ml) and a JE-5 rotor at 2500 r.p.m. and a flow rate of 110 ml/min in Hank's balanced salt solution supplemented with 2% human albumin. Elutriated monocytes were >95% pure as determined by morphology and antigenic phenotype.

Culture of Cell Lines and In Vitro *Stimulation*

All human cell lines were derived from patients with leukemia or lymphoma (11). The continuous human and animal cell lines were either taken from the stock of the cell bank (DSM, German Collection of Microorganisms and Cell Cultures) (12) or were generously made available for this study by the originating investigators. Cells were grown under optimal conditions in 50 ml or 260 ml tissue culture flasks or 24-well plates (Nunc) in their appropriate media (RPMI 1640, McCoy's 5A, Leibowitz's L-15, Iscove's MDM, Dulbecco's MEM, or MEM alpha; Gibco BRL) supplemented with 5–20% heat-inactivated (at 56°C for 45 min) fetal bovine serum (Sigma, Deisenhofen, Germany) at 37°C in a humidified atmosphere of 5% CO_2 in air. The cells were examined daily in the culture flasks under an inverted microscope. Cultures were incubated without antibiotics in order to avoid sublimal bacterial infection. Only mycoplasma-free cultures were used; freedom of mycoplasma contamination was checked routinely by DAPI staining and cultivation on agar. The cells were harvested in their logarithmic growth phase with viabilities exceeding 90% as determined by trypan blue dye exclusion. Cell pellets were kept frozen at −20°C or processed immediately.

In vitro stimulation of cell lines was carried out with two protein kinase C (PKC) activators, the pharmacological 12-*O*-tetradecanoylphorbol 13-acetate (TPA; Sigma) and the natural Bryostatin 1 (Bryo 1; kindly provided by Prof G.R. Pettit, Tempe, AZ, USA), the vitamin A-analogue all-*trans* retinoic acid (ATRA; Sigma), and the calcium transport regulator 1,25-dihydroxyvitamin D_3 (Vit. D3; Sigma). The inducers were first dissolved in ethanol or DMSO at 10^{-3} M and then further diluted in RPMI 1640 medium so that the final concentrations of the solvent were maximally 0.01% in the experiments. The cells were exposed to 10^{-7} M solutions of the reagents for up to 4 days. PB monocytes and some cell lines were stimulated with 100 ng/ml lipopolysaccharide (LPS; Sigma) and 200 U/ml interferon-γ (IFN-γ; Boehringer Mannheim, Mannheim, Germany) for up to 24 h.

RNA Isolation and Northern Blotting

Total cellular RNA was isolated using the guanidinium isothiocyanate-cesium chloride method (13). Northern blots were prepared by separating 10 μg of total RNA in an agarose gel containing 1% formaldehyde. The RNA was transferred to a nylon membrane (Nytran, Schleicher and Schüll, Dassel, Germany) and cross-linked with 1200 J ultraviolet light (UV Stratalinker 1800; Stratagene, Heidelberg, Germany). After 2 h of pre-hybridization the filters were hybridized with a nick-translated (Gibco BRL) or random primed (USB, Bad Homburg, Germany) [α-^{32}P]dCTP-labeled HMSE-1 probe overnight at 62°C. The filters were then washed stringently and exposed for autoradiography to X-ray films (Fuji RX) with intensifying screens at −80°C. Filters were rehybridized with a housekeeping gene as the control.

Probes

A 1746-bp *Eco*RI fragment cloned into pUC19 containing the partial coding sequence (nucleotides −10 to 1512 from the 3'-end of the HMSE gene) was kindly provided by Dr F. Zschunke (Göttingen, Germany) (2). A 238-bp fragment of the HMSE-1 cDNA from the 5'-end (nt −10 to 228, obtained after *Bam*HI digestion and extraction from the gel) was employed in some experiments. The β-actin probe pAct-1, a 1.25-kb *Pst*I fragment (cloned in pBR322) from the cDNA of hamster β-actin (obtained from Dr J.W.G. Janssen, Ulm, Germany) was used as control.

Determination of RNA Half-Life

The half-life of the MSE mRNA was determined by exposure of the cells to 10 μg/ml actinomycin D (Sigma), an inhibitor

of transcription, for 0–24 h before harvest of the cells and subsequent isolation of RNA.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Five micrograms of total RNA was used as a template for first strand cDNA synthesis using a reverse transcriptase preamplification system kit (SuperScript; Gibco BRL) in a final volume of 20 μl PCR buffer (containing 20 mM Tris-HCl of pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mg/ml BSA) supplemented with 0.5 μg of oligo dT primer. After heating the mixture at 70°C for 10 min, 200 U of Moloney murine leukemia virus reverse transcriptase and 1 μl of 10 mM dNTP mix were added to the reaction system. The reaction mixture was then incubated at 42°C for 50 min, at 90°C for 5 min and then quickly chilled on ice. After brief centrifugation, 2 U RNase H was added to the reaction mixture for 20 min at 37°C. RNA samples from some cell lines were treated with DNase I (RNase-free from Boehringer Mannheim) prior to reverse transcription. The reaction was incubated at 37°C for 1 h and then at 95°C for 5 min and immediately cooled on ice. Five microlitres of the reverse transcriptase reaction mixture containing the first strand cDNA was diluted with PCR buffer (10 X: 500 mM KCl, 15 mM $MgCl_2$, 100 mM Tris-HCl pH 8.3, 0.001% gelatin) containing 20 pmol of each upstream and downstream primer, 10 nmol of dNTP mix and 1.25 U of Taq DNA polymerase (Amersham-Buchler, Braunschweig, Germany). The primers used in the experiment were designed according to sequence data published previously (2): sense 5'-GGCAGTTACTCTCAGAGCTA-3' (sequence nucleotides 92–111, MSE-P1) and antisense 5'-CTTCCACAGGAGTGAC-ATGGC-3' (sequence nucleotides 960–940, MSE-P2). Oligonucleotide primers were prepared on an automated DNA synthesizer (Cylcone Plus, Millipore, Eschborn, Germany). The PCR was then performed with a DNA thermal cycler (Perkin Elmer Cetus, Heidelburg, Germany) for 32 cycles under the following conditions: 30 s at 94°C for denaturation, 30 s at 55°C for annealing, and 2 min at 72°C for extension. The amplified PCR products were electrophoresed in 1.2% agarose gels, stained with ethidium bromide and observed under ultraviolet light. Gels were blotted onto nylon filters using the Southern technique as described below. In order to assess the quality of reverse transcribed RNA and successful PCR-amplification, aliquots from the same products obtained from reverse transcription were amplified in parallel using the following two β-actin primers: sense 5'-ATGGATGATGATA-TCGCCGCG-3' and antisense 5'-CTAGAAGCATTTGCGGT-GGAC-3'.

Polymerase Chain Reaction and Single-Strand Conformation Polymorphism (PCR-SSCP)

PCR-SSCP analysis was modified from the previous description (14). In brief, 6 μl reverse transcribed cDNA was amplified by PCR (30 cycles; the same conditions as under RT-PCR) in the presence of 10 μCi [α-^{32}P]dCTP using the two primers MSE-P1 and MSE-P2 (see above). From the amplification product 4 μl were diluted 1 : 25 with 0.1% SDS, 10 mM EDTA and heated at 95°C after adding 1 volume sequencing stop solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol, 20 mM NaOH). Then, 2–4 μl of this mixture was loaded onto a 6% non-denaturing polyacrylamide gel containing 90 mM Tris-borate, 2 mM EDTA (pH 8), and 10% glycerol. Electrophoresis was performed at 25 W for 4 h with air cooling. Subseqently the gel was dried and exposed to an X-ray film for autoradiography overnight at room temperature.

DNA Sequencing

RT-PCR products were purified using Jetsorb (Genomed, Bad Oeynhausen, Germany). RT-PCR products were sequenced using the Taq DyeDeoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems, Weiterstadt, Germany). The primers used were MSE-P1 as sense, antisense 5'-GGTTCTTGGCCAA-TGGAGACA-3' (sequence nucleotides 526–506, MSE-P3), antisense 5'-GGCTGGATCTTCATTCACAGC-3' (sequence nucleotides 1526–1506, MSE-P4), and MSE-P2 as antisense at a concentration of 4 pmol per sequence reaction. The sequencing was performed with the DNA thermal cycler for 25 cycles under the following conditions: 30 s at 96°C for denaturation, 15 s at 50°C for annealing, and 4 min at 60°C for extension. The sequence reactions were purified as described by the manufacturer. The sequence reactions were electrophoresed using the Applied Biosystem's 373A DNA Sequencer.

Southern Blotting

High molecular weight DNA was extracted from frozen PBS-washed leukemic cells using standard methods (13). For the different experiments 20 μg of DNA was digested overnight with 100 U of one of the restriction enzymes *Hin*dIII (Pharmacia, Freiburg, Germany), *Bam*HI, *Eco*RI, *Pst*I (Gibco BRL), *Msp*I, *Hpa*II, or *Cfo*I (Boehringer Mannheim). To 1/10 of the reaction mix 200 ng lambda DNA (Pharmacia) was added as control. The digested DNA was extracted with phenol, precipitated in ethanol and separated in a 0.7% agarose gel. The DNA was fixed with 0.2 N HCl and then denatured with 0.5 M NaOH/1.5 M NaCl. After neutralization with 0.5 M Tris-HCl (pH 7.0)/3 M NaCl the DNA was blotted with 20 × SSC on nylon membranes and then treated and hybridized as described above under Northern blot analysis.

Pulsed Field Gel Electrophoresis

Pulsed field gel electrophoresis (PFGE) is an electrophoretic technique alternative to standard Southern analysis that uses alternating pulses of current directed at angles through an agarose gel to separate large DNA restriction fragments. Digested genomic DNA was separated on a 1.5% agarose gel using the Chef-Dr II PFGE system (Bio-Rad, München, Germany) according to the recommendations of the manufacturer. In brief, the gel was run for 5 h at 14°C applying a 200 V inverting field. The switching cycle increased from 1 to 4 s over these 5 h. Following PFGE the gel was treated similarly to a standard Southern blot.

Isoelectric Focusing

Enzyme extraction, separation by IEF and visualization of esterase isoenzymes have been described in detail elsewhere (10). In brief, enzymes were extracted by repeated cycles of freezing–thawing and solubilized by addition of Triton X 100 (Serva, Heidelberg, Germany). After centrifugation, aliquots of supernatant containing the enzyme preparation (extracted from equal numbers of cells) were separated by analytical IEF on horizontal thin-layer polyacrylamide gels (4.8% acryl/bisacrylamide, pH range 2–11 of the ampholyte Servalyt;

Serva) using an LKB Multiphor system (Pharmacia). Isoenzymes were visualized on the gels by submersion in a staining solution containing α-naphthyl acetate (Sigma) as substrate and Fast Blue RR (Serva) as the coupling diazonium salt. Addition of 40 mM NaF to the staining bath inhibited selectively the MSE band at about pH 6.0 (3). Bands on autoradiographed RNA films after Northern blot analysis or RT-PCR Southern blotting and on dried IEF gels were quantified densitometrically (LKB Ultroscan Densitometer; Pharmacia).

RESULTS

Expression of MSE

Detection of Protein by IEF and of mRNA by Northern Blotting. MSE protein was demonstrated by IEF on polyacrylamide gels as a distinct band with an isoeletric point of about pH 6.0. This band could be selectively inhibited by NaF. In the Northern blot analysis a single band corresponding to an MSE transcript of 2.0 kb was detected. Expression of MSE protein and mRNA were examined in all samples, i.e. normal cell populations, primary leukemia cells, and leukemia cell lines, by IEF and Northern blotting, respectively (Tables 1 and 2; Figures 1 and 2).

Of the purified normal cell populations ($n = 10$ from different individuals), only the PB monocyte preparations consistently displayed MSE protein and mRNA, whereas PB T cells, PB granulocytes, and tonsillar B cells were all negative.

Twenty-two PB or BM acute (myelo)monocytic leukemia samples, diagnosed as such on morphological–cytochemical (FAB M4 or M5) and immunophenotypical grounds (CD14+), were tested. mRNA expression was found in 14/20 cases tested; 11/14 cases analyzed showed the MSE band in the IEF.

MSE expression was examined in 188 cell lines (Table 2). All 112 lymphoid leukemia and lymphoma cell lines were negative at the Northern mRNA and protein level (29 pre B-cell leukemia, 17 B-cell leukemia, 21 T-cell leukemia, 13 myeloma, 8 Burkitt, 8 Hodgkin, and 16 non-Hodgkin lymphoma cell lines). In the category 'myeloid leukemia cell lines' ($n = 32$) four and five cell lines were MSE-positive in the IEF and Northern analysis, respectively. One out of 20 cell lines with megakaryocytic and/or erythroid features showed the MSE bands in the IEF and Northern gels. The MSE isoenzyme and the MSE transcript were found in 20/24 cell lines.

Detection of mRNA by RT-PCR. To assess the detection sensitivity of the IEF, Northern and RT-PCR analysis, positive cells were diluted with MSE-negative cells. In preliminary experiments we showed that it was possible with IEF to visualize an MSE-positive population in a mixture consisting of 1% positive cells (cell line THP-1) and 99% negative cells (RC-2A). The dilution experiments performed here revealed that Northern blotting had a maximal sensitivity of 2.5% posi-

Table 1 Expression of MSE mRNA and Protein in Primary Human Leukemia Cells

Patient no.	Surface Marker Expression[a]					FAB Subtype	MSE Expression				
	CD13	CD14	CD15	CD33	CDw65		Protein IEF[b]		mRNA Northern[b]		mRNA RT-PCR[c]
1	70	56	31	90	80	M5			++	[2.89×][d]	
2	10	5	75	35	70	M5			−		
3	44	75	5	79	66	M4	(+)	[0.71×][d]			
4	50	70	70	90	87	M5	+	[0.95×]	+	[0.55×]	+
5	75	60	37	76	60	M4eo			−		
6	24	34	20	76	20	M4			+	[1.24×]	
7	24	40	30	60	68	M4			(+)	[0.10×]	
8	12	52	44	20	80	M5a	−		−		−
9	82	55	47	81	49	M5	−		−		−
10	71	85	11	85	83	M4	+	[1.01×]	+	[1.06×]	
11	37	34	26	83	63	M4	(+)	[0.59×]	(+)	[0.40×]	+
12	6	44	34	56	56	M5	(+)	[0.70×]	(+]	[0.25×]	+
13	34	69	16	75	76	M5a	−		−		+
14	34	47	20	80	86	M5b	+	[1.19×]			+
15	30	55	12	80	80	M4	++	[4.43×]	(+)	[0.24×]	+
16	37	62	0	36	52	M4	(+)	[0.37×]	(+)	[0.36×]	
17	82	70	13	70	45	M4eo			+	[0.48×]	
18	34	60	14	90	72	M4eo	(+)	[0.61×]	(+)	[0.10×]	
19	82	47	48	71	35	M4eo			(+)	[0.08×]	
20	21	19	32	73	66	M4/M5			−		
21	14	33	77	82	87	M5	++	[6.22×]	++	[5.61×]	
22	16	57	23	50	70	M5	++	[5.74×]	+	[0.18×]	

[a]Expression of these surface markers (percentage of positive cells) was examined by indirect immunofluorescence staining and either microscopic or flow cytometric analysis using the monoclonal antibodies My7 (CD13), VIM-13 or UCHM1 (CD14), VIM-D5 (CD15), My9 (CD33), VIM-2 (CDw65); other markers examined are not listed but confirmed the diagnosis.
[b]Intensity of bands on IEF gels and Northern gels; the intensity of expression (thickness of the band) was graded in comparison with those from normal control monocyte samples: −, negative; (+), weaker; +, same intensity; ++, overexpression (stronger band).
[c]Intensity of bands on Southern gels hybridized with the HMSE-1 probe after RT-PCR: −, negative; +, positive.
[d]Intensity of expression (thickness of bands in IEF and Northern) analyzed densitometrically in comparison with normal unstimulated monocytes; data are given as X-fold of the normal values.

Table 2 Expression of MSE mRNA and Protein in Continuous Human Leukemia–Lymphoma Cell Lines

	Cell Line	Origin[a]	Protein	mRNA	
			IEF[b]	Northern[b]	RT-PCR[c]
Pre-B Leukemia Cell Lines					
1	1E8	Pre B-ALL	–	–	
2	207	Pre B-ALL	–	–	+
3	380	ALL	–	–	(+)
4	697	Pre B-ALL	–	–	–
5	ALL-1	Pre B-ALL	–	–	
6	BAY-91	ALL	–	–	–
7	BV-173	CML-BC	–	–	–
8	EU-1	ALL	–	–	
9	HAL-01	Pre B-ALL	–	–	(+)
10	HPB-NULL	ALL	–	–	
11	KARPAS-353	Pre B-ALL	–	–	
12	KOPN-8	ALL	–	–	
13	LAZ-221	ALL	–	–	
14	LILA-1	Pre B-ALL	–	–	
15	LK-63	Pre B-ALL	–	–	
16	MIK-ALL	Pre B-ALL	–	–	
17	NALM-1	CML-BC	–	–	
18	NALM-6	ALL	–	–	(+)
19	NALM-16	ALL	–	–	
20	OM9;22	ALL	–	–	
21	PC-53	Pre B-ALL	–	–	–
22	PRE-ALP	Pre B-ALL	–	–	
23	RCH-ACV	Pre B-ALL	–	–	
24	REH	ALL	–	–	+
25	RS4;11	ALL	–	–	+
26	SUP-B15	Pre B-ALL	–	–	
27	SUP-B27	Pre B-ALL	–	–	
28	TAHR-87	AUL	–	–	
29	TOM-1	Pre B-ALL	–	–	
B Leukemia Cell Lines					
1	BALL-1	ALL	–	–	
2	BALM-1	B-ALL	–	–	
3	BALM-6	B-ALL	–	–	
4	BALM-8	B-ALL	–	–	
5	BONNA-12	HCL	–	–	–
6	EH	HCL	–	–	(+)
7	EHEB	B-CLL	–	–	++
8	ESKOL	HCL	–	–	–
9	HAIR-M	HCL	–	–	
10	HC-1	HCL	–	–	
11	HK	HCL	–	–	
12	JVM-2	B-PLL	–	–	++
13	JVM-3	B-PLL	–	–	
14	JVM-13	B-PLL	–	–	+
15	KARPAS-231	B-ALL	–	–	
16	MN-60	B-ALL	–	–	–
17	WIEN-133	B-ALL	–	–	
T Leukemia Cell Lines					
1	BE-13	T-ALL	–	–	
2	CCRF-CEM	ALL	–	–	
3	CML-T1	CML-BC	–	–	–
4	DU-528	T-ALL	–	–	
5	HPB-ALL	T-ALL	–	–	
6	JURKAT	T-ALL	–	–	
7	KE-37	T-ALL	–	–	
8	LOUCY	T-ALL	–	–	
9	MDS	CMMoL	–	–	
10	MKB-1	AML	–	–	
11	MOLT-3	ALL	–	–	(+)
12	MOLT-13	T-ALL	–	–	
13	MOLT-15	AMoL	–	–	
14	MOLT-16	T-ALL	–	–	(+)
15	MOLT-17	T-ALL	–	–	
16	MO-T	HCL	–	–	

Table 2 *(Continued)*

Cell Line		Origin[a]	Protein	mRNA	
			IEF[b]	Northern[b]	RT-PCR[c]
T Leukemia Cell Lines (Continued)					
17	MT-1	ATL	–	–	(+)
18	P12/ICHIKAWA	ALL	–	–	
19	PEER	T-ALL	–	–	+
20	PF-382	ALL	–	–	–
21	SKW-3	CLL	–	–	–
Myeloma Cell Lines					
1	EJM	Myeloma	–	–	–
2	IM-9	Myeloma	–	–	–
3	KARPAS-620	Plasma cell leukemia	–	–	
4	KARPAS-707	Myeloma	–	–	–
5	L-363	Plasma cell leukemia	–	–	–
6	LP-1	Myeloma	–	–	
7	MM-1	Myeloma	–	–	–
8	MM-S1	Myeloma	–	–	(+)
9	NCI-H929	Myeloma	–	–	–
10	OPM-2	Myeloma	–	–	–
11	U-266	Myeloma	–	–	
12	U-1958	Plasma cell leukemia	–	–	
13	U-1996	Myeloma	–	–	+
Burkitt Lymphoma Cell Lines					
1	BJAB	Burkitt	–	–	
2	CA-46	Burkitt	–	–	(+)
3	DAUDI	Burkitt	–	–	+
4	DG-75	Burkitt	–	–	
5	EB-1	Burkitt	–	–	
6	NAMALWA	Burkitt	–	–	
7	NAMALWA-IPN.45	Burkitt	–	–	
8	ROS-17	Burkitt	–	–	+
Hodgkin Lymphoma Cell Lines					
1	CO	Hodgkin (nodular sclerosis)	–	–	
2	HDLM-1	Hodgkin (nodular sclerosis)	–	–	
3	HDLM-2	Hodgkin (nodular sclerosis)	–	–	(+)
4	HDLM-3	Hodgkin (nodular sclerosis)	–	–	
5	KM-H2	Hodgkin (mixed cellularity)	–	–	–
6	L-428	Hodgkin (nodular sclerosis)	–	–	+
7	L-540	Hodgkin (nodular sclerosis)	–	–	
8	SUP-HD1	Hodgkin (nodular sclerosis)	–	–	
Non-Hodgkin Lymphoma Cell Lines					
1	DEL	Malignant histiocytosis	–	–	
2	DOHH-2	B NHL (immunoblastic)	–	–	
3	HBL-1	B NHL (diffuse large cell)	–	–	
4	HT-58	B NHL (centroblastic)	–	–	(+)
5	KARPAS-299	T NHL (histiocytic)	–	–	+
6	KARPAS-422	B NHL	–	–	(+)
7	MC-116	B NHL (undifferentiated)	–	–	++
8	MH-1	Malignant histiocytosis	–	–	(+)
9	PFI-285	T NHL	–	–	–
10	RL	B NHL (undifferentiated)	–	–	
11	SCC-3	NHL (diffuse large cell)	–	–	–
12	ST-4	T NHL (convoluted type)	–	–	+
13	SUP-T1	T NHL (lymphoblastic)	–	–	
14	U-698-M	B NHL (lymphoblastic)	–	–	+
15	WSU-NHL	B NHL (histiocytic)	–	–	
16	WSU-WM	Waldenström	–	–	+
Myeloid Leukemia Cell Lines					
1	EM-2	CML-BC	–	–	+
2	EM-3	CML-BC	–	(+)	+
3	EOL-1	AML-eosino	+	+	+
4	EOL-3	AML-eosino	+	(+)	+
5	GDM-1	CML-BC	–	–	–
6	GF-D8	AML M1	–	–	++

Table 2 *(Continued)*

	Cell Line	Origin[a]	Protein	mRNA	
			IEF[b]	Northern[b]	RT-PCR[c]
Myeloid Leukemia Cell Lines (Continued)					
7	GM-153	AML	–	–	–
8	GM/S0	CML-BC	–	–	
9	HL-60	AML M2	–	–	(+)
10	HMC-1	Mast cell leukemia	–	–	(+)
11	KASUMI-1	AML M2	–	–	
12	KBM-7	CML-BC	–	–	++
13	KCL-22	CML-BC	–	–	–
14	KG-1	AML	–	–	(+)
15	KG-1A	AML	–	–	(+)
16	KMT-2	Umbilical cord blood	–	–	(+)
17	KOPM-28	CML-BC	–	–	++
18	KU-812	CML-BC	–	–	+
19	KU-812F	CML-BC	–	–	+++
20	KY-821	AML	–	–	+++
21	KYO-1	CML-BC	–	–	++
22	MOLM-6	CML-BC	–	–	+
23	MOLM-7	CML-BC	–	–	
24	MOLM-8	CML-BC	–	–	+
25	MR-87	AML	–	–	
26	NB-4	AML M3	(+)	(+)	
27	OCI-AML-5	AML	–	–	(+)
28	PL-21	AML M3	+	(+)	++
29	TI-1	AML M2	–	–	–
30	TS9;22	CML-BC	–	–	
31	UCSD/AML-1	AML	–	–	+
32	YS9;22	CML-BC	–	–	+
Monocytic Leukemia Cell Lines					
1	CTV-1	AML M5	–	–	+
2	DD	Histiocytic lymphoma	–	–	++
3	JOSK-I	AML M4	+++	+++	+++
4	JOSK-K	AML M5	++	++	+++
5	JOSK-M	CML-BC	++	++	++
6	JOSK-S	AML M5	+++	+++	++
7	KBM-3	AML M4	+	+++	+++
8	KBM-5	CML-BC	(+)	+++	+++
9	ML-2	AML M4	(+)	(+)	+
10	MOBS-1	AMoL	+	+++	+++
11	MONO-MAC-6	AML M5	+++	+++	+++
12	MV4-11	AML M5	(+)	(+)	+++
13	NOMO-1	AMoL	+++	+++	
14	OCI-AML-1	AML M4	–	–	+
15	OCI-AML-2	AML M4	+	++	+++
16	OCI-AML-3	AML M4	+	+++	+++
17	PLB-985	AML M4	+	+	++
18	RC-2A	AML M4	–	–	+
19	RW-LEU-4	CML-BC	+	+	+++
20	SKM-1	AMoL	++	+++	+++
21	THP-1	AML M5	+++	+++	+++
22	TK-1B	AML M4	+++	++	
23	U-937	Histiocytic lymphoma	(+)	(+)	
24	X-376	AML	+++	++	+++
Megakaryocytic-Erythroid Leukemia Cell Lines					
1	CHRF-288-11	AML M7	–	–	++
2	CMK	AML M7	–	–	+++
3	DAMI	AML M7	–	–	+
4	F-36P	AML M6	–	–	++
5	F-36EGM	AML M6	–	–	++
6	HEL	AML M6	–	–	(+)
7	K-562	CML-BC	–	–	+
8	KMOE-02	AML M6	–	–	(+)
9	LAMA-84	CML-BC	–	–	+++
10	M-07e	AML M7	–	–	++
11	MB-02	AML M7	–	–	
12	MEG-01	CML-BC	–	–	(+)

Table 2 *(Continued)*

Cell Line		Origin[a]	Protein	mRNA	
			IEF[b]	Northern[b]	RT-PCR[c]
Megakaryocytic-Erythroid Leukemia Cell Lines (Continued)					
13	MEGAL	AML M7	–	–	++
14	MKPL-1	AML M7	–	–	+
15	MOLM-1	CML-BC	–	–	++
16	OCI-M1	AML M6	–	–	+
17	OCI-M2	AML M6	–	–	–
18	T-33	CML-BC	–	–	
19	TF-1	AML M6	–	–	+
20	UT-7	AML M7	+	+++	+++

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AMoL, acute (myelo)monocytic leukemia; ATL, adult T-cell leukemia; AUL, acute undifferentiated leukemia; CLL, chronic lymphocytic leukemia; CML-BC, chronic myeloid leukemia in blast crisis; CMMoL, chronic myelomonocytic leukemia; HCL, hairy cell leukemia; NHL, non-Hodgkin's lymphoma; PLL, prolymphocytic leukemia.

[a]Cell lines were assigned to the respective categories based on their origin and their phenotypic and functional characteristics (e.g. surface markers, receptor gene rearrangements, expression of hemoglobin, etc.); original diagnoses (types and subtypes of malignancies) are given as far as indicated in the original publications (see also (11)).

[b]Intensity of bands on IEF gels and Northern gels; the intensity of expression (thickness of the band) was graded in comparison with those from normal control monocyte samples: –, negative; (+), weaker; +, same intensity, ++, overexpression (stronger band); +++, strong overexpression (very strong band).

[c]Intensity of bands on Southern gels hybridized with the HMSE-1 probe after RT-PCR; –, negative; (+), weakly positive; +, ++, +++, different degrees of positivity.

tive cells (PLB-985) in a background of 97.5% negative cells (KG-1). RT-PCR was 50–250 times more sensitive than Northern as the RT-PCR product of MSE was reproducibly detectable at a 1 : 2000 to 1 : 10 000 dilution. These results confirm similar levels of sensitivity in dilution experiments done to detect the enzyme myeloperoxidase (MPO): 1–3% for Northern and 0.05–0.1% for RT-PCR (15). The more sensitive RT-PCR method was carried out, both to confirm the results obtained by Northern blotting, and to further characterize any trace expression of cell lines negative at the protein and Northern mRNA level. The RT-PCR generated a unique and accurately sized product of 868 bp. The identity of PCR products from the cell lines was confirmed by Southern blots of the PCR products (Figure 3). The intensity of expression shown by RT-PCR is, of course, a gross estimate and not an exact quantification (since we did not perform quantitative PCR), but provides nevertheless a clear and reproducible picture of MSE trace expression.

One hundred and fourteen cell lines were examined for MSE mRNA expression by RT-PCR using primers MSE-P1 and -P2, followed by Southern blotting and hybridization with the HMSE-1 probe (Table 2). Seventy-two cell lines were positive; upon significantly longer exposure of the films to the radioactive filter a further 17 of the 42 initially negative cell lines showed weak bands. Of the 49 Northern-negative lymphoid leukemia and lymphoma cell lines, 29 (59%) were positive, most of them weakly or very weakly. Systemic contaminations were excluded as negative control cell lines and negative controls (H_2O) were consistently negative in all experiments; furthermore, the results were confirmed in 24 cell lines using the primer pair MSE-P1 and -P3. No particular pattern of positivity or negativity was seen among the various categories. Eighteen of 22 Northern-negative myeloid leukemia cell lines displayed bands in the RT-PCR Southern blots. All 21 monocytic cell lines investigated were RT-PCR-positive including four cell lines that were both IEF- and Northern-negative. With one exception all samples ($n = 18$) from cell cultures with megakaryocytic-erythroid features were Northern-negative, but RT-PCR-positive. Scanning densitometry of the RT-PCR Southern blots indicated that MSE mRNA was approximately 10–50-fold and 3–10-fold more abundant in monocytic cell lines (e.g. JOSK-I, JOSK-K, JOSK-S, U-937) than in lymphoid leukemia (e.g. JVM-13, NALM-6, REH) and erythroid-megakaryocytic cell lines (e.g. F-36P, LAMA-84, OCI-M1), respectively.

Overexpression of MSE

Previously, we noted a striking variability in the staining intensity of the MSE protein band in the IEF among different monocytic leukemia samples which, however, was not evident in normal controls. Compared with the bands from normal PB monocyte populations a certain percentage of the leukemic specimens showed increased expression of the isoenzyme. As cell extracts were prepared from the same number of cells and equal amounts of mRNA (10 μg) were applied in the gels, we compared the intensity of MSE, IEF, and Northern bands between normal and leukemic samples by densitometric scanning.

Leukemia Cell Lines. The extent of MSE mRNA and protein expression varied greatly among the 26 positive cell lines. Northern blot and IEF analysis showed that the steady-state levels of MSE transcripts and isoenzyme bands were elevated in 16/26 (62%) and in 9/25 (36%) cell lines, respectively, relative to normal purified monocytes (Figures 1, 2, 4, and 5). We considered any abundant MSE expression as 'overexpression' when cases showed in the densitometric scanning at least twice the amount of Northern mRNA or IEF protein found in the normal unstimulated monocytes. According to these criteria 16 cell lines overexpressed MSE mRNA and nine cell

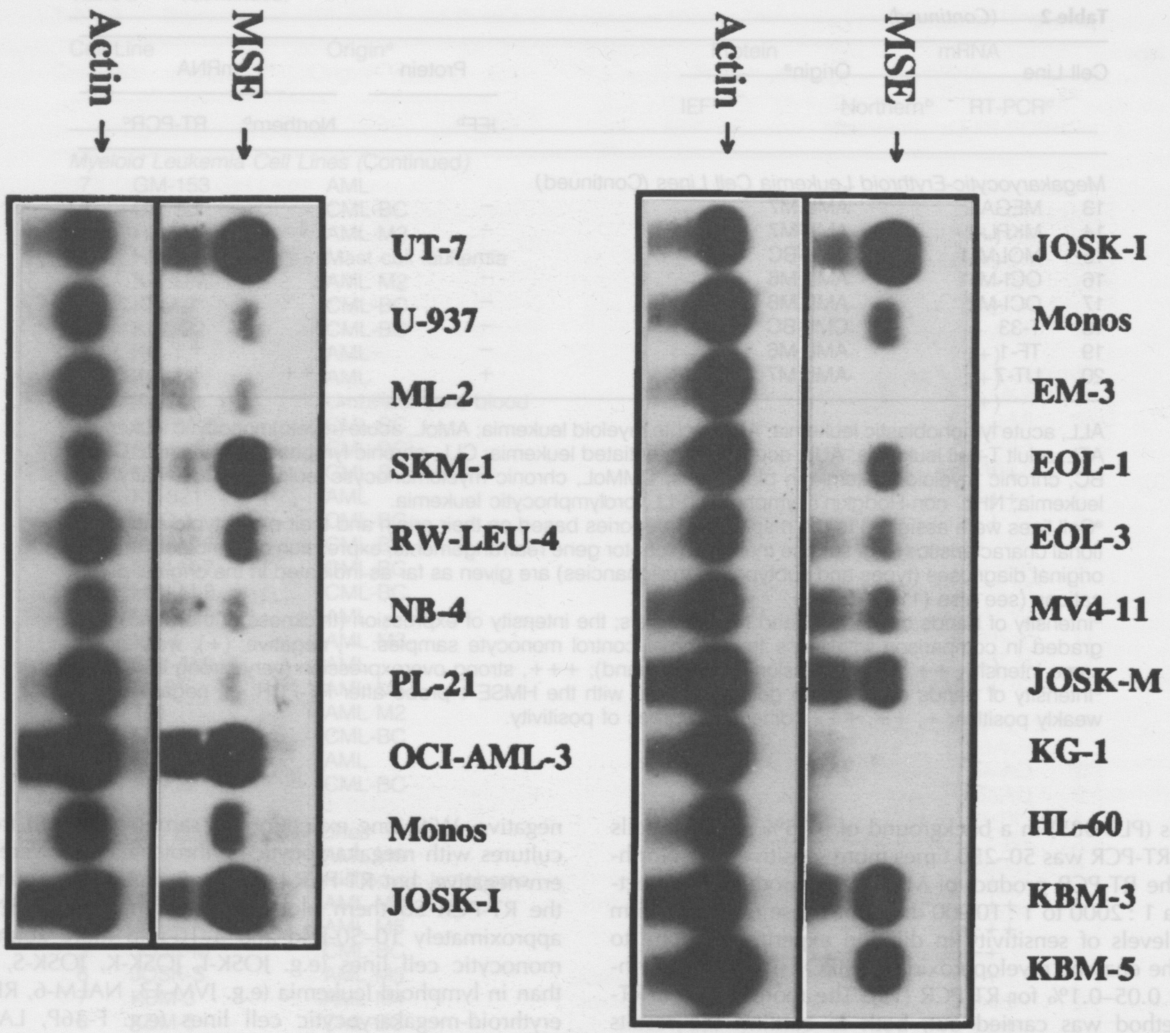

Figure 1 Analysis of MSE mRNA expression in a panel of leukemia cell lines and normal PB monocytes (Monos). Total cellular RNA from the cell lines indicated was analyzed by Northern blotting and hybridized with the HMSE-1 cDNA probe. The MSE message has a size of 2.0 kb. Inspection of the ethidium bromide-stained gel confirmed integrity of the RNA. Hybridization with the β-actin probe was used as an internal control for the loading of similar amounts of RNA

lines overexpressed the protein. The protein levels were not closely related to mRNA levels in some cell lines. The levels of protein expression in seven cell lines overexpressing RNA message fell into the range of 80–200% of that of normal monocytes. The intensities of the IEF bands of 16 cell lines were in the range of 50–170% of those of normal monocytes. Low MSE expression might be caused by deficiencies at various levels, but are more likely based on the relative immaturity of the cells, i.e. cells were arrested at developmental stages where physiologically this gene product is not yet expressed.

Primary Leukemia Cells. Two out of the 14 (14%) mRNA-positive cases expressed MSE at levels that were 2.8–5.6-fold higher than the MSE mRNA levels found in normal monocytes (Table 1). Abnormally high MSE protein levels were detected in 3/11 (27%) MSE-positive leukemic samples (range 4.4–6.2-fold) (Table 1).

Induced Expression of MSE

Leukemia Cell Lines. In order to analyze whether MSE expression can be modulated *in vitro*, positive and negative cell lines were stimulated with ATRA, TPA, Vit. D3 or Bryo 1 and LPS + IFN-γ. ATRA upregulated steady-state levels of MSE transcripts in positive cell lines ($n = 3$); TPA was not very effective and either up- or downregulated, albeit only slightly, the mRNA and protein levels in positive cell lines ($n = 4$) (Table 3; Figures 6 and 7). Vit. D3 and Bryo 1 were not effective in altering the MSE expression, neither up- nor downregulation in positive cell lines nor newly induced expression in negative cell lines (HL-60, NB-4). LPS + IFN-γ caused only minor, insignificant changes (<25%) in MSE mRNA and protein expression in seven leukemia cell lines. Neither ATRA, TPA, nor LPS + IFN-γ induced new MSE transcripts or protein in the initially negative cell lines HL-60, KG-1, and TI-1. The morphological appearance of cell lines in which MSE expression could not be induced or altered was, nevertheless, clearly modified attesting to the efficiency of the inducers

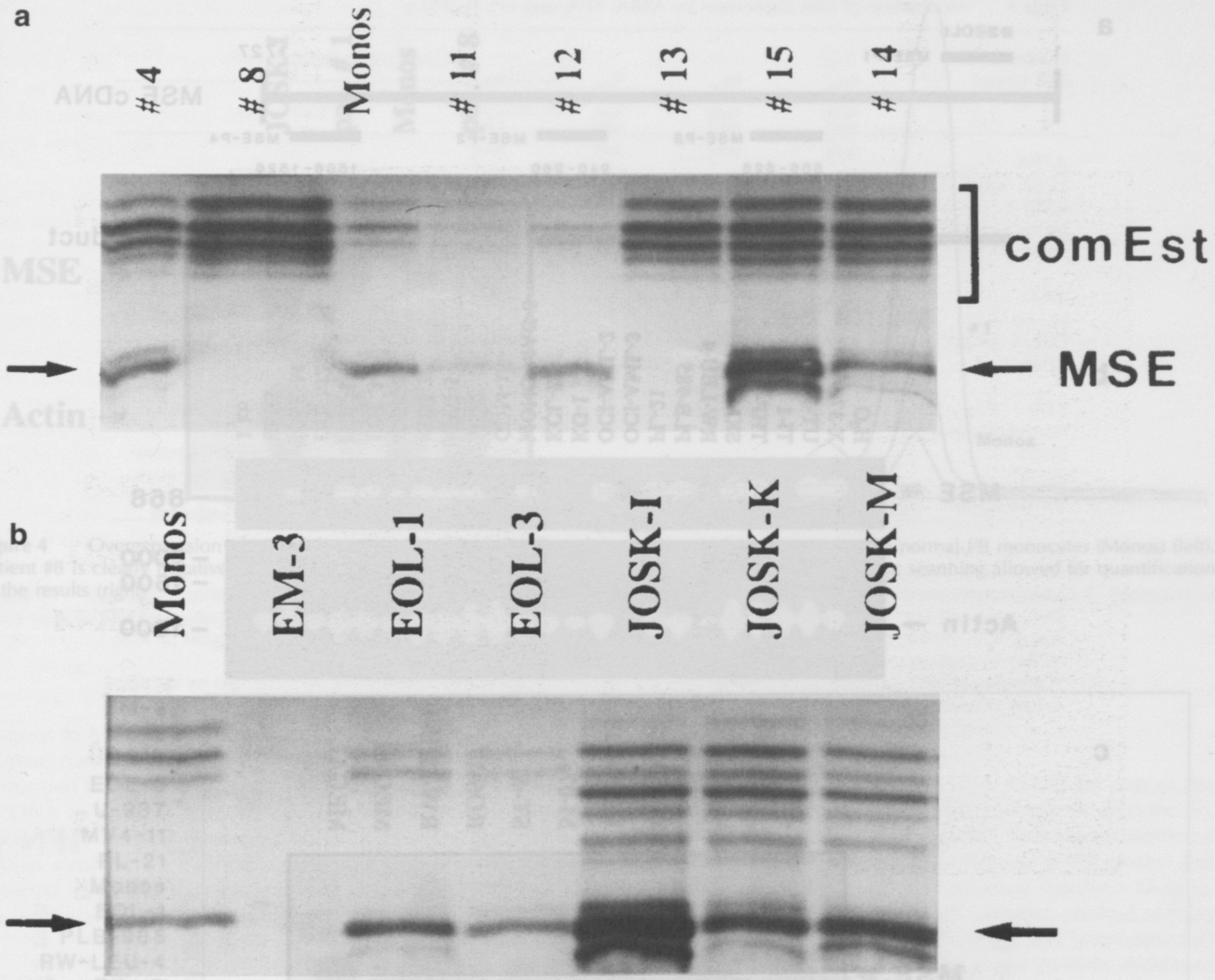

Figure 2 Expression of MSE protein in primary monocytic leukemia samples (a) and in leukemia cell lines (b) in comparison with normal PB monocytes (Monos). Enzymes were extracted, solubilized, separated by IEF on polyacrylamide gels and visualized by α-naphthyl acetate staining. The MSE isoenzyme is focused as a distinct single or double band at about pH 6.0 (indicated by arrows). This band could be selectively inhibited by NaF (not shown). The group of IEF bands with higher isoelectric points (pH 6.3–7.9, the upper bands in the figure) have been termed *common esterases* (Com Est) (9), but are expressed independently of the MSE isoenzyme; compare for example isoenzyme patterns of EOL-1, EOL-3, JOSK-I, and JOSK-K and patients #8, #12, and #13. EM-3 and patients #8 and #13 are clearly MSE-negative. Aliquots of enzyme extracts from equal numbers of cells were separated by IEF. Thus, the intensity of the MSE bands could be compared relative to the level of expression by normal monocytes

used: adherence to the plastic flask in TPA- or Bryo 1-exposed cultures; enlargement of the cytoplasma in TPA-, Bryo 1- or ATRA-treated cells; cellular aggregation in cultures with LPS.

Normal Monocytes. Exposure of normal monocytes to LPS + IFN-γ enhanced both MSE mRNA and protein levels by a factor of 1.5–2. Culture in serum-free medium (M-SFM) with or without ATRA showed a downregulation of MSE mRNA expression in normal monocytes to 20% at 48 h, 1% at 72 h and 0.3% at 96 h of the control values at 0 h, respectively. Thus ATRA was not effective.

Characterization of the MSE Gene

Half-Life of MSE mRNA. The half-life ($t_{\frac{1}{2}}$) of the MSE transcript was determined by incubating the JOSK-I, NOMO-1 and PLB-985 cells in the presence of the transcription inhibitor actinomycin D. The $t_{\frac{1}{2}}$ of MSE mRNA was about 5–6 h.

Single Gene and Gene Amplification. Genomic DNA of the cell lines JOSK-I, KG-1, MOBS-1, NB-4, NOMO-1, OCI-AML-2, OCI-AML-3, PLB-985, SKM-1, TAHR-87, THP-1, and UT-7 were digested with the restriction enzymes *Eco*RI, *Hin*dIII or *Pst*I which have cleavage sites in the human MSE gene (16) and probed with the HMSE-1 cDNA or with a 238-bp fragment from the 5'-end (nt −10–228, obtained after *Bam*HI digestion and extraction from the gel) of the HMSE-1 cDNA. Genomic DNA from different cell lines cut with the same enzyme (*Hin*dIII) showed identical banding patterns in Southern blots (Figure 8); the intensities of the various bands were equal or very similar in all cell lines despite the fact that these cell lines transcribed quite different amounts of mRNA.

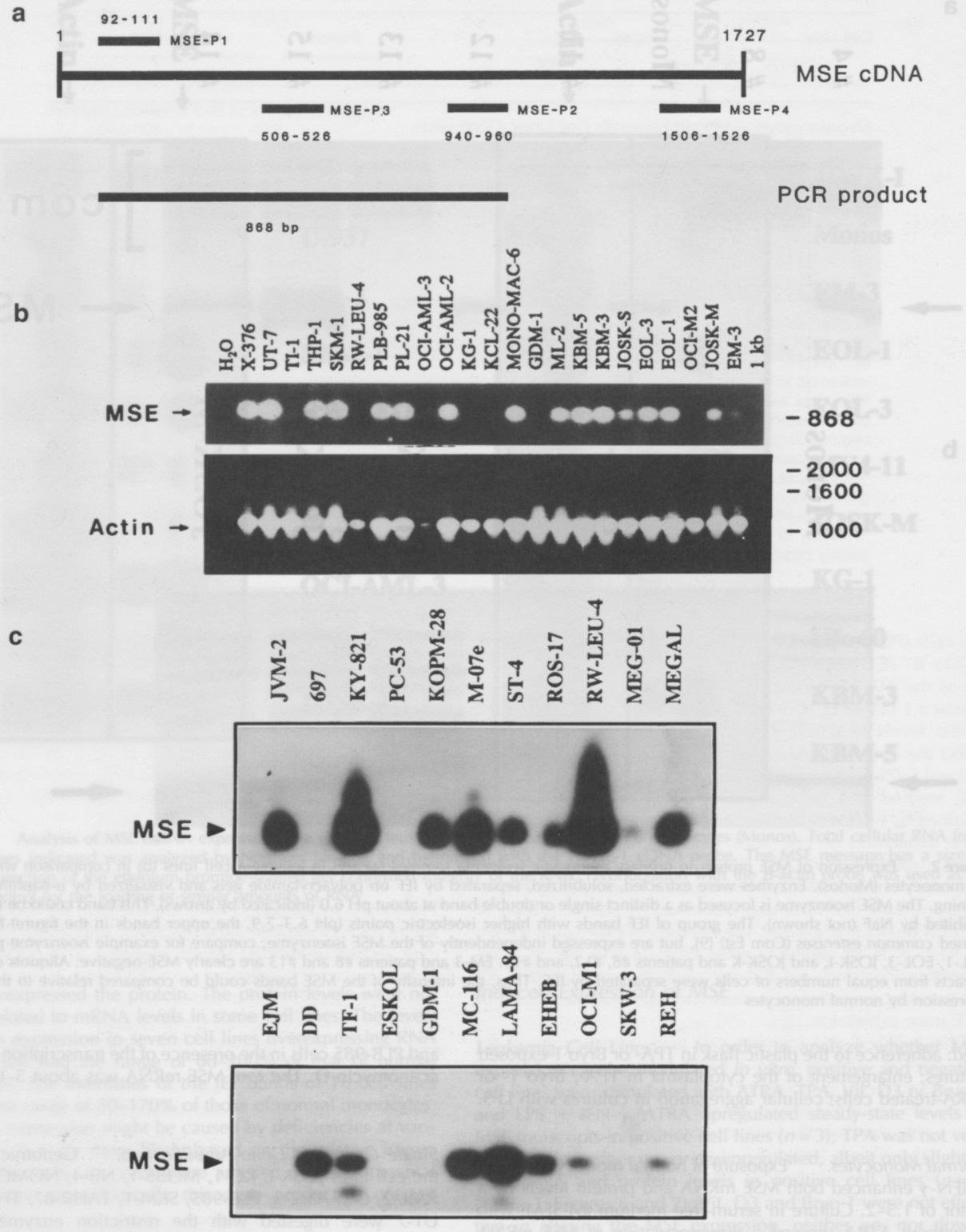

Figure 3 RT-PCR analysis of MSE transcripts in leukemia–lymphoma cell lines using ethidium bromide staining and hybridization after Southern blotting with the HMSE-1 cDNA probe. (a) Schematic diagram of the HMSE-1 cDNA showing the primers MSE-P1 and -P2 used for RT-PCR detection of MSE mRNA and the primers MSE-P1 through -P4 employed for DNA sequencing. (b) Agarose gel electrophoresis of the PCR products (868 bp) amplified using primer pair P1/P2 in a 32-cycle PCR. Molecular size markers were run in the outside lane (denoted as 1 kb ladder). Lane marked H_2O without template RNA represents the negative control. PCR products of the same RT reaction using β-actin primers were used as controls for the quality of the RNA and successful PCR amplification. The cell lines OCI-AML-3 and RW-LEU-4 are here negative which might have been caused by insufficient reverse transcription and/or amplification (see the weak actin band); on repeated experiments these two cell lines were clearly positive. (c) The products were transferred to nylon membranes by the method of Southern and hybridized with the HMSE-1 probe. Compare the weak expression in lymphoid cell lines (e.g. EHEB, REH) *vs.* the strong signals in monocytic cell lines (e.g. RW-LEU-4)

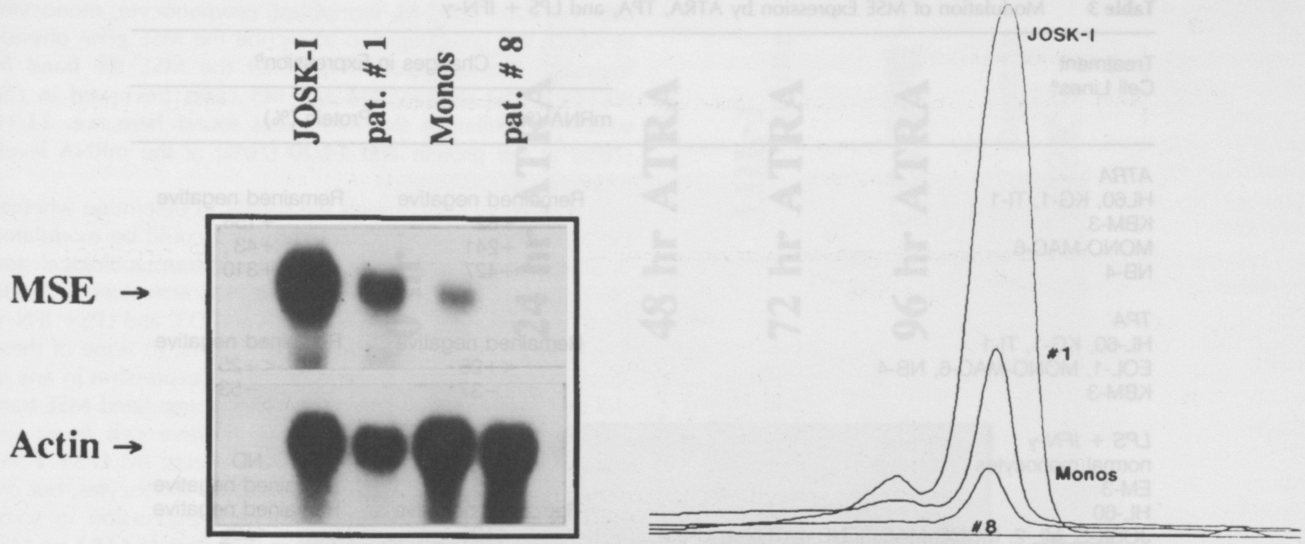

Figure 4 Overexpression of MSE mRNA in the cell line JOSK-I and the patient #1 in comparison with normal PB monocytes (Monos) (left). Patient #8 is clearly negative. The filter was rehybridized with a β-actin cDNA probe. Laser densitometric scanning allowed for quantification of the results (right)

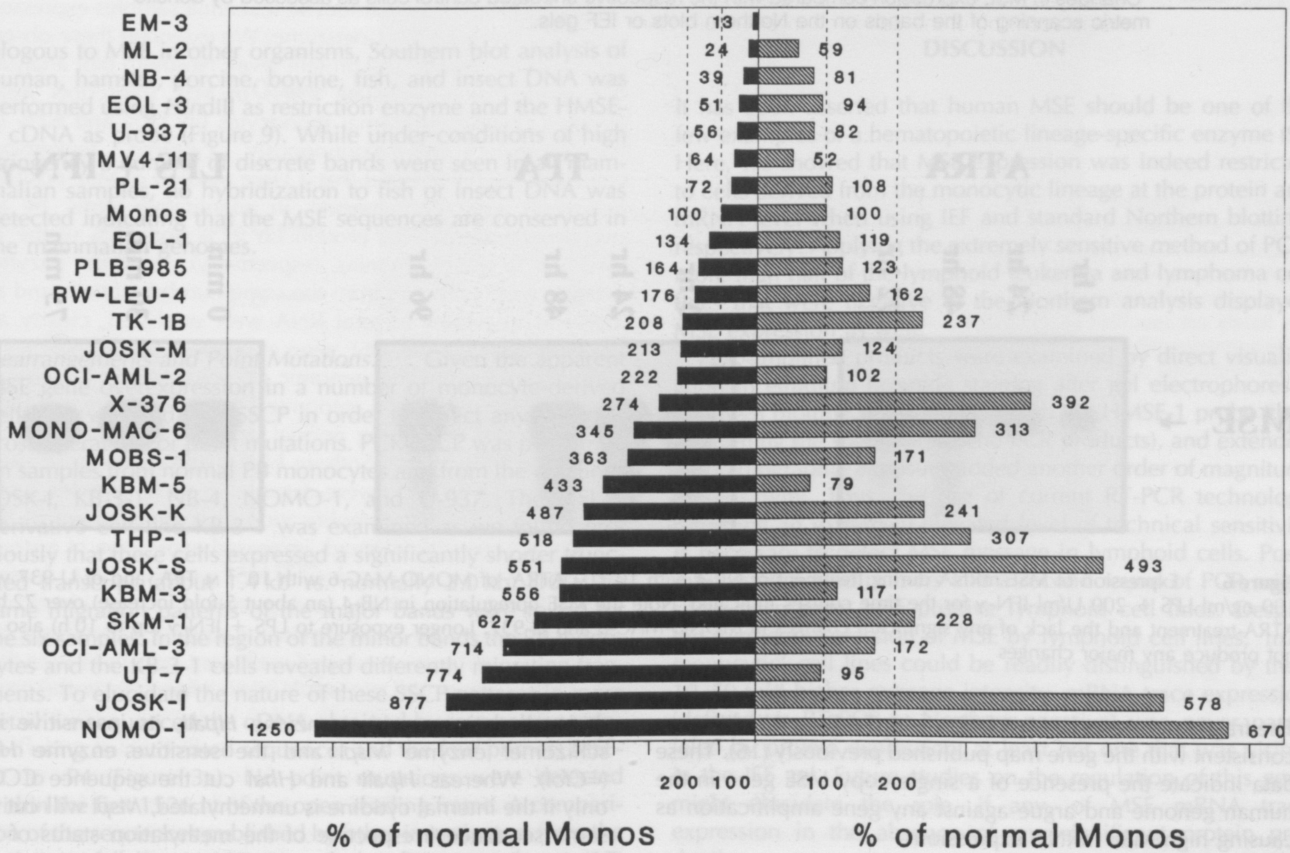

Figure 5 Overexpression of MSE mRNA and protein in leukemia cell lines. The intensity of the bands on Northern blots and IEF gels were measured by laser densitometry and related to the values found for normal PB monocytes (Monos) which were set as 100%. Overexpression was defined as 200% or higher of the mRNA or protein levels of the Monos. Thus, 16/26 and 9/25 leukemia cell lines overexpressed MSE mRNA and protein, respectively (EM-3 was mRNA+, but protein-negative). The levels of signals from different blots or gels were not compared with the levels from other experiments as different exposure times or staining periods might have been used; instead, positive control standards (JOSK-1 and Monos) were included to which the densitometry data were related. (■), mRNA; (▨), protein

Table 3 Modulation of MSE Expression by ATRA, TPA, and LPS + IFN-γ

Treatment Cell Lines[a]	Changes in Expression[b]	
	mRNA (%)	Protein (%)
ATRA		
HL60, KG-1, TI-1	Remained negative	Remained negative
KBM-3	+82	+158
MONO-MAC-6	+241	+43
NB-4	+427	+310
TPA		
HL-60, KG-1, TI-1	Remained negative	Remained negative
EOL-1, MONO-MAC-6, NB-4	<+25	<+25
KBM-3	−37	−53
LPS + IFN-γ		
normal monocytes	+111	ND
EM-3	−25	Remained negative
HL-60	Remained negative	Remained negative
JOSK-S, ML-2, MONO-MAC-6, NB-4, THP-1, U-937	<±25	<±25

ND, not done.
[a]Cells were exposed to 10^{-7} M ATRA, 10^{-7} M TPA or 100 ng/ml LPS + 200 U/ml IFN-γ; ATRA- or TPA-treated cells were cultured for 96 h; RNA and protein of LPS-treated cells were harvested after 4 h and 24 h, respectively. There were no changes in MSE expression in cell lines cultured continuously in their respective media; however, culture of normal monocytes for 96 h led to a clear downregulation of MSE mRNA expression.
[b]Changes in MSE expression compared with the respective untreated control cells as assessed by densitometric scanning of the bands on the Northern blots or IEF gels.

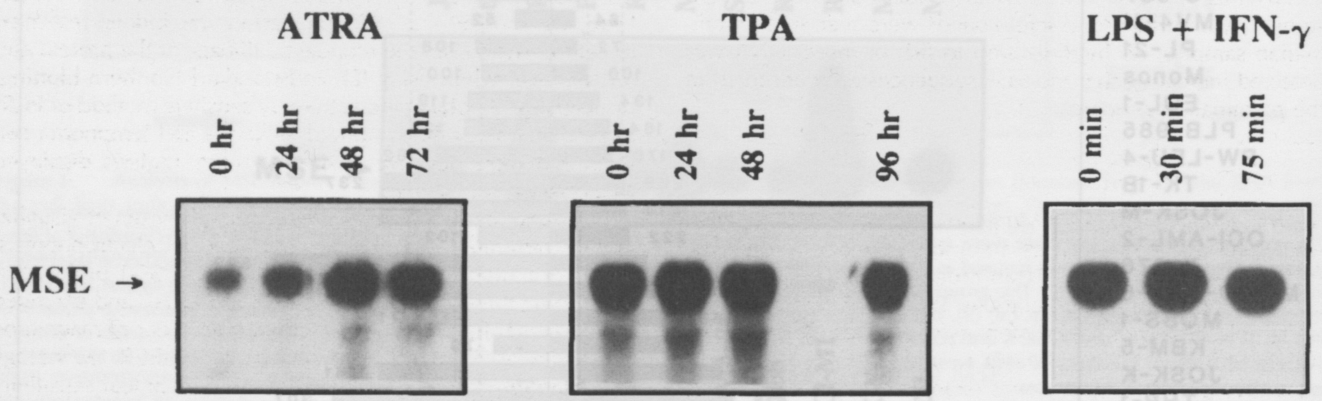

Figure 6 Expression of MSE mRNA during treatment of NB-4 with 10^{-7} M ATRA, of MONO-MAC-6 with 10^{-7} M TPA, and of U-937 with 100 ng/ml LPS + 200 U/ml IFN-γ for the time courses indicated. Note the MSE upregulation in NB-4 (an about 5-fold increase) over 72 h of ATRA-treatment and the lack of any significant changes in MONO-MAC-6 and U-937. Longer exposure to LPS + IFN-γ (up to 10 h) also did not produce any major changes

The fragments seen after restriction with *Eco*RI or *Hin*dIII were consistent with the gene map published previously (16). These data indicate the presence of a single copy MSE gene in the human genome and argue against any gene amplification as causing high MSE mRNA expression.

DNA Methylation. Differences in DNA methylation were determined in cell lines with different degrees of MSE expression: 697 (negative in RT-PCR, Northern, IEF); HL-60 (RT-PCR+, Northern- and IEF-negative); PLB-985 (RT-PCR+, Northern+, IEF+). For Southern blots and hybridization with the HMSE-1 probe, DNA was restricted to completion with the methylation-sensitive enzyme *Hpa*II, its insensitive isoschizomer enzyme *Msp*I and the sensitive enzyme *Hha*I (=*Cfo*I). Whereas *Hpa*II and *Hha*I cut the sequence CCGG only if the internal cytidine is unmethylated, *Msp*I will cut the same sequence irrespective of the methylation status of the nucleotides. No differential restriction patterns of *Hpa*II, *Msp*I or *Hha*I were detected between the three cell lines 697, HL-60, and PLB-985 suggesting that demethylation of cytidine residues in the sequence CpG of the MSE gene is not correlated with its expression.

Expression in Various Species. To detect sequences hom-

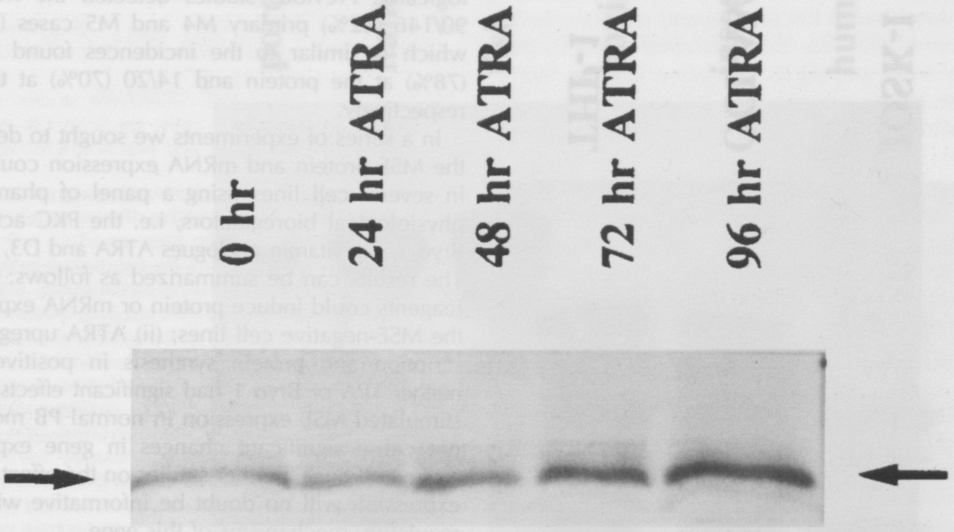

Figure 7 Expression of MSE protein in the NB-4 leukemia cell line exposed to 10^{-7} M ATRA for the time periods indicated. Treatment of NB-4 cells with ATRA upregulated the MSE protein expression (arrow) about 4-fold

ologous to MSE in other organisms, Southern blot analysis of human, hamster, porcine, bovine, fish, and insect DNA was performed using *Hin*dIII as restriction enzyme and the HMSE-1 cDNA as probe (Figure 9). While under conditions of high stringency a number of discrete bands were seen in all mammalian samples, no hybridization to fish or insect DNA was detected indicating that the MSE sequences are conserved in the mammalian genomes.

Rearrangements and Point Mutations. Given the apparent MSE gene overexpression in a number of monocyte-derived cell lines we used PCR-SSCP in order to detect any genomic gross alterations or point mutations. PCR-SSCP was performed on samples from normal PB monocytes and from the cell lines JOSK-I, KB-3-1, NB-4, NOMO-1, and U-937. The HELA-derivative cell line KB-3-1 was examined as we found previously that these cells expressed a significantly shorter truncated transcript (about 1.4 kb *vs.* normally 2.0 kb) (10). The same migration patterns of the major bands were found for the six samples. In the region of the minor bands the PB monocytes and the KB-3-1 cells revealed differently migrating fragments. To elucidate the nature of these SSCP patterns in more detail we sequenced the cDNA obtained by reverse transcription using an automated sequencer and the four primers MSE-P1 to -P4 (Figure 3a). No point mutations were detected within the first 1526 nt of the open reading frame. A comparison of the sequences published by other investigators from the leukemia cell line U-937 (2) and alveolar macrophages (17) with our data showed a differential occurrence of a nucleotide triplet in the various samples. This triplet encompassed nt 892–894 (CAG coding for the neutral polar amino acid glutamine). While the cell line U-937 studied elsewhere (2) and our KB-3-1 lacked the triplet, these nucleotides were present in the alveolar macrophages (17), the PB monocytes, the cell lines JOSK-I, NB-4, NOMO-1, and in our U-937.

DISCUSSION

It has been asserted that human MSE should be one of the few examples of a hematopoietic lineage-specific enzyme (9). Here, we showed that MSE expression was indeed restricted to cells derived from the monocytic lineage at the protein and mRNA level when using IEF and standard Northern blotting, respectively. Applying the extremely sensitive method of PCR, more than half of the lymphoid leukemia and lymphoma cell lines that were negative in the Northern analysis displayed MSE expression as well.

PCR-amplified products were examined by direct visualization in ethidium bromide staining after gel electrophoresis. Southern blotting, hybridization with the HMSE-1 probe (thus confirming the specificity of the PCR products), and extended autoradiographic exposure added another order of magnitude of sensitivity. Thus, the use of current RT-PCR technology reflecting an extremely elevated level of technical sensitivity is necessary to detect MSE message in lymphoid cells. Possibly, using nested primers and a second round of PCR might show even more MSE-positive lymphoid cell lines. Despite the frequent expression of MSE by lymphoid cell lines, 'truly monocytic' cell lines could be readily distinguished by their 10–50-fold higher message intensity. mRNA trace expression (defined as RT-PCR+, Northern-negative) was never paralleled by protein expression, at least not one that was visible in the IEF gel. Future studies on the regulation of this gene might elucidate the role, if any, of MSE mRNA trace expression in the absence of any significant protein production.

The lack of MSE protein or mRNA (Northern) expression in some monocyte-derived cell lines and primary samples might be explained as follows: (i) an inheritable monocyte esterase deficiency with an autosomal dominant mode of transmission has been reported recently; the incidences were 0.8%, 1.7%, and 3.9% for normal individuals and for patients with either non-malignant or malignant diseases, respectively (18); (ii) a

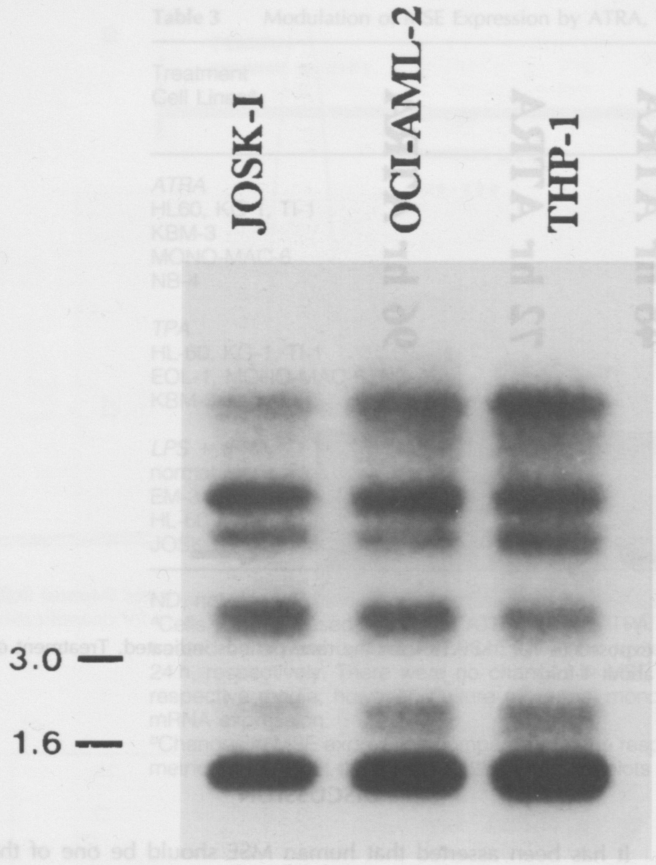

Figure 8 Restriction pattern of the MSE gene in high molecular weight DNA isolated from several MSE mRNA-positive and -negative leukemia cell lines. After endonuclease digestion with *Hin*dIII the DNA fragments were separated by pulsed field gel electrophoresis, Southern blotted, and hybridized to the HMSE-1 probe. The sizes of the bands are specified in the figure. The bands from all cell lines have approximately the same intensity; there were no gross alterations of the restriction patterns

misdiagnosis (i.e. acute myeloid leukemia without a monocytic component *vs.* acute myelomonocytic/monocytic leukemia) of the cases from which the continuous cell lines had been established and of our fresh leukemia samples cannot be entirely excluded; regarding our primary leukemia specimens the eligibility criteria included only cases that were CD14+ and belonged morphologically to the FAB categories M4 or M5; with regard to the cell lines we were left to rely on the original publications detailing the features of the seeding material; (iii) some cell lines appear to have lost characteristic features still found on the original cells, e.g. most continuous monocyte-derived cell lines are no longer CD14+ despite strong expression of this surface marker on the initial population (12); significant differences between cell lines and the respective primary cells were reported for other surface antigens, morphological appearance, etc. (11); (iv) this 'dedifferentiation' during the establishment of the cell lines might have led to an arrest at a more immature stage of differentiation and some of the freshly explanted leukemic cells might correspond to rather immature normal counterparts; it will be interesting to assign by *in situ* hybridization the precise stage of differentiation (CFU-M, monoblast, promonocyte, monocyte) at which the cells begin to transcribe the MSE gene physiologically. Previous studies detected the MSE IEF band in 90/146 (62%) primary M4 and M5 cases (reviewed in (3)) which is similar to the incidences found here, i.e. 11/14 (78%) at the protein and 14/20 (70%) at the mRNA level, respectively.

In a series of experiments we sought to determine whether the MSE protein and mRNA expression could be modulated in several cell lines using a panel of pharmacological and physiological bioregulators, i.e. the PKC activators TPA and Bryo 1, the vitamin analogues ATRA and D3, and LPS+ IFN-γ. The results can be summarized as follows: (i) none of these reagents could induce protein or mRNA expression in any of the MSE-negative cell lines; (ii) ATRA upregulated MSE transcription and protein synthesis in positive cell lines; (iii) neither TPA or Bryo 1 had significant effects; (iv) LPS+ IFN-γ stimulated MSE expression in normal PB monocytes, but did not cause significant changes in gene expression in some seven cell lines. Further studies on the effect of ATRA on MSE expression will no doubt be informative with respect to the regulatory mechanisms of this gene.

As documented previously, there is a considerable variability in the staining intensities of MSE in leukemic cells (19). The comparison of bands from normal PB monocyte populations with laser densitometric scanning showed that a certain percentage of the monocytic leukemia samples expressed increased levels of this isoenzyme. We extended these observations at the mRNA and protein level; Northern blot analysis confirmed that steady-state levels of MSE transcripts were elevated relative to normal monocytes (in 14% and 62% of the primary and continuously cultured leukemias, respectively); IEF showed that a large fraction of MSE-positive leukemias were distinguished, in quantitative terms, by an overabundance of MSE protein (in 27% and 36% of the *in vivo* and *in vitro* leukemias, respectively). As all the enzyme extracts were prepared from the same number of cells and as identical quantities of total RNA were analyzed, clearly an overexpression of the MSE gene appears to occur.

To determine whether genomic alterations might be responsible for high expression, genomic DNA of negative cell lines and cell lines expressing normal or overabundant levels of MSE were subjected to Sothern blot analysis. No gross alterations of the MSE gene were detected in these samples, arguing against a possible amplification or rearrangement of the gene and suggesting, instead, increased expression of a structurally unaltered gene. Analysis of MSE genomic DNA, reverse transcribed DNA, mRNA, and protein revealed identically sized bands and the same isoelectric points, respectively, for all normal and fresh or cultured malignant cells indicating that any abnormalities did not result from differences in the gross DNA, RNA, or protein structures.

Then, a two-step strategy based on PCR-SSCP and PCR-directed sequencing was used to define any minute alterations or point mutations of the gene. While no point mutations could be detected in the cDNA, sequence comparison between published data and our results unveiled the presence of a base triplet (CAG at nt 892–894 coding for glutamine) in our normal and malignant specimens and the lack of these nucleotides in the U-937 derived HSSE-1 sequence (2) and in a HeLa-subclone aberrantly expressing this gene. The significance of this finding is unclear at the present time; it might possibly represent an insignificant polymorphism. In any event, in our U-937 these nucleotides were clearly present.

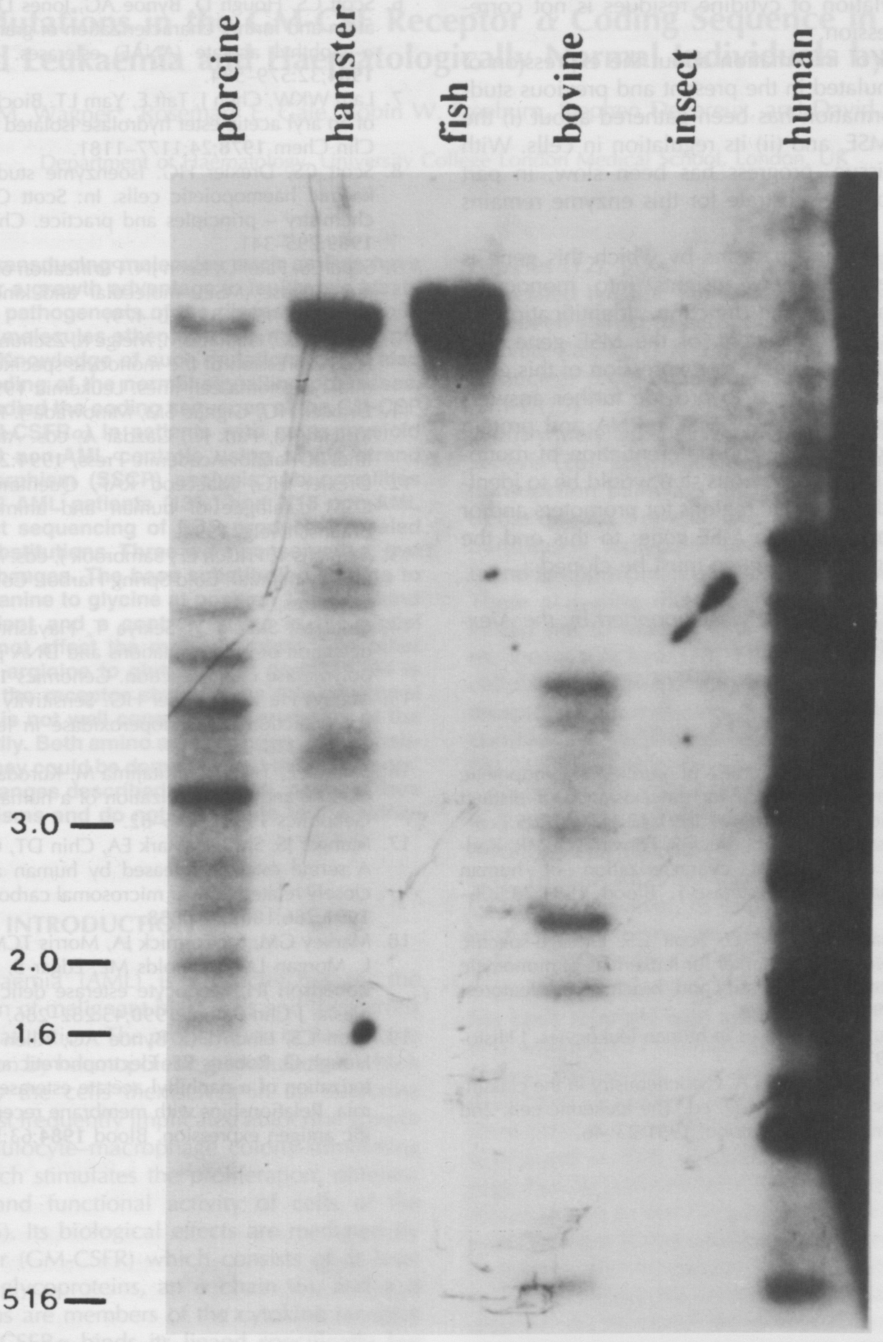

Figure 9 Conservation of the MSE gene in mammalian species. Genomic DNA from cell lines of different species was cut with *Hin*dIII, size-separated by agarose gel electrophoresis, Southern blotted and hybridized with the HMSE-1 probe. The cell lines used were as follows: AM-C6SC8 (porcine kidney), BHK-21 (hamster kidney cells), D-11 (rainbow trout liver), KNR-028 (bovine embryonal kidney), MB-020 (insect, cabbage moth larvae), OCI-AML-2 (human AML M4)

Precisely what the mechanisms are that led to overabundant levels of MSE mRNA and protein is open to further experiments, but our preliminary analysis has already excluded some possibilities. It remains to be determined whether the high proportion of blast cells overexpressing MSE is related to an abnormal regulation of the MSE gene due to the leukemic process (possibly caused by alterations, e.g. point mutations, in regulatory elements controlling the level of gene expression) or is triggered by a more physiological mechanism already present in normal cells. In other words, does the MSE overexpression reflect an abnormality or a process that is normal for a particular stage of monocyte differentiation or activation?

Cross-reactivity was found with other mammalian species suggesting that the MSE gene is evolutionarily conserved. The lack of expression in many other human cell types (urinary bladder, kidney, breast, skin, cervix, ovary, and colon) (10) favors an exclusive role in the hematopoietic system. Analysis of DNA methylation of the MSE gene did not show differential restriction patterns of methylation-sensitive restriction enzymes between MSE-positive and -negative cell lines sug-

gesting that demethylation of cytidine residues is not correlated with MSE expression.

While a great deal of information about the expression of MSE has been accumulated in the present and previous studies, only limited information has been gathered about (i) the cellular function of MSE, and (ii) its regulation in cells. With respect to the first issue, progress has been slow, in part because the physiological substrate for this enzyme remains unknown.

Further studies on the mechanisms by which this gene is regulated should lead to new insights into monocyte–macrophage differentiation and function. Identification of regulators, e.g. transcription factors, of the MSE gene is a prime objective of future studies. Overexpression of this gene by transfection should enable us to provide further answers on the up- and downregulation of MSE mRNA and protein during activation, proliferation, and differentiation of monocytes–macrophages. The next obvious step would be to identify and characterize the genomic regions for promoters and/or other regulatory elements of the MSE gene; to this end the thus far elusive 5'-region of the gene must be cloned.

Acknowledgements. Dr Z.B. Hu was supported by the Alexander von Humboldt-Foundation.

REFERENCES

1. Patel D, Scott CS. Inhibitor studies of purified haemopoietic (myeloid) cell esterases: evidence for the existence of distinct enzyme species. Biochem Pharmacol **1991**;42:1577–1585.
2. Zschunke F, Salmassi A, Kreipe H, Buck F, Parwaresch MR, Radzun HJ. cDNA cloning and characterization of human monocyte/macrophage serine esterase-1. Blood **1991**;78:506–512.
3. Drexler HG, Gignac SM, Patel D, Scott CS. Lineage-specific monocytic esterase, a distinct marker for leukemias of monocytic origin: cytochemical, isoenzymatic and biochemical features. Leuk Lymphoma **1991**;4:295–312.
4. Li CY, Lam KW, Yam LT. Esterases in human leukocytes. J Histochem Cytochem **1973**;21:1–12.
5. Flandrin G, Daniel MT, Crockard A. Cytochemistry in the classification of leukemias. In: Catovsky D, ed. The leukemic cell, 2nd edn. Edinburgh: Churchill Livingstone, **1991**:23–46.
6. Scott CS, Hough D, Bynoe AG, Jones DB, Roberts BE. Fractionation and further characterization of granulocytic and monocytic α-naphthyl acetate (ANAE) esterases. J Histochem Cytochem **1984**;32:579–584.
7. Lam WKW, Chen J, Taft E, Yam LT. Biochemical characterization of an aryl acetic ester hydrolase isolated from human monocytes. Clin Chem **1978**;24:1177–1181.
8. Scott CS, Drexler HG. Isoenzyme studies of normal and leukaemic haemopoietic cells. In: Scott CS, ed. Leukaemia cytochemistry – principles and practice. Chichester: Ellis Horwood, **1989**;295–341.
9. Scott CS, Patel D, Keen JN. Purification of human monocyte-specific esterase (MSE): molecular and kinetic characteristics. Br J Haematol **1992**;81:470–479.
10. Uphoff CC, Gignac SM, Metge K, Zschunke F, Radzun HJ, Drexler HG. Expression of the monocyte-specific esterase gene in leukemia–lymphoma cell lines. Leukemia **1993**;7:58–62.
11. Drexler HG, Gignac SM, Minowada J. Hematopoietic cell lines. In: Hay RJ, Park JG, Gazdar A, eds. Atlas of human tumor cell lines. Orlando: Academic Press, **1994**:213–250.
12. Drexler HG, MacLeod RAF, Quentmeier H, Steube K, eds. DSM catalogue of human and animal cell lines, 4th edn. Braunschweig, **1994**.
13. Maniatis T, Fritsch EF, Sambrook J, eds. Molecular cloning, a laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, **1982**.
14. Orita M, Suzuki Y, Sekiya T, Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics **1989**;5:874–879.
15. Ma W, Hu ZB, Drexler HG. Sensitivity of different methods for the detection of myeloperoxidase in leukemia cells. Leukemia **1994**;8:336–342.
16. Shibata F, Takagi Y, Kitajima M, Kuroda T, Omura T. Molecular cloning and characterization of a human carboxylesterase gene. Genomics **1993**;17:76–82.
17. Munger JS, Shi GP, Mark EA, Chin DT, Gerard C, Chapman HA. A serine esterase released by human alveolar macrophages is closely related to liver microsomal carboxyl-esterase. J Biol Chem **1991**;266:18832–18838.
18. Markey GM, McCormick JA, Morris TCM, Alexander HD, Nolan L, Morgan LM, Reynolds ME, Edgar S, Bell AL, McCaigue MD, Robertson JH. Monocyte esterase deficiency in malignant neoplasia. J Clin Pathol **1990**;45:282–286.
19. Scott CS, Linch DC, Bynoe AG, Allen C, Hogg N, Ainley MJ, Hough D, Roberts BE. Electrophoretic and cytochemical characterization of α-naphthyl acetate esterase in acute myeloid leukemia. Relationships with membrane receptor and monocyte-specific antigen expression. Blood **1984**;63:579–587.