Journal of Cellular Biochemistry 78:519–532 (2000)

ARTICLES

Human Monocyte/Neutrophil Elastase Inhibitor (*MNEI*) is Regulated by PU.1/Spi-1, Sp1, and NF-κB

Weilan Zeng and Eileen Remold-O'Donnell*

The Center for Blood Research and the Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115

Abstract Human monocyte/neutrophil elastase inhibitor (MNEI) is a specific inhibitor of the neutrophil azurophil granule proteases including elastase. To understand the physiological mechanisms that regulate expression of *MNEI*, we dissected a 1.0 kb region upstream of exon 1. On transient transfection, promoter activity of *MNEI*-luciferase constructs was highest in U937 myeloid cells, followed by K562 hematopoietic cells, followed by HeLa cervical carcinoma cells, indicating that the *MNEI* promoter is most active in myeloid cells and is also active in non-myeloid cells. Three transcription factor binding elements, which confer the majority of activity, are located within the first 180 base pairs of the promoter, one of which, located at −128, was active in U937 and K562 cells but inactive in non-myeloid HeLa cells. The three proximal elements were identified by transient transfection, mutation, gel shift and competition assays as Sp1 at −170, PU.1/Spi-1 at −128, and Sp1 at −66. The *trans*-acting factors that bind and control these elements were detected, and their identity confirmed by antibody supershift assays. Further upstream at −821, an additional regulatory element was identified controlled by NF-κB, which supports the highest levels of *MNEI* transcriptional activity. In U937 cells, reporter gene expression by the *MNEI*-luciferase construct that included the NF-κB element was two- to three-fold greater than the construct without the element. In addition, treatment of myeloid cells with lipopolysaccharide, a complex glycolipid of gram-negative bacteria, activated NF-κB to bind the −821 element, together suggesting that enhancement of expression of the anti-inflammatory *MNEI* gene is linked to innate immune responses to bacterial infection. J. Cell. Biochem. 78:519–532, 2000.

Key words: monocytes; neutrophils; promoter; regulation of gene transcription; protease; protease inhibitor

Human monocyte/neutrophil elastase inhibitor (MNEI) is a fast-acting specific inhibitor of the three proteases of myeloid cell azurophil granules: elastase (also called neutrophil elastase), cathepsin-G, and proteinase-3. These closely related enzymes are synthesized by promyelocytes, packaged in azurophil (primary) granules, and carried in circulating neutrophils and monocytes [Fouret et al., 1989; Grisolano et al., 1994; Zimmer et al., 1992]. In a process that protects against microbial infection, the granule proteases are released into endocytized vacuoles or extracellularly when the phagocytic cells encounter microorganisms at extravascular sites. In contrast, excess granule protease, particularly elastase, acts as a major agent of inflammatory damage by degrading antibodies, cell surface receptors, and matrix proteins including elastin and inducing the production of inflammatory cytokines [Doring, 1994; Johnson et al., 1988].

MNEI, a 42 kDa serpin superfamily protein of known sequence [Remold-O'Donnell et al., 1992], is a prevalent component of neutrophils, monocytes, and macrophages [Remold-O'Donnell et al., 1989]. MNEI reacts rapidly and stoichiometrically with free elastase to form an inactive complex [Remold-O'Donnell, 1985], thereby neutralizing the protease and restoring protease:protease inhibitor balance. Recently, MNEI was shown to also inhibit cathepsin-G and proteinase-3 with high affinity and to react negligibly with other proteases [Remold-O'Donnell et al., 1998; Sugimori et al., 1995]. We have been exploring the potential of recombinant MNEI as a therapy for neutrophil protease-induced inflammatory lung damage, a major pathological process, for example, in the

Grant sponsor: National Institutes of Health; Grant number: HL41579.

*Correspondence to: Eileen Remold-O'Donnell, The Center for Blood Research, 800 Huntington Avenue, Boston, MA 02115. E-mail: remold@cbr.med.harvard.edu

Received 1 January 2000; Accepted 24 February 2000

inherited disease cystic fibrosis. In preclinical studies, recombinant MNEI delivered to the airways of rats prevented multi-parameter lung damage caused by instillation of neutrophil elastase or airway secretions of cystic fibrosis patients [Rees et al., 1999].

Unlike the biochemical and pharmacological properties, the natural regulatory mechanisms that control expression and function of MNEI are poorly defined. To understand the physiological regulation and in vivo action of MNEI, we characterized the *MNEI* gene and promoter, especially the regulatory elements that control *MNEI* expression. MNEI is encoded in humans by a single gene, *MNEI*, which spans 9.5 kB on the short arm of chromosome 6 at 6p24-pter [Evans et al., 1995; Zeng et al., 1998].[1] *MNEI* contains seven exons, and the 3′-untranslated region includes alternative polyadenylation signals [Zeng et al., 1998], which appear to serve a regulatory role [Kordula et al., 1993]. In the upstream region, the coding sequence begins within exon two, and the transcription start site, identified by primer extension, corresponds to a 60 bp exon one. In the present study, we examine the promoter region of *MNEI* by dissecting a 1.0 kb region 5′ of the transcription start site to identify critical sites that control promoter activity and *trans*-acting nuclear factors that bind the *MNEI* promoter and regulate its transcriptional activity.

MATERIALS AND METHODS

Cell Culture

U937 monocyte-like cells [Sundstrom and Nilsson, 1976] from a strain (U937/DYL) maintained in this laboratory since 1985 [Remold-O'Donnell, 1985] were grown in DMEM with high glucose (Mediatech, Herndon, VA) with 10% fetal calf serum. K562 erythroid/myeloid cells [Lozzio and Lozzio, 1975] and Daudi, a B-cell line [Klein et al., 1968] (American Type Culture Collection (ATCC), Rockville, MD), were grown in RPMI 1640 with 10% fetal calf serum; THP-1 monocyte-like cells [Tsuchiya et al., 1980] (ATCC) were grown in the same medium with 50 μM 2-mercaptoethanol (Sigma, St. Louis, MO). HeLa cervical carcinoma cells, strain S3, provided by Dr. Philip Auron, Harvard Medical School, were grown in DMEM with 10% fetal calf serum in 10 cm diameter dishes and passaged by treatment with 25 mM EDTA in phosphate-buffered saline (PBS). All media have 100 units/ml penicillin and 100 μg/ml streptomycin. Where mentioned, LPS (*Escherichia coli* 0111:B4; Sigma) was added to U937 and THP-1 cell cultures (1 or 10 μg/ml) for 2 h prior to cell harvest.

MNEI-Luciferase Constructs

MNEI promoter fragments were generated by PCR using a genomic clone as template [Zeng et al., 1998] and were cloned into the *Bgl* II site of firefly luciferase pGL-3 basic vector (Promega, Madison, WI), which contains neither promoter nor enhancer. The constructs extended from different upstream sites to +25 bp relative to the *MNEI* transcription start site [Zeng et al., 1998]. The constructs were sequenced to verify correct insertion and orientation. Mutated constructs were generated by Quik-Change Site-Directed Mutagenesis reagents (Stratagene, La Jolla, CA) and confirmed by sequencing (Table 1). Plasmid DNA was purified by Qiagen tip-500 DNA reagents (Qiagen, Chatsworth, CA).

Transfection

Non-adherent cell lines U937, K562, and Daudi in log phase growth were harvested for transfection after being supplemented with fresh medium on the preceding day. The cells (4×10^6) were combined with 20 μg of experimental *MNEI* plasmid or pGL-3 basic vector in 400 μl complete media in 0.4 cm electroporation cuvettes. Electroporation was carried out with the Gene pulser II (Bio-Rad, Hercules, CA) at 1070 microfarad and 360 V (U937 cells), 280 V (K562 cells), or 240 V (Daudi cells) [Baum et al., 1994]. pRL-TK (3 μg) encoding *Renilla* luciferase driven by the herpes simplex virus thymidine kinase promoter was co-transfected with the experimental plasmids and pGL-3 as normalizing control for transfection efficiency in most experiments. Following transfection, 12 ml media was added, and the cells were cultured for 24 h, harvested by pelleting, and washed once with PBS containing Ca^{2+} and Mg^{2+}. The cells were lysed in 100 μl PBS with Ca^{2+} and Mg^{2+} by three cycles of freezing and thawing, and the lysates were clarified by centrifugation.

[1]The *MNEI* gene has also been called *ELANH2* and *P12* [Evans et al., 1995].

TABLE I. Description of Wild-Type and Mutated *MNEI* Oligonucleotides and Constructs

Oligonucleotide and construct	Sequence[a]	Location	Length
−138	5′-AGCCACGCTCACTTCTGCTTGCACTTA-3′	−140 to 114	27 bp
M-138 and $Mp138_{PU}$	5′-AGCCACGCT*TTAAA*CTGCTTGCACTTA-3′	−140 to 114	27 bp
$PU.1_{consensus}$	5′-GGGCTGCTTGAGGAAGTATAAGAAT-3′	—	25 bp
−180	5′-AAAGAAGCCGCGCCCCTGAGGAG-3′	−176 to −154	23 bp
M-180 and Mp180	5′-AAAGAAGCCGC*AAA*CCTGAGGAGGGC-3′	−176 to −151	26 bp
$Sp1_{consensus}$	5′-ATTCGATCGGGGCGGGGCGAGC-3′	—	22 bp
$AP\text{-}2_{consensus}$	5′-GATCGAACTGACCGCCCGCGGCCCGT-3′	—	26 bp
−66	5′-AGGGCCGCCGCCCCGCCCGCGGCTGCTG-3′	−73 to −46	28 bp
1M-66 and $1Mp138_{GC}$	5′-GGCCGCCGCCCCACCCGC-3′	−71 to −54	18 bp
2M-66 and $2Mp138_{GC}$	5′-GGCCGCCGCC*AA*GCCCGC-3′	−71 to −54	18 bp
3M-66 and $3Mp138_{GC}$	5′-GGCCGCCG*AA*CCGCCCGC-3′	−71 to −54	18 bp
−829	5′-TAGCTGGAGGGGAATTTCACTTAGGAT-3′	−829 to −803	27 bp
M-829	5′-TAGCTGGAGG*CCC*ATTTCACTTAGGAT-3′	−829 to −803	27 bp
$NF\text{-}\kappa B_{consensus}$	5′-AGTTGAGGGGACTTTCCCAGGC-3′	—	22 bp

[a]Mutated nucleotides are indicated by italics.

HeLa cells were transfected by the calcium phosphate method (reagents from 5 prime → 3 prime). The cells were passaged 1 day prior to transfection to 40–50% confluence (≈10^6 cells per 10 cm dish), and fresh media was added 2 h before transfection. Plasmids (20 μg) with or without 3 μg pRL-TK (Promega) as described above were added, and the HeLa cells were cultured with complete transfection components for 24 h, washed twice with DMEM, and cultured in fresh medium for an additional 24 h. For harvest, the HeLa cells were detached with EDTA and pelleted; they were lysed by freezing and thawing as described above for non-adherent cells.

Luciferase Assay

Lysates of transfected cells (5 μl/assay) were analyzed for firefly luciferase and Renilla luciferase by addition of Fire-Lite and Ren-Lite substrates (Packard, Meriden, CT) and quantitation of the enzymatic products with the Top-Count luminescence counter (Packard). At least three independent experiments were performed for each plasmid. Firelight luciferase activity in each experiment was calculated as the average/mean of at least duplicate transfections without or with normalization based on the activity of co-transfected Renilla luciferase. Comparison of multiple experiments indicated that normalization did not alter mean results, and luciferase activities from both types of quantitation were pooled after conversion to relative units as defined in figure legends.

Preparation of Nuclear Extracts

Nuclear extracts of U937, K562, and THP-1 cells were prepared using a modified method [Andrews and Faller, 1991; Dignam et al., 1983]. Log phase cells (5–10 × 10^6) were harvested, washed twice with cold PBS; disrupted by incubation at 4°C for 10 min in 400 μl hypotonic buffer A (0.05% Nonidet P-40, 10 mM HEPES buffer, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM dithiothreitol) containing proteinase inhibitors (2 mM diisopropyl fluorophosphate, 50 μg/ml leupeptin, 4 μg/ml pepstatin). The nuclei were harvested by pelleting at 3,000–4,000 rpm for 2 min in a microfuge and were extracted in 40 μl buffer C (10 mM HEPES buffer, pH 7.9, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM dithiothreitol) containing proteinase inhibitors by incubation at 4°C for 30–60 min with occasional gentle tapping. Cell debris was pelleted in a microcentrifuge at 15,000 rpm at 4°C for 10–20 min, and the clarified nuclear extracts were stored in aliquots at −80°C. Protein concentration was determined using the Bradford reagent

(Bio-Rad). Preparations of nuclear extract of HeLa cells were obtained from Promega.

Electrophoretic Mobility Shift Assays (EMSA)

Complementary DNA oligonucleotides (Oligos Etc., Wilsonville, OR) in 2 mM Tris · HCl, pH 7.5, 1 mM $MgCl_2$, 5 mM NaCl, 0.1 mM DTT) were annealed by heating at 100°C for 2–5 min and slowly cooling at ≈22°C. Double-stranded oligomers (≈25 ng) were labeled with [γ-^{32}P]ATP (3,000 Ci/mmol) and T_4 polynucleotide kinase (Promega) and purified by acrylamide gel electrophoresis. For gel shift assays, nuclear extracts (5–8 μg) were incubated for 20 min at 4°C with 2–5 × 10^4 cpm of ^{32}P-labeled probe in 12.5 μl of 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 5 mM $MgCl_2$, 5 mM NaCl, 1 mM DTT, 1 mM EDTA containing 1 μg of poly(dI-dC). For competition assays, unlabeled competitor DNA was incubated with the nuclear extracts at 4°C for 20 min prior to addition of labeled oligomer. For supershift assays, 2 μg of specific rabbit (PU.1, AP-2, NF-κB p65) or goat (Sp1, NF-κB p50) antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) were added in 1 μl after the binding reaction, and the mixture was incubated at ≈22°C for 30 min. The products were resolved on 5% non-denaturing acrylamide gels (100 V, 0.5 × TBE buffer, ≈22°C); the gels were then dried and exposed to autoradiographic film.

RESULTS

Promoter Activity of the 5′ Region of Human *MNEI* Examined in Cells of Different Origin

To identify *cis*-elements and *trans*-acting factors important in human *MNEI* promoter activity, we linked six cloned fragments of the immediate 5′ flanking region of *MNEI* genomic DNA ranging in size from 1,000 to 40 bp upstream of the transcription start site to the luciferase reporter gene to generate the plasmids p1000, p800, p500, p180, p100, and p40. These unidirectional deletion constructs and the promoterless vector pGL-3 were examined by transfection of cell lines of varying origin. When transiently transfected into U937 monocyte-like cells, K562 myeloid/erythroid cells, and HeLa epithelial-like cells, the six *MNEI* plasmids displayed varying levels of promoter activity (>4-fold enhancement of luciferase activity relative to pGL-3). Comparison across cell lines revealed significant quantitative differences in the promoter activity of individual *MNEI* constructs when transfected into different cells. This quantitative difference is shown for p180, which was the most active of these constructs in each of the cell lines. The highest promoter activity of p180, 106-fold enhancement relative to the pGL-3, was found when the construct was transfected into U937 cells, followed by 87-fold in K562 cells and 57-fold in HeLa cells (Fig. 1A). In contrast, negligible promoter activity (three to five fold enhancement relative to pGL-3) was found for *MNEI* construct p180 transfected into Daudi B-lymphoid cells (Fig. 1A). The positive control for Daudi cell transfection was the activity of Renilla luciferase driven by the TK promoter of the co-transfected plasmid pRL-TK. The finding that the *MNEI* constructs were active in the myeloid cell lines U937 and K562 and also in non-myeloid HeLa cells but inactive in Daudi cells is consistent with the known expression of MNEI protein in the former three cell lines and its absence in Daudi cells [Remold-O'Donnell et al., in preparation] (see also Discussion).

Localization of Regulatory Elements in the *MNEI* Promoter

The comparison of the luciferase activities of the six *MNEI* step-deletion plasmids within different transfected cell lines revealed an activity pattern that, with one exception, was similar for U937, K562, and HeLa cells (Fig 1B). In each of the three cell lines, p1000, the longest *MNEI* plasmid, showed near maximal promoter activity, and p800 showed decreased activity, suggesting that a positive acting element functional in the three cell lines is located between −1,000 and −800. Of these constructs, p180 showed the highest promoter activity, suggesting that major elements that control the *MNEI* promoter are located in the first 180 bp upstream of the transcription start site. Compared to p180, p100 showed markedly decreased activity, indicating that sequences critical for *MNEI* promoter activity are located between −180 and −100. The smallest construct, p40, which contains no identifiable motif other than a TATA-like sequence (TATAAGAG) at −22 bp, had low but reproducible activity in the three cell lines, suggesting that the TATA motif is functional. Negative acting elements are apparently present upstream of −180 (see decreased activity of p800 and p500 compared to p180); these were not further ex-

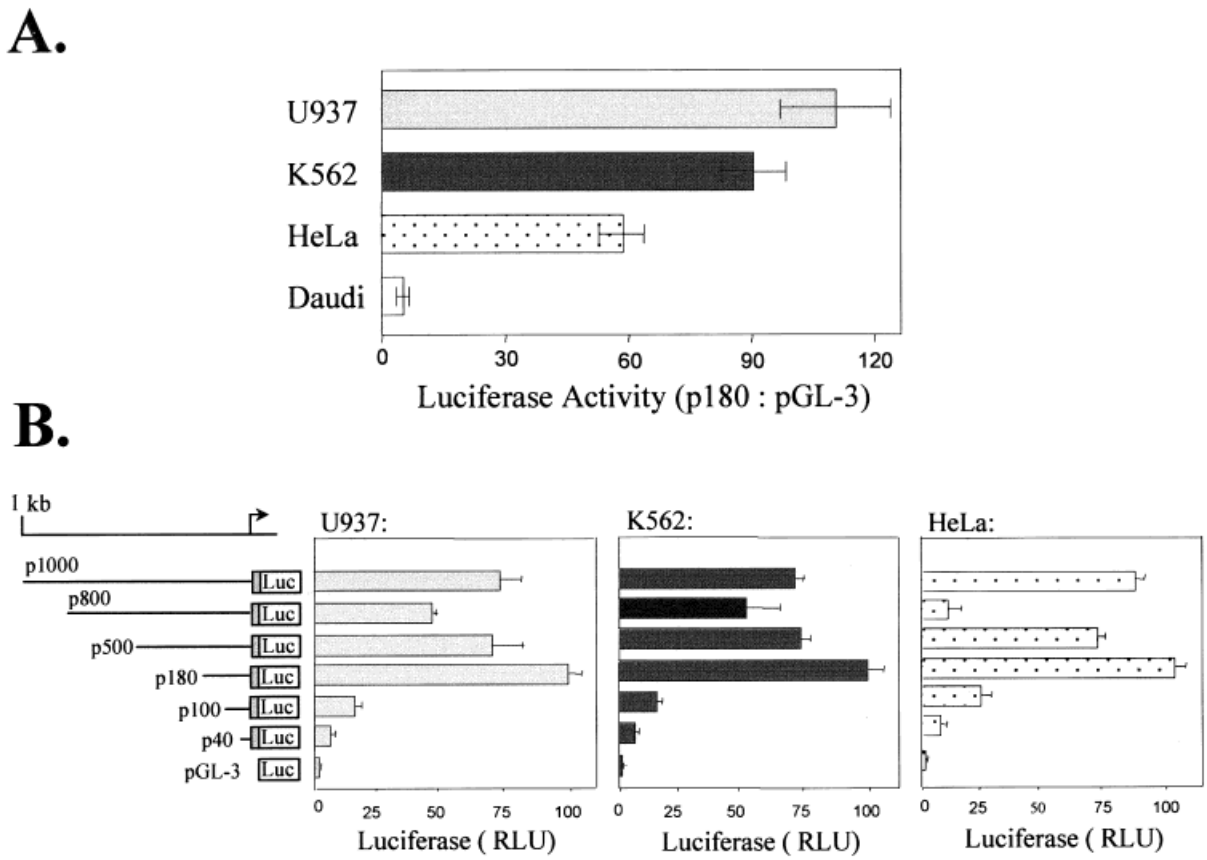

Fig. 1. Promoter function of *MNEI*-luciferase constructs in cells of different lineage. **A**: Quantitative comparison of one *MNEI* construct, p180 (described below), transiently transfected into U937 (myeloid), K562 (erythroid/myeloid), HeLa (epithelial-like), and Daudi (B-lymphoid) cells. Luciferase activities, assayed after 24 h (48 h for HeLa cells), are expressed as fold stimulation, i.e., the ratio of *MNEI*-p180 activity to the promoterless vector pGL3. Values are the mean ± SEM of eight to 12 experiments with duplicate transfections except for Daudi cells (four experiments). **B**: Comparative luciferase activity of a series of *MNEI* step-deletion constructs transiently transfected into U937 (left), K562 (middle), and HeLa cells (right). The *MNEI* regions encompassed by the plasmids begin at varying upstream sites shown schematically at the left and indicated by the name, e.g., p1000 begins at −1,000, and extends to +25 relative to the transcription start site. Luciferase activities are expressed as RLU (relative light units) ± SEM with the activity of p180 for each cell line set as 100 units. Results are the mean ± SEM of four to seven experiments with duplicate transfections.

amined. The single difference in the activity pattern for the three cell lines, the weaker activity of p800 in HeLa cells, was also not further examined. In the remainder of the study, we concentrated on characterizing the positive acting elements between −1,000 and −800 and between −180 and −100.

For detailed localization of the regulatory elements between −180 and −100, four additional step deletion plasmids, p162, p149, p138, p123, were generated. When transfected into U937, K562, and HeLa cells, decreased activity was found for p162 relative to p180 in all three cell lines (Fig. 2), indicating that a positive regulatory element that functions in the three cell lines is located in the −180 to −162 region. In contrast, the construct p138 was significantly more active than p100 in the myeloid cell lines U937 and K562 (Fig. 2, left), but p138 and p100 showed identical activity in HeLa cells (Fig. 2, right), indicating a regulatory element in this region active only in the myeloid cell lines. The myeloid cell regulatory element was further localized to the region between −138 and −123 bp by three-way comparison of the activities of p138, p123, and p100 in U937 and K562 cells (Fig. 2, left panel).

Characterization of PU.1/Spi-1 Regulation of *MNEI* Expression by an Element at −128

On searching the *MNEI* sequence from −138 to −123 for a myeloid-specific binding motif, we

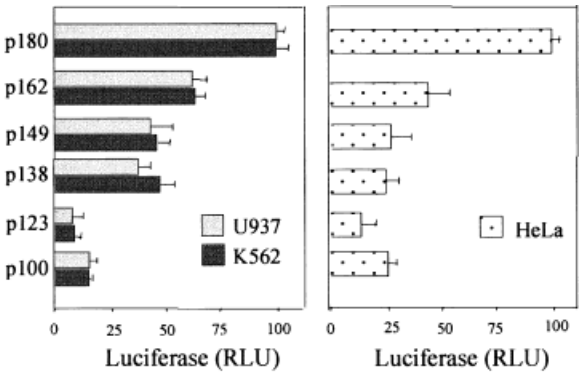

Fig. 2. Analysis of promoter activity of proximal region (−180 to −100 bp) step-deletion constructs. The indicated *MNEI*-luciferase constructs (named based on the number of nucleotides upstream of the transcription start site) were transiently transfected into U937 cells and K562 cells (**left**) and HeLa cells (**right**). Luciferase activities, the results of three experiments with duplicate transfections, are expressed as mean RLU ± SEM with p180 activity set as 100 units for each cell line. Note the decreased promoter activity of p162 relative to p180 in the three cell lines. Note that the promoter activity of p100 is less than p138 in U937 and K562 cells (left), but that p100 and p138 have equal activity in HeLa cells (right).

noted the sequence 5′-TTCT-3′ beginning at −128, which corresponds on the non-coding strand to a potential PU.1 binding element. PU.1/Spi1 has been identified as a major transcription factor in regulation of gene expression in myeloid cells [McKercher et al., 1996; Scott et al., 1994]. To characterize this putative element and its *trans*-acting binding factor(s) in myeloid cells, electrophoretic mobility shift assays (EMSAs) were performed. Radiolabeled double stranded oligonucleotide probe including the −128 putative element of *MNEI* (probe −138; sequence in Table 1) produced a major shifted band with U937 cell nuclear protein (Fig. 3A, lane 3) that had identical mobility to the shifted band of radiolabeled consensus PU.1 probe (probe $PU.1_{consensus}$; lane 1). Probe −138 produced no binding band with nuclear protein of HeLa cells (lane 7), which lack PU.1 [Galson and Housman, 1988; Kominato et al., 1995]. In competition experiments, the shifted band of probe −138 was abrogated by excess non-labeled self oligonucleotide and by excess $PU.1_{consensus}$ oligonucleotide (lanes 4 and 5), but was unaffected by excess oligonucleotide M-138 with mutation of the putative PU.1 motif (lane 6). The identity of the U937 cell-derived protein that binds to probe -138 was confirmed as PU.1 by quantitative supershift of

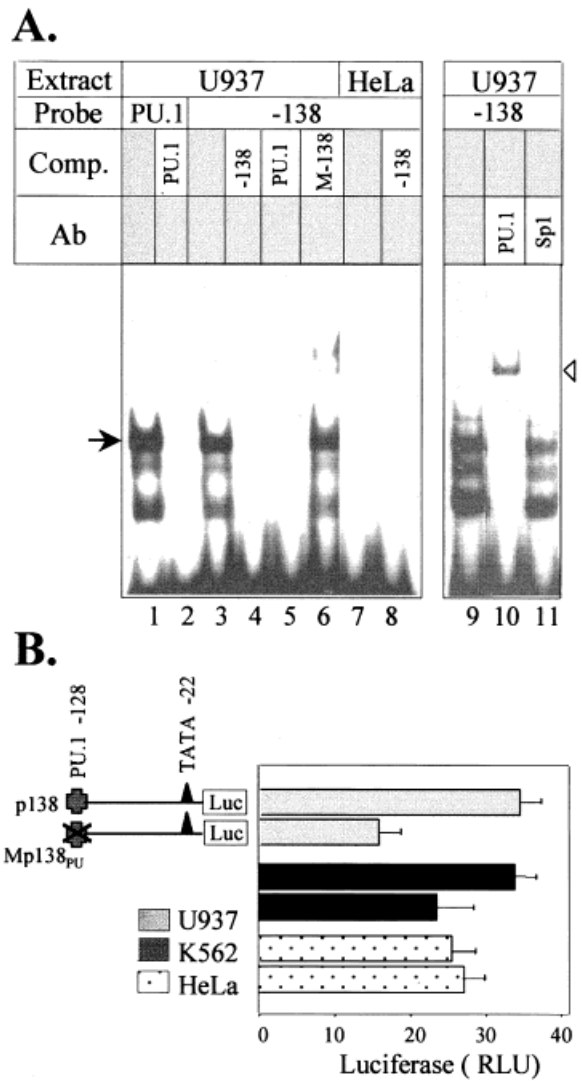

Fig. 3. Demonstration of binding of myeloid cell PU.1 to the *MNEI* promoter by EMSA and functional assay. **A**: EMSA demonstrates binding of PU.1 to *MNEI* probe −138. Shown in the **left panel** are U937 cell nuclear extracts incubated with $PU.1_{consensus}$ probe (lanes 1 and 2) or *MNEI* probe −138; (lanes 3–6) and HeLa cell nuclear extract incubated with probe −138 (lanes 7 and 8) without or with 500-fold excess non-labeled competitor oligonucleotides: $PU.1_{consensus}$ (lanes 2 and 5), −138 (lane 4), or M−138 in which the putative PU.1 binding element is mutated (lane 6). The arrow on the left indicates co-migrating mobility-shifted bands in lanes 1, 3, and 6, but not in lane 7. In the **right panel**, probe −138 was incubated with U937 nuclear extract without (lane 9) or with antibodies to PU.1 (lane 10) or Sp1 (lane 11). The arrowhead on the right indicates the supershifted band formed by PU.1 antibody. **B**: Mutation analysis. The *MNEI*-luciferase construct p138 and Mp138 generated by mutation of the PU.1 binding motif (Table 1) were transfected into U937, K562, and HeLa cells. Shown are mean luciferase activities (±SEM) of three experiments with duplicate transfections expressed as RLU (with p180 set as 100 units). Note that p138 containing the Wild-type PU.1 binding element showed greater promoter activity than Mp138 in U937 cells and K562 cells, but p138 and Mp138 had equal promoter activity in HeLa cells.

the band on incubation with PU.1 antibodies (lane 10), but not by Sp1 antibodies (lane 11).

To confirm the function of the PU.1 element, a plasmid, $Mp138_{PU}$, was generated from construct p138 with the same mutation as probe M-138 of the PU.1 core motif (Table 1) and was transiently transfected into the three cell lines. The luciferase activity of Mp138 was decreased relative to p138 in U937 cells and K562 cells (Fig. 3B). In contrast, the two constructs Mp138 and p138 were equally active in HeLa cells (S3 strain; Fig. 3B), which lack PU.1 [Kominato et al., 1995], confirming the presence of a PU.1 element at −128 that enhances *MNEI* promoter activity in myeloid cell lines.

Characterization of An Sp1 Element at −170 and Its Role in Regulation of *MNEI* Expression

Based on the greater luciferase activity of p180 relative to p162 in the three cell lines (Fig. 2), we designed an *MNEI* probe from −176 to −154 and performed gel shift assays to identify the element in this region responsible for promoter activity. The probe, −180, which includes a GC-rich sequence (potential Sp1 binding GC box) beginning at −170 (Table 1), produced a major shifted band with nuclear protein from K562 cells (Fig. 4A, lane 1), U937 cells (lane 2) and HeLa cells (lane 8), and these bands were abrogated by excess of self oligonucleotide (lanes 3 and 9) and $Sp1_{consensus}$ (lanes 4 and 10), but not by oligonucleotide M-180 with mutation at −164/162 of a potential GC box (lanes 5 and 11). Excess of $AP\text{-}2_{consensus}$ oligonucleotide, which is also a GC-rich sequence, partially decreased the binding of HeLa cell nuclear factor to the *MNEI* −180 probe (lane 12). Incubation with specific antibodies to Sp1 quantitatively supershifted the U937 band (lane 6) and HeLa cell band (lane 13), and antibodies to AP-2 had no effect (lanes

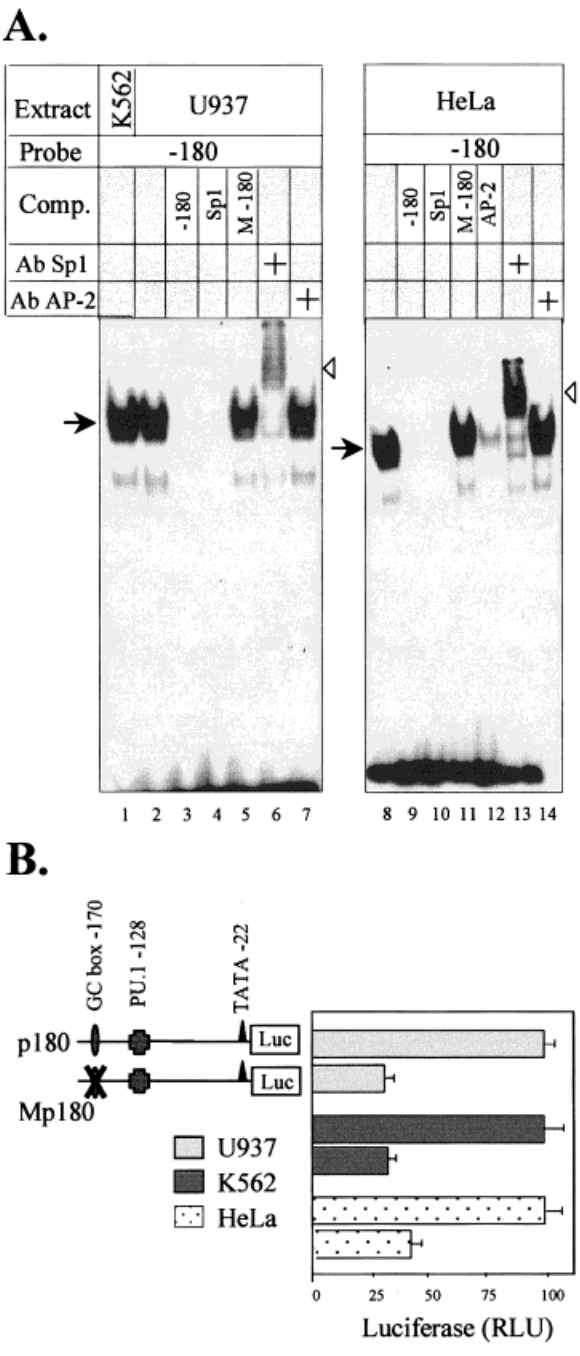

Fig. 4. Demonstration of Sp1 binding to a GC box at −170 bp by EMSA and functional assay. **A**: EMSA. Shown are nuclear extracts from K562 (lane 1), U937 (lanes 2–7), and HeLa cells (lanes 8–14) incubated with *MNEI* probe −180 without or with 500-fold excess non-labeled competitor oligonucleotide −180 (lanes 3 and 9), $Sp1_{consensus}$ (lanes 4 and 10), oligonucleotide M-180 with mutation of the putative GC box (lanes 5–11), or $AP\text{-}2_{consensus}$ (lane 12). The arrows on the left of the panels indicate the shifted bands. Note the abrogation of probe −180 shift by excess self and $Sp1_{consensus}$ and weak inhibition by excess $AP\text{-}2_{consensus}$. Similar findings were obtained with K562 extracts (data not shown). In supershift analysis, probe −180 was incubated with nuclear extracts of U937 (lanes 6 and 7) or HeLa cells (lanes 13 and 14) and antibodies to Sp1 (lanes 6 and 13) or AP-2 (lanes 7 and 14). The arrowheads at the right of the panels indicate the supershifted bands formed with Sp1 antibody. **B**: Mutation analysis. The *MNEI*-luciferase construct p180 and the mutated construct Mp180 were transfected into U937, K562, and HeLa cells. Shown are mean luciferase activities ± SEM of three experiments expressed as RLU as in Figures 1B, 2, 3. Note that Mp180 in which the GC box is mutated showed decreased promoter activity in the three cell lines.

7 and 14), verifying the identity of the *trans*-acting factor that binds the −170 *MNEI* element as Sp1.

To confirm the function of the Sp1 binding site at −170, a plasmid, Mp180, was generated from p180 with the same mutation as oligonucleotide M-180. When transiently transfected into U937, K562, and HeLa cells, Mp180 generated decreased luciferase activity relative to p180 in all three cell lines (Fig. 4B), verifying the existence of a GC box binding, *trans*-active, protein Sp1 that contributes to regulation of full activity of the *MNEI* promoter in both myeloid and non-myeloid cells.

Characterization of Second Sp1 Element at −66 and Its Role in Regulation of *MNEI* Expression

Sequence analysis of the proximal 5′ flanking region revealed another putative regulatory element upstream of the TATA box. This sequence, which consists of two overlapping inverted GC-box motifs within a GC-rich region, begins at −66 bp, a common location for functional GC boxes. Although a construct containing this motif and the TATA box had only ≈20% of full promoter activity (Fig. 1B), we nonetheless tested the region by electrophoretic mobility shift assay for regulatory activity. Probe −66 encompassing −73 to −46 bp (Table 1) produced a major shifted band with nuclear protein from U937 cells (Fig. 5A, lane 1), which was abrogated by excess self (lane 2) and excess $Sp1_{consensus}$ (lane 3). Three oligonucleotides with mutations of the GC box were tested; 1M-66 was partially effective, and 2M-66 and 3M-66 were ineffective, in preventing nuclear factor binding to the −66 probe (lanes 4–6). Similar results were obtained for HeLa cell nuclear extracts, except that all three mutated oligonucleotides failed to inhibit nuclear factor binding to the *MNEI* −66 probe (data not shown). The identity of the U937/HeLa cell-derived protein that binds probe −66 was confirmed as Sp1 by the demonstration that Sp1 antibodies, but not AP-2 or PU.1 antibodies, quantitatively supershifted the band (lanes 7–9).

Three plasmids were generated in which the same mutations of the proximal GC box core sequence at −66 bp were introduced in p138 (sequences in Table 1). The three mutated plasmids, particularly $2Mp138_{GC}$ and $3Mp138_{GC}$, showed remarkably decreased luciferase activity relative to Wild-type p138 when transfected

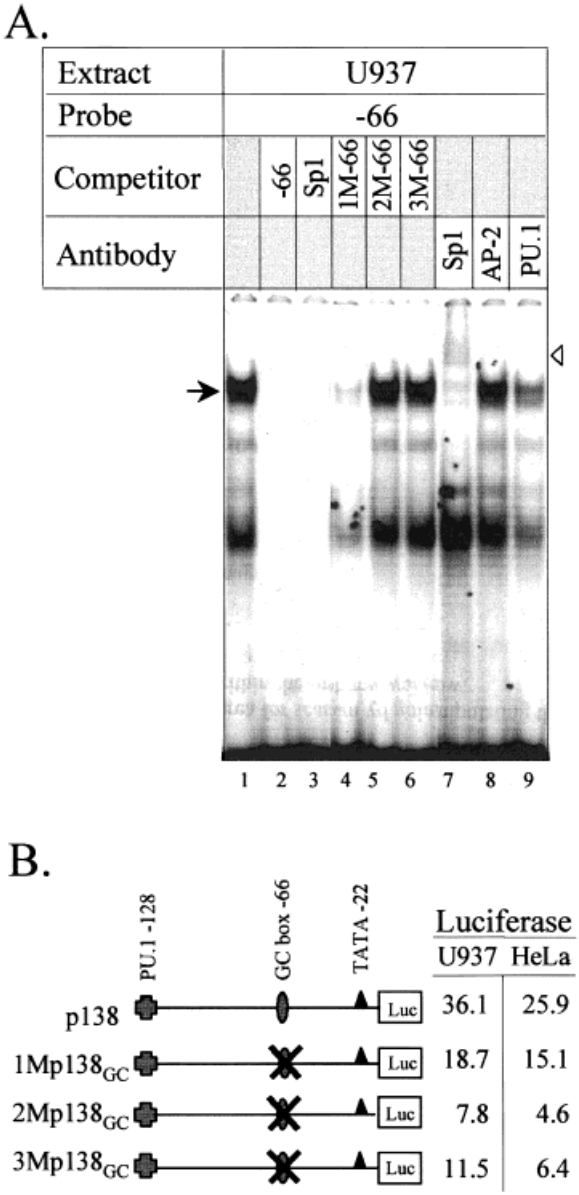

Fig. 5. Demonstration of Sp1 binding to a proximal GC box at −66 by EMSA and functional analysis. **A**: EMSA. U937 nuclear extract was incubated with probe −66 without (lane 1) or with 500-fold excess non-labeled -66 (lane 2), $Sp1_{consensus}$ (lane 3) or the mutated oligonucleotides 1M-66, 2M-66, 3M-66 (defined in Table 1; lanes 4–6). The arrow on the left indicates the shifted band in lanes 1, 5, and 6. Supershift analysis shows the effect of antibodies to Sp1 (lane 7, supershift band indicated by arrowhead on the right), AP-2 (lane 8, no effect), and PU.1 (lane 9, no effect). **B**: Mutation analysis. U937 and HeLa cells were transfected with the Wild-type construct p138 and the constructs $1Mp138_{GC}$, $2Mp138_{GC}$, and $3Mp138_{GC}$ with mutations (Table 1) of the GC box at −66. Luciferase activities (mean ± SEM of three experiments) are expressed as RLU with the p180 values for each cell line as 100 units.

into U937 and HeLa cells (Fig. 5B), suggesting that interaction of Sp1 with this GC box is important for *MNEI* promoter activity in both myeloid and non-myeloid lines. These findings indicate that the proximal Sp1 element at −66 has substantial impact on promoter function.

Characterization of NF-κB Regulation of *MNEI* Expression by an Element at −821

To characterize the distal positive acting element between −1,000 to −800 (Fig. 1B), an additional Wild-type plasmid, p829, was constructed, which included a putative NF-κB motif not present in p800. When these constructs were transfected into U937 and HeLa cells, luciferase activity was more than doubled for p829 compared to p800 to the highest level of all *MNEI* constructs (20% higher than p180; Fig. 6A). Mutational analysis was also performed by generation of a plasmid Mp829 by mutation of the NF-κB motif, which begins at −821. On transfection, luciferase activity was markedly decreased for Mp829 relative to p829 in U937 cells and HeLa cells (Fig. 6A), strongly suggesting the presence of a functional NFκB-like element.

NF-κB is the prototype of a family of transcription factors important in coordinate control of gene expression, the induction of which involves conversion of inactive cytosolic precursors to active dimers that translocate to the nucleus [Lenardo and Baltimore, 1989]. The apparent effect of an NF-κB element of *MNEI* to increase luciferase reporter gene expression was, in one respect, surprising because the transfected cells had not been treated with an inducing stimulus. As the first step toward identifying the factor that binds and controls the −821 *MNEI* element, we performed electrophoretic mobility assays with nuclear extracts prepared from U937 cells that had either been left unstimulated or treated with lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, which is known to induce NF-κB in monocytic cells [Donald et al., 1995; Mackman et al., 1991; Pomerantz et al., 1990]. When incubated with NF-κB consensus probe, nuclear extracts from untreated and LPS-treated U937 cells both produced a prominent shifted band (Fig. 6B, lanes 1–3), suggesting that the U937 cells used in this study contain an active NF-κB like molecule within their nuclei. Nuclear extracts from untreated K562 cells also contained a factor that bound NF-κB consensus probe (data not shown).

To perform mobility shift assays with the specific *MNEI* probe −829, we used nuclear extracts from another monocyte-like cell line, THP-1, for which expression of active NF-κB has been shown to be LPS-sensitive [Donald et al., 1995; Mackman et al., 1991]. When incubated with *MNEI* probe −829, nuclear extracts from unstimulated THP-1 cells failed to produce a shifted band (Fig. 6B, lane 4), but extracts of LPS-treated THP-1 cells produced a dose-dependent broad shifted band, possibly representing multiple components (lanes 5 and 6). Formation of the shifted *MNEI* −829 band was abrogated by excess of self oligonucleotide (lane 7) and NF-κB$_{consensus}$ (lane 8), but not by M-829 oligonucleotide with the same mutation as Mp829 of the NF-κB core sequence (lane 9). Moreover, the protein complex of the *MNEI*−829 probe was supershifted on incubation with antibodies to NF-κB p50 (lane 10) and partially supershifted on incubation with NF-κB p65 antibodies (lane 11), confirming that the transcription factor in LPS-treated THP-1 nuclear extracts that binds the *MNEI* −821 element is NF-κB or an NF-κB family member.

DISCUSSION

We present here data identifying four functional transcription factor binding elements in the promoter of human *MNEI*. A regulatory element controlled by transcription factor PU.1/Spi-1 and two non-contiguous elements controlled by Sp1 were identified within 180 bp immediately upstream of the transcription start site. An additional regulatory site located further upstream at −821 was also identified, which is controlled by NF-κB.

On transient transfection of *MNEI*-luciferase constructs, promoter activity was highest in U937 cells, a monocyte-like line, followed by K562 cells, a less mature line chronic myelogenous leukemia cell line capable of myeloid, erythroid, or megakaryocytic differentiation [Andersson et al., 1979; Cheng et al., 1994; Lozzio and Lozzio, 1975; Yu et al., 1989]. Lesser, but still substantial, promoter activity was noted on transfection of the *MNEI* constructs into HeLa cervical carcinoma cells, indicating that the *MNEI* promoter, although most active in myeloid cells, is clearly not myeloid-specific. These findings are consistent

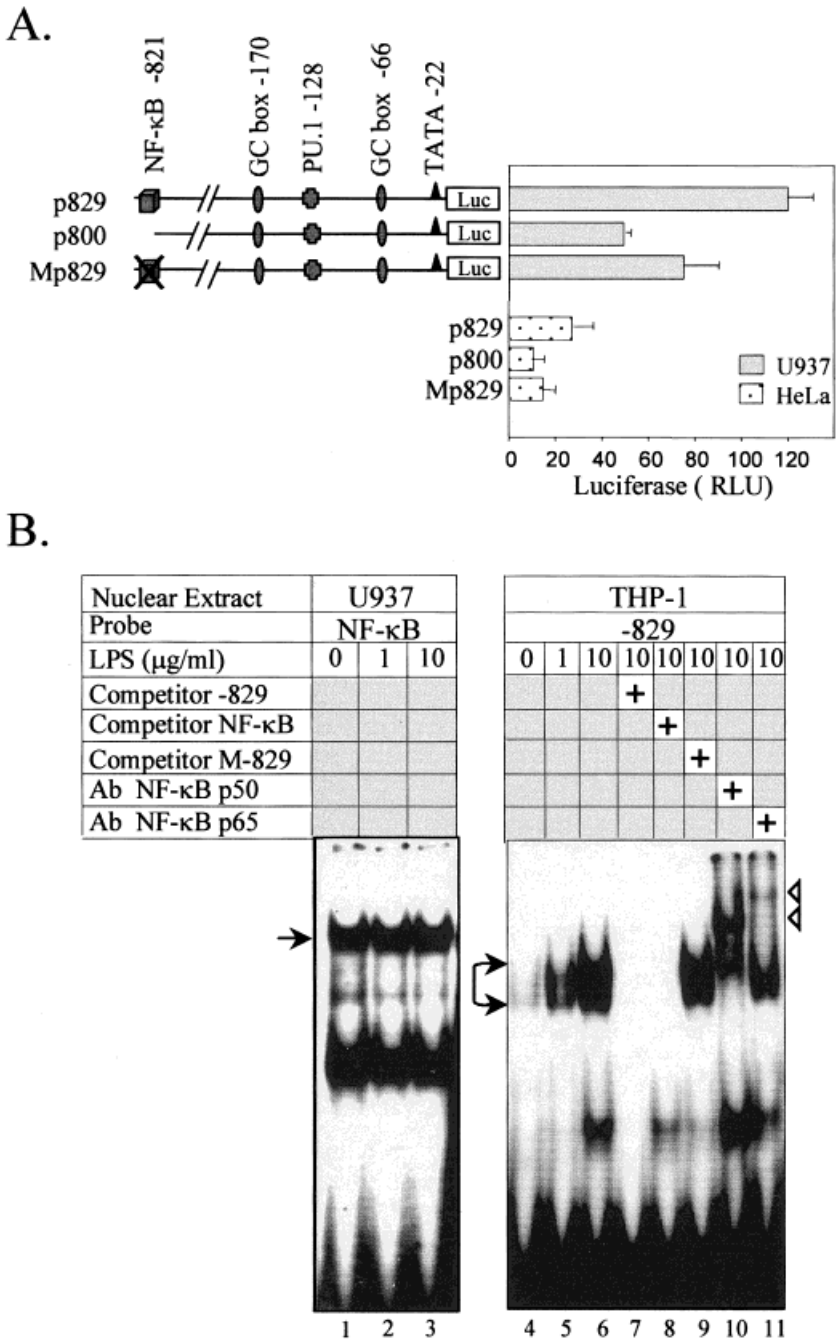

Fig. 6. Demonstration of NF-κB binding to a distal *MNEI* element by mutation analysis and EMSA. **A**: Mutation analysis. U937 and HeLa cells were transfected with p829, p800, and the mutated construct Mp829. Note that the luciferase activity (RLU, three experiments) of p829 containing the putative NF-κB motif is greater than p800 and Mp829 in both cell lines. Note also that in U937 cells p829 activity is greater than p180, which was set as 100 RLU. **B**: EMSA. In the left panel, nuclear extracts prepared from U937 cells that had been incubated for 2 h without (lane 1) or with LPS at 1 μg/ml (lane 2) or 10 μg/ml (lane 3), were incubated with probe NF-κB$_{consensus}$. The arrow on the left indicates the shifted NF-κB complex formed with nuclear protein from untreated and treated U937 cells. In the right panel, nuclear extracts prepared from THP-1 cells that had been incubated for 2 h without (lane 4) or with LPS at 1 μg/ml (lane 5) or 10 μg/ml (lanes 6–11) were incubated with *MNEI* probe −829 without or with 500-fold excess non-labeled −829 (lane 7), NF-κB$_{consensus}$ (lane 8), or M-829 in which the putative NF-κB element is mutated (lane 9). Note the absence in lane 4 and appearance in lanes 5 and 6 of a shifted band of −829 complex (arrow on the left of the panel). The identity of the nuclear factor in LPS-treated THP-1 cells that binds to *MNEI* probe -829 was confirmed as NF-κB by supershift of the band to new mobility positions by NF-κB p50 antibodies (lane 10) and NF-κB p65 antibodies (lane 11; supershift bands indicated by arrowheads on the right).

with the protein and RNA expression patterns recently documented for *MNEI* (summarized below). At the protein level, MNEI functions as a potent and highly specific inhibitor of the neutrophil azurophil granule proteases, elastase, cathepsin-G, and proteinase-3, all of which are tightly regulated gene products specific to early myelomonocytic cells [Fouret et al., 1989; Grisolano et al., 1994; Zimmer et al., 1992]. Thus, from the perspective of coordinated understanding of *MNEI* expression and function, there is particular need to study control exerted by the *MNEI* promoter in the context of myeloid cells.

Many characterized myeloid genes lack a TATA box, including granulocyte-macrophage colony stimulating factor receptor α (GM-CSF-R) [Hohaus et al., 1995], macrophage colony-stimulating factor receptor (M-CSFR) [Zhang et al., 1993], $p47^{phox}$ [Li et al., 1997] and Bruton's tyrosine kinase (Btk) [Himmelmann et al., 1996]. The *MNEI* promoter contains a TATA sequence located at a common position, −22 bp relative to the transcription start site [Zeng et al., 1998], and in this respect, it resembles the myeloid genes that contain a TATA box. These include IL1B, most abundantly expressed by monocytes [Kominato et al., 1995], as well as myeloperoxidase [Nuchprayoon et al., 1994], and the genes encoding the MNEI target proteases, neutrophil elastase [Oelgeschlager et al., 1996], proteinase-3 [Sturrock et al., 1992], and cathepsin-G [Grisolano et al., 1994]. Luciferase reporter gene expression was observed on transfection of a minimal *MNEI* construct, p40, that includes the TATA motif (Fig. 1B), strongly suggesting that the *MNEI* TATA sequence is functional. As previously noted, the TATA box and flanking sequence of *MNEI* is identical in 13 of 14 positions to the corresponding region of the genes for two MNEI target proteases, neutrophil elastase, and proteinase-3 [Zeng et al., 1998]. The implications of this correlation are unknown.

The transcription factor PU.1/Spi-1 is an Ets domain protein characterized as a major regulator of myeloid gene expression and restricted in expression primarily to myeloid and B cells [Galson et al., 1993; McKercher et al., 1996; Scott et al., 1994]. The present study identified a functional PU.1 motif in the *MNEI* promoter located at −128. An *MNEI* construct that includes the PU.1 motif increased luciferase reporter gene expression in U937 cells and K562 cells, indicating that the PU.1 element of *MNEI* is functional in early and mature myeloid cells. In contrast, the inclusion of the *MNEI* PU.1 element failed to increase transcriptional activity in HeLa cells, which are known to lack PU.1. In addition, an *MNEI* probe that included the PU.1 sequence bound a nuclear protein of U937 and K562 cells, but failed to bind HeLa cell nuclear proteins, confirming that the *MNEI* PU.1 motif at −128 is a myeloid-specific functional element. These findings, together with the ≈50% reporter gene expression activity remaining when the mutated construct $Mp138_{PU}$ is transfected into U937 and K562 (Fig. 3B), strongly suggest that the transcription factor PU.1 contributes to, but is not absolutely required for, *MNEI* expression in myeloid cells.

Another promoter, which like *MNEI*, is regulated by, but not absolutely dependent on, PU.1, is *Bruton's tyrosine kinase (Btk)* [Himmelmann et al., 1996; Muller et al., 1996]. *Btk* promoter constructs were transcriptionally active in K562 myeloid cells, B cell lines and, at lower levels, in HeLa cells [Himmelmann et al., 1996]. Consistent with these findings, *Btk* is expressed in fetal liver cells of PU.1 gene-deleted mice, but its levels are decreased several fold relative to Wild-type mice [Muller et al., 1996].

The most frequent PU.1/Spi-1 binding motif contains the core sequence 5′-GGAA-3′ [Ray-Gallet et al., 1995]. Less frequently, myeloid promoters have been identified with the PU.1 core sequence, 5′-AGAA-3′, for example, *IL-1B* [Kominato et al., 1995] and *CD11b* [Pahl et al., 1993]. The *MNEI* 5′-TTCT-3′ core sequence corresponds to the last named PU.1 sequence in inverted orientation.

The transcription factor Sp1 is universally and preferentially expressed in hematopoietic cells [Tenen et al., 1997]. Recent studies increasingly document the contribution of Sp1 to regulation of tissue-specific expression of TATA-less myeloid genes [Chen et al., 1993; Eichbaum et al., 1997; Zhang et al., 1994] in which case Sp1 interacts through co-activators with the basal transcription apparatus [Pugh and Tjian, 1990]. Sp1 was also identified as an enhancer in the neutrophil elastase and proteinase-3 genes [Nuchprayoon et al., 1999]. In the *MNEI* promoter, two GC box motifs with functional Sp1 binding activity were identified

in the 180 bp proximal region, one upstream and one downstream of the PU.1 element. *MNEI* reporter constructs that included the upstream Sp1 binding site at −170 supported robust *MNEI* promoter activity. The proximal Sp1 binding site at −66 bp did not support strong transcriptional activity in the absence of upstream elements; however, mutation and deletion of this element indicated that this region also is important for *MNEI* promoter activity.

Another regulatory element identified in the *MNEI* promoter is NF-κB, located far upstream of the transcription start site at −821 and separated from the cluster of proximal elements, $Sp1_{-170}$, $PU.1_{-128}$, $Sp1_{-66}$, and TATA, by apparent negative regulatory elements that decrease *MNEI* promoter activity (Fig. 1B). Characterized NF-κB elements consist of dissimilar pentameric half sites [Urban et al., 1991]; the −821 *MNEI* site has the sequence 5′-GGGGAATTTCA-3′. Inclusion of this site in a transfected *MNEI* construct increased luciferase expression by U937 cells more than twofold to the highest level of all *MNEI* constructs (p829, Fig. 6A).

Treatment of THP-1 monocyte-like cells with LPS, a complex glycolipid of gram-negative bacteria, revealed stringently regulated activation and binding of NF-κB family protein(s) to the −821 *MNEI* element. The protein component of the complex consists, at least in part, of NF-κB p50/p65 heterodimers as it was supershifted by either p50 or p65 antibodies. Cumulatively, these findings suggest that *MNEI* expression is increased in response to gram-negative bacterial infection by the action of LPS to activate an NF-κB family member and induce its binding to the −821 element.

Regulation of *MNEI* promoter activity in myeloid cells in response to LPS is interesting in view of the recent study in mice demonstrating the critical role of the MNEI target, neutrophil elastase, in the response to bacterial infection. Specifically, mice depleted at the gene level of neutrophil elastase were found to be hypersensitive to gram-negative bacterial infection [Belaaouaj et al., 1998]. These findings suggest that the innate response of myeloid cells to LPS-expressing bacteria includes not only destruction of the bacteria by neutrophil elastase, but also increased activity of the *MNEI* promoter to ensure sufficient MNEI for inhibition of excess elastase. Indeed, substantially increased levels of MNEI are found in airway fluids of patients with cystic fibrosis [Remold-O'Donnell et al., unpublished findings], suggesting that production and/or release of MNEI is enhanced as part of the local response to infection and inflammation.

MNEI was discovered and characterized based on its elastase inhibitory activity as a product of monocytes, macrophages and neutrophils, where it is present at high levels, constituting ≥0.1% of total protein [Remold-O'Donnell, 1985]. The present study indicates that the promoter activity of *MNEI* is not specific to these cells. Indeed, findings obtained on examining MNEI protein expression with recently developed reagents are completely consistent with the promoter findings. MNEI protein was detected by Western blot, not only in myeloid cells, but also in HeLa and other cell lines and in platelets and primary T lymphocytes and B-lymphocytes [Remold-O'Donnell et al., manuscript in preparation]. In the same study, Daudi cells, a Burkitt lymphoma cell line [Klein et al., 1968], in contrast to primary B lymphocytes, were negative for *MNEI* expression by Western and Northern blots, consistent with the absence of transcriptional activity when *MNEI*-luciferase constructs were transfected into Daudi cells (Fig. 1). In the single study in non-myeloid cells, MNEI released from tumor cells was shown to be responsible for immunomodulatory activity toward infiltrating lymphocytes [Packard et al., 1995], strongly suggesting that investigation of the regulation and functional role of MNEI in non-myeloid cells is also warranted.

Overall, this analysis contributes toward understanding physiological regulation and action of *MNEI* by identifying three proximal region transcription factor binding elements, Sp1, PU.1, and Sp1, that control short distance promoter function and comprise the minimal regulatory region. The fourth identified transcription factor binding element, NF-κB, located further upstream at −821, was shown to mediate enhanced *MNEI* promoter activity in monocytic cells in response to LPS, suggesting increased expression of *MNEI* in inflammatory reactions. The identification of PU.1, Sp1, and NF-κB as regulatory elements in the *MNEI* promoter suggests that this anti-inflammatory gene is controlled by the same transcriptional factors prominent in inflammatory responses. These data will be the basis for correlating promoter activity with altered *MNEI* expres-

sion in physiological and pathological settings and in the context of pharmacological treatments of inflammation.

ACKNOWLEDGMENTS

We thank Philip E. Auron, Nawarat Wara-Aswapati, Alla Tsytsykova, and Anne Sturrock for advice, helpful suggestions and reagents; we are grateful to Drs. Auron, Alvin E. Davis, III, and Anne E. Goldfeld, for critical evaluation of the manuscript. Hong Gao and Jessica Cooley are thanked for help with experiments and generation of figures.

REFERENCES

Andersson LC, Jokinen M, Gahmberg CG. 1979. Induction of erythroid differentiation in the human leukaemia cell line K562. Nature 278:364–365.

Andrews NC, Faller DV. 1991. A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. Nucleic Acids Res 19:2499.

Baum C, Forster P, Hegewisch-Becker S, Harbers K. 1994. An optimized electroporation protocol applicable to a wide range of cell lines. Biotechniques 17:1058–1062.

Belaaouaj A, McCarthy R, Baumann M, Gao Z, Ley TJ, Abraham SN, Shapiro SD. 1998. Mice lacking neutrophil elastase reveal impaired host defense against gram negative bacterial sepsis. Nat Med 4:615–618.

Chen HM, Pahl HL, Scheibe RJ, Zhang DE, Tenen DG. 1993. The Sp1 transcription factor binds the CD11b promoter specifically in myeloid cells in vivo and is essential for myeloid-specific promoter activity. J Biol Chem 268: 8230–8239.

Cheng T, Wang Y, Dai W. 1994. Transcription factor egr-1 is involved in phorbol 12-myristate 13- acetate-induced megakaryocytic differentiation of K562 cells. J Biol Chem 269:30848–30853.

Dignam JD, Lebovitz RM, Roeder RG. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res 11:1475–1489.

Donald R, Ballard DW, Hawiger J. 1995. Proteolytic processing of NF-kappa B/I kappa B in human monocytes. ATP- dependent induction by pro-inflammatory mediators. J Biol Chem 270:9–12.

Doring G. 1994. The role of neutrophil elastase in chronic inflammation. Am J Respir Crit Care Med 150:S114–117.

Eichbaum Q, Heney D, Raveh D, Chung M, Davidson M, Epstein J, Ezekowitz RAB. 1997. Murine macrophage mannose receptor promoter is regulated by the transcription factors PU.1 and SP1. Blood 90:4135–4143.

Evans E, Cooley J, Remold-O'Donnell E. 1995. Characterization and chromosomal localization of ELANH2, the gene encoding human monocyte/neutrophil elastase inhibitor. Genomics 28:235–240.

Fouret P, du Bois RM, Bernaudin JF, Takahashi H, Ferrans VJ, Crystal RG. 1989. Expression of the neutrophil elastase gene during human bone marrow cell differentiation. J Exp Med 169:833–845.

Galson DL, Housman DE. 1988. Detection of two tissue-specific DNA-binding proteins with affinity for sites in the mouse beta-globin intervening sequence 2. Mol Cell Biol 8:381–392.

Galson DL, Hensold JO, Bishop TR, Schalling M, D'Andrea AD, Jones C, Auron PE, Housman DE. 1993. Mouse beta-globin DNA-binding protein B1 is identifical to a proto-oncogene, the transcription factor Spi-1/PU.1, and is restricted in expression to hematopoietic cells and the testis. Mol Cell Biol 13:2929–2941.

Grisolano JL, Sclar GM, Ley TJ. 1994. Early myeloid cell-specific expression of the human cathepsin G gene in transgenic mice. Proc Natl Acad Sci U S A 91:8989–8993.

Himmelmann A, Thevenin C, Harrison K, Kehrl JH. 1996. Analysis of the Bruton's tyrosine kinase gene promoter reveals critical PU.1 and SP1 sites. Blood 87:1036–1044.

Hohaus S, Petrovick MS, Voso MT, Sun Z, Zhang DE, Tenen DG. 1995. PU.1 (Spi-1) and C/EBP alpha regulate expression of the granulocyte-macrophage colony-stimulating factor receptor alpha gene. Mol Cell Biol 15:5830–5845.

Johnson RJ, Couser WG, Alpers CE, Vissers M, Schulze M, Klebanoff SJ. 1988. The human neutrophil serine proteinases, elastase and cathepsin G, can mediate glomerular injury in vivo. J Exp Med 168:1169–1174.

Klein E, Klein G, Nadkarni JS, Nadkarni JJ, Wigzell H, Clifford P. 1968. Surface IgM-kappa specificity on a Burkitt lymphoma cell in vivo and in derived culture lines. Cancer Res 28:1300–1310.

Kominato Y, Galson DL, Waterman WR, Webb AC, Auron PE. 1995. Monocyte expression of the human prointerleukin 1 beta gene (IL1B) is dependent on promoter sequences which bind the hematopoietic transcription factor Spi-1/PU.1. Mol Cell Biol 15:58–68.

Kordula T, Dubin A, Schooltink H, Koj A, Heinrich PC, Rose-John S. 1993. Molecular cloning and expression of an intracellular serpin: An elastase inhibitor from horse leucocytes. Biochem J 293:187–193.

Lenardo MJ, Baltimore D (1989): NF-kappa B: A pleiotropic mediator of inducible and tissue-specific gene control. Cell 58:227–229.

Li SL, Valente AJ, Zhao SJ, Clark RA. 1997. PU.1 is essential for p47(phox) promoter activity in myeloid cells. J Biol Chem 272:17802–17809.

Lozzio CB, Lozzio BB. 1975. Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. Blood 45:321–334.

Mackman N, Brand K, Edgington TS. 1991. Lipopolysaccharide-mediated transcriptional activation of the human tissue factor gene in THP-1 monocytic cells requires both activator protein 1 and nuclear factor kappa B binding sites. J Exp Med 174:1517–1527.

McKercher SR, Torbett BE, Anderson KL, Henkel GW, Vestal DJ, Baribault H, Klemsz M, Feeney AJ, Wu GE, Paige CJ, Maki RA. 1996. Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. Embo J 15:5647–5658.

Muller S, Sideras P, Smith CI, Xanthopoulos KG. 1996. Cell specific expression of human Bruton's agammaglobulinemia tyrosine kinase gene (Btk) is regulated by Sp1- and Spi-1/PU.1-family members. Oncogene 13:1955–1964.

Nuchprayoon I, Meyers S, Scott LM, Suzow J, Hiebert S, Friedman AD. 1994. PEBP2/CBF, the murine homolog of the human myeloid AML1 and PEBP2 beta/CBF beta proto-oncoproteins, regulates the murine myeloperoxidase and neutrophil elastase genes in immature myeloid cells. Mol Cell Biol 14:5558–5568.

Nuchprayoon I, Shang J, Simkevich CP, Luo M, Rosmarin AG, Friedman AD. 1999. An enhancer located between the neutrophil elastase and proteinase 3 promoters is activated by Sp1 and an Ets factor. J Biol Chem 274: 1085–1091.

Oelgeschlager M, Nuchprayoon I, Luscher B, Friedman AD. 1996. C/EBP, c-Myb, and PU.1 cooperate to regulate the neutrophil elastase promoter. Mol Cell Biol 16:4717–4725.

Packard BZ, Lee SS, Remold-O'Donnell E, Komoriya A. 1995. A serpin from human tumor cells with direct lymphoid immunomodulatory activity: mitogenic stimulation of human tumor-infiltrating lymphocytes. Biochim Biophys Acta 1269:41–50.

Pahl HL, Scheibe RJ, Zhang DE, Chen HM, Galson DL, Maki RA, Tenen DG. 1993. The proto-oncogene PU.1 regulates expression of the myeloid-specific CD11b promoter. J Biol Chem 268:5014–5020.

Pomerantz RJ, Feinberg MB, Trono D, Baltimore D. 1990. Lipopolysaccharide is a potent monocyte/macrophage-specific stimulator of human immunodeficiency virus type 1 expression. J Exp Med 172:253–261.

Pugh BF, Tjian R. 1990. Mechanism of transcriptional activation by Sp1: evidence for coactivators. Cell 61: 1187–1197.

Ray-Gallet D, Mao C, Tavitian A, Moreau-Gachelin F. 1995. DNA binding specificities of Spi-1/PU.1 and Spi-B transcription factors and identification of a Spi-1/Spi-B binding site in the c-fes/c-fps promoter. Oncogene 11: 303–313.

Rees DD, Rogers RA, Cooley J, Mandle RJ, Kenney DM, Remold-O'Donnell E. 1999. Recombinant human Monocyte/Neutrophil elastase inhibitor protects rat lungs against injury from cystic fibrosis airway secretions. Am J Respir Cell Mol Biol 20:69–78.

Remold-O'Donnell E. 1985. A fast-acting elastase inhibitor in human monocytes. J Exp Med 162:2142–2155.

Remold-O'Donnell E, Chin J, Alberts M. 1992. Sequence and molecular characterization of human monocyte/neutrophil elastase inhibitor. Proc Natl Acad Sci U S A 89:5635–5639.

Remold-O'Donnell E, Cooley J, Mandle R. 1998. MNEI is an efficient inhibitor of neutrophil proteases. Am J Resp Crit Care Med 157:606A.

Remold-O'Donnell E, Nixon JC, Rose RM. 1989. Elastase inhibitor. Characterization of the human elastase inhibitor molecule associated with monocytes, macrophages, and neutrophils. J Exp Med 169:1071–1086.

Scott EW, Simon MC, Anastasi J, Singh H. 1994. Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. Science 265:1573–1577.

Sturrock AB, Franklin KF, Rao G, Marshall BC, Rebentisch MB, Lemons RS, Hoidal JR. 1992. Structure, chromosomal assignment, and expression of the gene for proteinase-3. The Wegener's granulomatosis autoantigen. J Biol Chem 267:21193–21199.

Sugimori T, Cooley J, Hoidal JR, Remold-O'Donnell E. 1995. Inhibitory properties of recombinant human monocyte/neutrophil elastase inhibitor. Am J Respir Cell Mol Biol 13:314–322.

Sundstrom C, Nilsson K. 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int J Cancer 17:565–577.

Tenen DG, Hromas R, Licht JD, Zhang DE. 1997. Transcription factors, normal myeloid development, and leukemia. Blood 90:489–519.

Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K. 1980. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int J Cancer 26:171–176.

Urban MB, Schreck R, Baeuerle PA. 1991. NF-kappa B contacts DNA by a heterodimer of the p50 and p65 subunit. Embo J 10:1817–1825.

Yu G, Smithgall TE, Glazer RI. 1989. K562 leukemia cells transfected with the human c-fes gene acquire the ability to undergo myeloid differentiation. J Biol Chem 264: 10276–10281.

Zeng W, Silverman GA, Remold-O'Donnell E. 1998. Structure and sequence of human M/NEI (monocyte/neutrophil elastase inhibitor), an Ov-serpin family gene. Gene 213:179–187.

Zhang DE, Hetherington CJ, Chen H-M, Tenen DG. 1993. The macrophage transcription factor PU.1 directs tissue-specific expression of the macrophage colony-stimulating factor receptor. Mol Cell Biol 14:373–381.

Zhang DE, Hetherington CJ, Tan S, Dziennis SE, Gonzalez DA, Chen HM, Tenen DG. 1994. Sp1 is a critical factor for the monocytic specific expression of human CD14. J Biol Chem 269:11425–11434.

Zimmer M, Medcalf RL, Fink TM, Mattmann C, Lichter P, Jenne DE. 1992. Three human elastase-like genes coordinately expressed in the myelomonocyte lineage are organized as a single genetic locus on 19pter. Proc Natl Acad Sci U S A 89:8215–8219.