Transcriptional and Posttranscriptional Modulation of Human Neutrophil Elastase Gene Expression

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Human neutrophil elastase (NE), a 29-Kd potent serine protease stored in azurophilic granules of mature neutrophils, is coded for by the NE gene, a single copy gene with 5 exons spanning a 6-kb segment of chromosome 11 at q14. With the knowledge that the NE gene expression is limited to early myeloid cell differentiation, mechanisms modulating expression of the NE gene were evaluated in the HL-60 promyelocytic leukemia cell line, a model of early bone marrow precursor cells. Consistent with the presence of NE messenger RNA (mRNA) transcripts in undifferentiated HL-60 cells, nuclear transcription run-on analyses showed that HL-60 cells actively transcribed the NE gene. However, the transcription rate of the NE gene was relatively low, only 40% of the myeloperoxidase gene, a gene expressed in parallel with NE. When induced toward the mononuclear phagocytic lineage with phorbol 12-myristate 13-acetate (PMA), HL-60 cells exhibited marked suppression of NE gene transcription, declining to 17% of the resting rate within 2 days. Induction toward mononuclear phagocytic lineage differentiation caused no change in NE mRNA transcript half-life (T1/2), but mRNA levels decreased markedly over time, with levels undetectable 1.5 days after PMA stimulation. In contrast, when induced toward the myelocytic lineage with dimethyl sulfoxide, the rate of NE gene transcription increased 1.9-fold within 5 days. Interestingly, the mRNA transcript levels increased 2.5-fold by 5 days despite the fact that induction toward myelocytic lineage differentiation was accompanied by a marked reduction of NE mRNA transcript T1/2. Together, these observations suggest that the NE gene expression during bone marrow differentiation is modulated mainly at the transcriptional level, with some posttranscriptional modulation contributing, particularly during myelocytic lineage differentiation.

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HUMAN NEUTROPHIL elastase (NE; EC 3.4.21.37), a 29-Kd powerful serine protease stored in the azurophilic (primary) granules of mature neutrophils, is released when the neutrophil is activated or degranulates. NE plays a central role in the pathogenesis of pulmonary emphysema through its ability to destroy the alveolar walls of the lung. The NE protein is coded for by a single copy gene composed of 5 exons and 4 introns spanning a 6-kb segment of chromosome 11 at q14.29 Interestingly, despite the fact that neutrophils carry large amounts of NE and are capable of synthesizing a variety of proteins,12,10 the NE gene is not expressed in mature neutrophils. In this regard, in situ hybridization studies have shown that NE messenger RNA (mRNA) transcripts are present only in bone marrow myelocytic precursor cells, mostly promyelocytes.12 Consistent with this observation, differentiation of the HL-60 promyelocytic leukemia cell line13-18 induced with phorbol 12-myristate 13-acetate (PMA) toward the mononuclear phagocytic lineage results in a reduction in NE mRNA transcript levels, whereas differentiation induced with dimethyl sulfoxide (Me2SO) toward the myelocytic lineage causes an increase in NE transcript levels.11

With this background, the present study is directed toward evaluating the levels of control of NE gene expression that result in the sharply controlled, lineage-specific modulation of NE mRNA levels in bone marrow cells. To accomplish this, we have used the HL-60 cell line to examine NE gene transcription and mRNA stability consequent to mononuclear phagocytic or myelocytic differentiation. The observations suggest that, although there likely is some posttranscriptional modulation, the majority of control of NE gene expression is at the transcriptional level.

MATERIALS AND METHODS

Source of cells and cell culture. The HL-60 promyelocytic leukemia cell line (American Type Culture Collection [ATCC], Rockville, MD; CCL 240), K-562 erythroleukemia cell line (ATCC CCL 243), HeLa cervical carcinoma cell line (ATCC CCL 2), and WI-26VA4 transformed lung fibroblast cell line (ATCC CCL 95.1) were maintained at 37°C in Dulbecco's Modified Eagle Medium (DMEM; Whittaker Bioproducts, Walkersville, MD) supplemented with 10% fetal bovine serum, 4 mM/L glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin (all from Biofluids, Inc, Rockville, MD). Fresh monocytes and T lymphocytes were isolated from peripheral blood of normal individuals as previously described19-20; after purification, the cell populations consisted of ≥ 90% monocytes or T lymphocytes, respectively, and were ≥ 95% viable by trypan blue exclusion.

To evaluate the regulatory mechanisms of NE gene expression during differentiation, the HL-60 cell line was used as a model of bone marrow precursor cells.27-18 HL-60 cells were induced to differentiate toward the mononuclear phagocytic lineage by PMA (80 nmol/L, up to 48 hours),16-18 or toward the myelocytic lineage by Me2SO (1.25% [vol/vol], up to 7 days)14-15,17,18 (PMA and Me2SO from Sigma Chemical Co, St Louis, MO). All experiments were performed when cells were in the exponential phase of growth; the induction of HL-60 cells for differentiation was initiated at a cellular density of 5 × 10⁶/mL in culture medium. The viability of HL-60 cells was always greater than 90% as assessed by trypan blue exclusion.

Quantification of NE gene transcription rate. The relative transcription rate of the NE gene was evaluated using nuclear transcription run-on analysis.21-23 Nuclei were isolated from 10⁶ cells of each cultured cell line or peripheral blood cells. To evaluate the effect of differentiation on the transcription level of the NE gene, HL-60 cells were stimulated with PMA (for 2, 15, and 48 hours) or with Me2SO (for 18 hours, 3 and 5 days) and nuclei from 10⁶ cells were
examined at each time point as well as those of the undifferentiated HL-60 cells (0 time). For cells in monolayers (HL-60 cells after incubation with PMA, HeLa and WI-26VA4 cell lines, and purified blood monocytes), the numbers of cells were determined by the numbers of viable cells (assessed by trypan blue exclusion) after treatment with trypsin to remove the cells from the plates. To obtain nuclei, cells were washed twice with Ca²⁺/Mg²⁺-free phosphate-buffered saline (PBS), pH 7.4 (Mediatech, Herndon, VA), and incubated in lysis buffer (10 mmol/L Tris-HCl, pH 7.4, 10 mmol/L KCl, 3 mmol/L MgCl₂, ice on for 10 minutes. The cells were then lysed with a Dounce homogenizer (10 strokes, B pestle) in the presence of Nonidet P-40 (0.1% final concentration; Sigma). The nuclei were recovered by centrifugation at 200g for 5 minutes, resuspended in 100 μL of storage buffer (20 mmol/L [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, HEPES] [Sigma], pH 7.4, 50% glycerol, 90 mmol/L KCl, 5 mmol/L MgCl₂, 5 mmol/L dithiothreitol), and stored over liquid nitrogen. To evaluate transcription, nuclei were thawed, incubated with 5 mmol/L ATP, 2 mmol/L CTP, 2 mmol/L UTP (all from Pharmacia-LKB, Piscataway, NJ), 250 μCi [α-³²P]GTP (> 400 Ci/mmol; Amersham Corp., Arlington Heights, IL), and 2 U of RNase inhibitor (RNasin; Promega, Madison, WI) at 37°C for 20 minutes. RNA was then isolated by the acid guanidinium thiocyanate-phenol-chloroform method, using RNAzol B (Cinna/Biotex Laboratories International Inc, Friendwood, TX) and resuspended in 10 mmol/L HEPES, pH 7.4, 5 mmol/L EDTA, 0.1% sodium dodecyl sulfate (SDS). ³²P-labeled nascent RNA was purified by centrifugation through Sephadex G-50 columns (5 Prime → 3 Prime Inc, West Chester, PA), denatured (65°C for 10 minutes), and hybridized to filter-bound denatured DNA targets in 1 mL of hybridization buffer (50 mmol/L sodium phosphate buffer, pH 6.5, 50% formamide, 1X Denhardt's solution [Ficoll, 0.2 mg/mL; polyvinylpyrrolidone, 0.2 mg/mL; bovine serum albumin, 0.2 mg/mL], 300 μg/mL yeast tRNA, and 0.1% SDS) at 42°C for 36 hours. To compare the transcription rate of the NE gene with that of other genes both before and after incubation with PMA or Me₂SO over various periods of time, equal cpm of ³²P-labeled RNA were used for each time point assessment. Plasmids containing the individual DNA targets (5 μg each) were denatured at 30 mmol/L NaOH for 10 minutes, neutralized with 2 mol/L ammonium acetate, blotted on nitrocellulose membrane (BA85; Schleicher and Schuell Inc, Keene, NH), and resuspended in 100 μL of storage buffer (20 mmol/L sodium phosphate buffer, pH 7.0, 1 mmol/L EDTA, 0.5% bovine serum albumin, 7% SDS at 65°C for 30 minutes, once in 0.5X SSC, 0.1% SDS at 65°C for 36 hours). Membranes were then washed twice in 2X SSC, 0.1% SDS at 23°C for 15 minutes, once in 0.5X SSC, 0.1% SDS at 23°C for 15 minutes, and once in 0.1X SSC, 0.1% SDS at 65°C for 20 minutes, subjected to autoradiography and autoradiograms scanned by a laser densitometer (UltraScan Laser Densitometer, Pharmacia-LKB).

To evaluate NE mRNA levels in HL-60 cells following differentiation after various incubation periods (with PMA for 0 to 48 hours; with Me₂SO for 0 to 7 days), HL-60 cells were washed and total RNA was isolated as described above. The levels of NE mRNA transcripts, as well as β-actin mRNA transcripts, were evaluated by Northern analysis, followed by densitometric scanning as described above, and the resulting autoradiograms were scanned by laser densitometry.

Stability of NE mRNA transcripts during differentiation of HL-60 cells. To assess the effect of differentiation on stability of NE mRNA transcripts, undifferentiated HL-60 cells and HL-60 cells incubated either with PMA (for 2 and 15 hours), or with Me₂SO (for 18 hours, 3 days, and 5 days) were exposed to actinomycin D (5 μg/mL; US Biochemicals, Cleveland, OH) for 0 to 24 hours. Total cellular RNA was then extracted, and NE mRNA levels were evaluated by Northern analysis, followed by densitometric scanning as described above. The T₁/₂ of NE mRNA transcripts in undifferentiated HL-60 cells as well as differentiated HL-60 cells was calculated by the method of least-squares.

RESULTS

Transcription levels of the NE gene. Nuclear run-on analyses showed that undifferentiated HL-60 cells actively transcribed the NE gene, in contrast with cells such as K-562, human blood monocytes, human T-lymphocytes, HeLa, or WI-26VA4 in which no transcription was observed (Fig 1A). In contrast, active transcription of the control β-actin gene was observed in all cell types. However, compared with the β-actin gene, the NE gene was transcribed at a lower level in undifferentiated HL-60 cells (Fig 1B). For example, while the relative NE transcription rate was 24% ± 4%* that of the β-actin gene, c-fos was 12% ± 2%, c-myc, 120% ± 13%, and myeloperoxidase, 62% ± 9%.

*All data are expressed as mean ± SEM, and all statistical comparisons were carried out using the two-tailed Student's t-test.

Evaluation of NE mRNA transcript levels. NE mRNA transcripts, and as a control, β-actin mRNA transcripts, were evaluated by Northern analysis. Briefly, total cellular RNA was isolated from HL-60 cells by the guanidinium thiocyanate-CCl₄ gradient method. RNA (10 μg for NE, 5 μg for β-actin mRNA detection) was subjected to formaldehyde agarose gel electrophoresis, transferred to nylon membranes (Nytran; Schleicher and Schuell), UV cross-linked (Stratalinker; Stratagene), and hybridized with the 6.65-kb NE cDNA probe (pPB15) or the β-actin cDNA probe (pHF6A-1) that had been ³²P-labeled by random priming. Hybridization was performed in 0.5 mol/L sodium phosphate buffer, pH 7.0, 1 mmol/L EDTA, 0.5% bovine serum albumin, 7% SDS at 65°C for 66 hours. Membranes were then washed twice in 2X SSC, 0.1% SDS at 23°C for 15 minutes, once in 0.5X SSC, 0.1% SDS at 23°C for 15 minutes, and once in 0.1X SSC, 0.1% SDS at 65°C for 20 minutes, subjected to autoradiography and autoradiograms scanned by a laser densitometer (UltraScan Laser Densitometer, Pharmacia-LKB).

To evaluate NE mRNA levels in HL-60 cells following differentiation after various incubation periods (with PMA for 0 to 48 hours; with Me₂SO for 0 to 7 days), HL-60 cells were washed and total RNA was isolated as described above. The levels of NE mRNA transcripts, as well as β-actin mRNA transcripts, were evaluated by Northern analysis, followed by densitometric scanning as described above, and the resulting autoradiograms were scanned by laser densitometry.
HL-60 cells were induced to differentiate toward the mononuclear phagocytic lineage with PMA, NE mRNA levels declined rapidly and became undetectable after 36 hours (Fig 2). In contrast, β-actin mRNA transcript levels slightly increased and remained at similar levels. The decline of the NE transcript level appeared to be primarily caused by a decrease in NE transcription rate. In this regard, the transcription rate of the NE gene declined after induction with PMA (after 2 hours, 54% ± 5% of control level; 15 hours, 47%; 48 hours, 17% ± 12%; respectively), while the β-actin transcription rate elevated transiently after 2 hours (132% ± 21% of control), but returned to the basal level thereafter (15 hours, 99%; 48 hours, 112% ± 15%; respectively) (Fig 3).

Interestingly, while the changes in transcription rate of the NE gene induced by PMA paralleled the decrease in NE mRNA transcript level, the NE transcripts actually remained stable. For example, while the T1/2 of NE mRNA transcripts in undifferentiated HL-60 cells was 10.1 hours ± 0.6 hour (degree of fit; r = −.88), when HL-60 cells were induced to differentiate toward the mononuclear phagocytic lineage with stimulation by PMA for 2 hours, the NE mRNA T1/2 transiently increased up to 15.1 hours (r = −.83; Fig 4). However, after 15 hours of PMA, the T1/2 returned to the basal level (10.4 hours, r = −.92).

**NE gene expression after myelocytic lineage differentiation.** Consistent with our previous observations, when HL-60 cells were induced to differentiate toward the myelocytic lineage with MeSO4, following a small decrease at 24 hours, NE mRNA transcript levels increased up to 188% ± 30% (3 days) and 211% ± 22% (5 days) of the initial level (P < .02 and P < .005; undifferentiated cells versus cells after 3 and 5 days of MeSO4 incubation, respectively) and remained elevated even after 7 days (Fig 5). In contrast, β-actin mRNA levels remained unchanged for 5 days, and then mildly decreased. As with the changes in NE transcript levels following differentiation toward the mononuclear phagocytic lineage, the changes in NE mRNA levels following myelocytic lineage differentiation appeared to be modulated by NE gene transcription, but in a direction opposite to that following mononuclear phagocytic differentiation. In this regard, during myelocytic lineage differentiation with MeSO4, although the NE gene transcription rate first showed a mild decrease to 72% ± 12% of the basal level at 18 hours, there was a marked increase to 208% ± 74% after 3 days that remained elevated at 191% ± 33% after 5 days of incubation (Fig 6). The β-actin gene transcription rate also showed a mild initial decline at 18 hours, but returned to the basal level where it remained after longer incubation with MeSO4. Interestingly, in contrast to the changes in NE mRNA T1/2 after mononuclear phagocytic differentiation, the T1/2 of NE mRNA transcripts showed a marked decrease from the baseline value of 10.1 hours after the induction of HL-60 cells to differentiate toward the myelocytic lineage with MeSO4 (4.1 hours at 18 hours [r = −.90]; 4.5 hours at 3 days [r = −.90]; 5.1 hours at 5 days [r = −.91]; Fig 7). Thus, as with mononuclear phagocytic differentiation, the changes in NE mRNA levels were modulated primarily at the level of transcription despite the decline in NE transcript stability.
**DISCUSSION**

NE is one of the most potentially harmful human proteolytic enzymes capable of functioning in the extracellular milieu, with a broad range of substrates, including the protein components of the connective tissue matrix that forms the architectural support of most organs.2,4,6,14 Expression of the NE gene is tightly controlled, being limited to bone marrow precursor cells at the promyelocyte stage.11,12 In this regard, NE is produced in promyelocytes and stored in primary granules of cells of the myelocytic lineage. After

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**Fig 2.** NE mRNA transcript levels evaluated by Northern analysis during differentiation of HL-60 cells toward the mononuclear phagocytic lineage induced by PMA. (A) Relative levels of NE and, as a control, β-actin mRNA transcripts after addition of PMA. The NE and β-actin mRNA levels in undifferentiated HL-60 cells were defined as 100%, respectively. Each data point represents the average of three independent experiments. (B) An example of northern hybridization after incubation of HL-60 cells with PMA. Top: levels of NE mRNA transcripts (1.3 kb). Incubation time is indicated above as 0 to 48 hours. Bottom: β-actin mRNA transcript (2.1 kb) levels as control.

**Fig 3.** Relative transcription rate of the NE gene during differentiation of HL-60 cells toward the mononuclear phagocytic lineage. Shown are the relative transcription rates of the NE and β-actin genes after incubation of HL-60 cells with PMA compared with that of undifferentiated cells defined as 100%. Each point represents the average of two separate determinations.

**Fig 4.** Stability of NE mRNA transcripts during mononuclear phagocytic lineage differentiation. Shown is the decline of NE mRNA transcript levels after inhibition of RNA synthesis with actinomycin D in undifferentiated HL-60 cells (○) and cells induced to differentiate toward the mononuclear phagocytic lineage with PMA for 2 hours (●) or 15 hours (■). NE mRNA levels are shown relative to that of the control cells before the addition of actinomycin D. The calculated T₁/2 of NE mRNA transcripts in each condition is indicated below.
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Fig 5. NE mRNA transcript levels evaluated by Northern analysis during differentiation of HL-60 cells toward the myelocytic lineage induced by Me₂SO. (A) Relative levels of NE and, as a control, β-actin mRNA transcripts after addition of Me₂SO. The NE and β-actin mRNA levels in undifferentiated HL-60 cells were defined as 100%, respectively. Each data point represents the average of three independent experiments, except at 6 days and 7 days. (B) An example of Northern hybridization after incubation of HL-60 cells with Me₂SO. Top: levels of NE mRNA transcripts (1.3 kb). Incubation time is indicated above as 0 days to 7 days. Bottom: β-actin mRNA transcript (2.1 kb) levels as control.

leaving the bone marrow, neutrophils cannot produce NE, but release NE after surface activation or when the neutrophil disintegrates. Using the HL-60 promyelocytic cell line as a model of myeloid differentiation, the present study shows that regulation of NE gene expression appears to be primarily at the transcriptional level.

In undifferentiated HL-60 cells, abundant NE mRNA transcripts are detectable, although the transcription rate of the NE gene is relatively low compared with that of the myeloperoxidase gene, a gene coding for another myelocytic protein stored in the primary granules of neutrophils. Interestingly, NE mRNA transcripts in undifferentiated HL-60 cells are relatively stable with a T_{1/2} of 10 hours, in contrast with the much shorter T_{1/2} (4.5 hours) of MPO mRNA transcripts. The relative stability of NE mRNA transcripts is consistent with the fact that they do not contain an AUUUA sequence in the 3' untranslated regions, a destabilizing signal that modulates the T_{1/2} of mRNA transcripts of some cytokines and proto-oncogenes, whereas MPO mRNA transcripts have three motifs of this sequence. In the context that undifferentiated HL-60 cells have relatively abundant amounts of NE mRNA transcripts in spite of the relatively low transcription rate of the NE gene, it is likely that in the undifferentiated state, posttranscriptional processes such as maintaining mRNA stability contribute significantly to the ability of these cells to produce NE.

However, expression of the NE gene is very different when HL-60 cells differentiate. As the cells differentiate toward the mononuclear phagocytic lineage, the level of NE mRNA transcripts declines and becomes undetectable. Importantly, this decline in mRNA transcript level is consistent with the decrease in the transcription rate of the gene, whereas the T_{1/2} of NE mRNA transcripts does not change appreciably. Thus, it appears that at least a significant proportion of the downregulation of NE gene expression in the HL-60 model of bone marrow precursor cells during mononuclear phagocytic lineage differentiation is controlled at the transcriptional level.

Like mononuclear phagocytic differentiation, the modulation of NE gene expression in myelocytic differentiation of HL-60 cells is also controlled primarily at the transcrip-
was clearly the dominant mechanism of NE gene expression.

The transcriptional level, but in the opposite direction. As in mononuclear phagocytic lineage differentiation, the change of the NE mRNA transcript levels in myelocytic lineage differentiation was parallel to that of the transcription rate of the NE gene, in this case increasing dramatically. Transcription was clearly the dominant mechanism of NE gene expression because the stability of the mature NE mRNA transcripts actually decreased during differentiation toward the myelocytic lineage. Thus, there appear to be opposing regulatory forces functioning during myelocytic differentiation, with the transcriptional processes dominating, as evidenced by the actual counts of viable cells and by the increasing amounts of recovered total RNA from whole cells in the present study, the apparent increase in NE mRNA transcripts with Me2SO is not caused by proliferation of HL-60 cells per se, because we have analyzed the equivalent amounts of total RNA extracted at each time point, and, more importantly, the levels of control β-actin transcripts in the identical amounts of total RNA remained similar without increase throughout the period of Me2SO incubation.

The transcriptional and posttranscriptional modulation of NE gene expression during bone marrow differentiation is of interest in comparison with that of the genes coding for other enzymes stored in the azurophilic granules of neutrophils such as myeloperoxidase and cathepsin G. The MPO gene is also expressed in the limited period of the early stage of myelocytic differentiation, although MPO mRNA transcripts disappear earlier than do NE mRNA transcripts during myelocytic differentiation in bone marrow. As for the NE gene, the expression of the MPO gene in HL-60 cells is modulated during differentiation at both the transcriptional and posttranscriptional levels. This may also be true for gene expression of cathepsin G, another serine protease of neutrophils. Although there is not much known about differentiation specific expression of the cathepsin G gene toward the myelocytic lineage, there is evidence that PMA causes transcriptional downregulation of the gene in the U-937 cells along with differentiation toward the mononuclear phagocytic lineage.

Fig 6. Relative transcription rate of the NE gene during differentiation of HL-60 cells toward the myelocytic lineage. Shown are the relative transcription rates of the NE and β-actin genes after incubation of HL-60 cells with Me2SO compared with that of undifferentiated cells defined as 100%. Each point represents the average of two separate determinations.

Fig 7. Stability of NE mRNA transcripts during myelocytic lineage differentiation. Shown is the decline of NE mRNA levels after inhibition of RNA synthesis with actinomycin D in undifferentiated HL-60 cells (○) and cells induced to differentiate toward the myelocytic lineage with Me2SO for 18 hours (●), 3 days (■), or 5 days (▲). NE mRNA levels are shown relative to that of the control cells before the addition of actinomycin D. The calculated T1/2 of NE mRNA transcripts in each condition is indicated below.
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...gene and the MPO gene share a 19-bp highly homologous (90%) sequence in their 5' flanking regions, and a similar pyrimidine-rich motif is also found in the 5' flanking region of the cathepsin G gene starting at the position of −53.47 Because the first part of this sequence (CCCCCTTC) is identical in these three genes, it is conceivable that these genes may be transcriptionally regulated, at least in part, in a related manner during myeloid differentiation. However, we could not determine the function of this conserved sequence as well as other sequences of the 5' flanking region of the NE gene in regards to NE gene transcription in HL-60 cells, because it was not possible to reproducibly transfect these HL-60 cells with plasmid vectors containing the NE promoter sequences linked to the chloramphenicol acetyltransferase reporter gene despite trying a variety of available methods, including calcium phosphate coprecipitation, diethyl aminoethyl (DEAE)-dextran, liposomes, polybrene, or electroporation (unpublished observations).

Recently, a cDNA encoding a novel serine protease “myeloblastin” has been characterized, showing a deduced amino acid sequence with 55% homology to NE.49 Interestingly, although the functional relevance between NE and myeloblastin is unknown, myeloblastin gene expression is downregulated during differentiation toward both the mononuclear phagocytic and myelocytic lineages in HL-60 cells. Furthermore, inhibition of myeloblastin gene expression is associated with proliferation arrest and differentiation of HL-60 cells toward the mononuclear phagocytic lineage. Although it is unknown whether inhibition of NE gene expression is associated with differentiation of HL-60 cells, it is conceivable that NE contributes per se to the regulation of proliferation and differentiation of bone marrow cells.50

Together, these observations of the modulation of lineage-specific NE gene expression in bone marrow cell differentiation offer possible clinical applications. First, for human disorders in which a relative excess of NE is playing a central role, such as pulmonary emphysema associated with α1-antitrypsin deficiency,51 one therapeutic strategy to modulate the amount of NE in the target organ could be to suppress the expression of the NE gene in bone marrow in the early stages of myeloid differentiation by developing agents that would mimic the downregulating effect of PMA. However, such an approach may carry risks if NE serves functions (as yet unknown) critical for normal biologic activities. Second, the fact that NE gene expression is tightly controlled, and limited to the promyelocytic stage of myeloid differentiation, provides a possible marker for the subclassification of acute leukemias based on the identification of the stage of arrest of myeloid cells during their differentiation.52

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REFERENCES

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