Neutrophil-specific Granule Deficiency Results from a Novel Mutation with Loss of Function of the Transcription Factor CCAAT/Enhancer Binding Protein ε

By Julie A. Lekstrom-Himes, Susan E. Dorman, Piroska Kopar, Steven M. Holland, and John I. Gallin

From the Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

Summary
Neutrophil-specific granule deficiency (SGD) is a rare disorder characterized by recurrent pyogenic infections, defective neutrophil chemotaxis and bactericidal activity, and lack of neutrophil secondary granule proteins. CCAAT/enhancer binding protein (C/EBP)ε, a member of the leucine zipper family of transcription factors, is expressed primarily in myeloid cells, and its knockout mouse model possesses distinctive defects, including a lack of neutrophil secondary granule proteins. Sequence analysis of the genomic DNA of a patient with SGD revealed a five-basepair deletion in the second exon of the C/EBPε locus. The predicted frame shift results in a truncation of the 32-kD major C/EBPε isoform, with loss of the dimerization domain, DNA binding region, and transcriptional activity. The multiple functional defects observed in these early neutrophil progenitor cells, a consequence of C/EBPε deficiency, define SGD as a defect in myelopoiesis and establish the requirement for C/EBPε for the promyelocyte-myelocyte transition in myeloid differentiation.

Key words: myelopoiesis • lactoferrin • granulocyte • immunodeficiency • neutrophil

Neutrophil-specific granule deficiency (SGD) is a rare congenital disorder marked by frequent and severe bacterial infections. The five reported cases consistently describe pleiotropic characteristics, including lack of secondary granule proteins and defensins, abnormalities in neutrophil migration and disaggregation, atypical nuclear morphology, and impaired bactericidal activity (1–11). More recent work has revealed additional granule abnormalities in the eosinophils of SGD patients, with absence of eosinophil-specific granule contents, including eosinophil cationic protein, eosinophil-derived neurotoxin, and major basic protein (12). Platelet disorders and associated bleeding diatheses, including the neutrophilic phagocytosis of platelets (13) and the absence of platelet-high-molecular-mass von Willebrand factor multimers stored in platelet α granules (14), have also been reported in SGD patients. In contrast to these seemingly genetically unrelated manifestations, these patients express normal levels of salivary lactoferrin (8, 15, 16), a characteristic specific granule marker absent in neutrophils in SGD, suggesting that the responsible defect involves myeloid-specific transcriptional regulation.

CCAAT/enhancer binding proteins (C/EBPs) comprise a family of transcription factors that are key regulators of cellular differentiation and function in a variety of tissues (17). The prototypic C/EBP is a modular protein consisting of one or more activation domains, a dimerization basic zipper domain and a DNA binding region (18). C/EBPs are least conserved in their activation domains and vary from dominant negative repressors to strong activators. C/EBPε, the newest member of the family, is expressed exclusively in cells of myeloid and T cell lineage (19–22). The human C/EBPε gene encodes four mRNA isoforms with varying splice patterns, driven from two alternative promoters, and from which are translated three protein isoforms (23). Analogous to what has been shown for C/EBPα and C/EBPβ (24, 25), in vitro transfection data suggest that the full length, 32-kD isoform of C/EBPε (C/EBPε32) possesses the fully active transcriptional activation domain, whereas the short, 14.2-kD isoform (C/EBPε14) lacks transcriptional activity (23).

Nearly 60% of C/EBPε knockout mice (26) succumb to low pathogenicity bacterial infections by 4–6 mo of age. Neutrophils from C/EBPε knockout mice have morphological features similar to human SGD neutrophils, including bilobed nuclei, absent specific and tertiary granule contents, and defective chemotaxis and bactericidal activity (27). The striking phenotypic similarities between SGD defects and the C/EBPε knockout model prompted a search for a C/EBPε knockout mutation in an SGD patient’s genomic locus.
Materials and Methods

Patient. Material from a previously described (5, 6) male patient lacking neutrophil-specific granules was studied. Research was conducted with informed consent under the guidelines of a National Institutes of Health (NIH) Internal Review Board-approved protocol, no. 92-I-99. The patient died from complications of pneumonia at age 20.

DNA, RNA, and Protein Extraction. Peripheral blood neutrophils were isolated as described (28), cryopreserved with dimethylformamide (Sigma Chemical Co.), and maintained at −140°C. Cell proteins were extracted as described (29). DNA extraction from cryopreserved fibroblasts proceeded as described (30). RNA was extracted from patient bone marrow aspirate using RNAzol reagent (Teltest) as per manufacturer's protocol. Normal human bone marrow RNA was purchased from Clontech.

PCR Amplification of Genomic Sequence. PCR reaction was performed using Platinum taq DNA polymerase (Life Technologies) per manufacturer's instructions and cycled as follows: 96°C for 12 min, followed by a three-step cycle—94°C for 30 s, 60°C for 30 s, and 72°C for 2 min—for 35–40 cycles. PCR products were gel purified and recovered using Gene Clean (Bio101). Products were sequenced with an ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Corp.). Primers were chosen from a published sequence available from EMBL/GenBank/DDBJ under accession no. U48865. Primer sets (upstream, downstream): B, 5'-AGC GCC CAT GCA AAA GGA AAG ACA, 5'-TCC ACC TAC CCC CAA GAG AAA GTT (bp 667–1186); C, 5'-CCC ACG GGA CCT ACT ACG (bp 1818–2343); F, 5'-CTC CCC GGC TGG CCC CTT TAC (bp 3133–3615); G, 5'-GGG GTG GGT GCT ACA AAA GAA ACT, 5'-TCA GGG AGG GGC AGG ACA (bp 1143–1553); H, 5'-ACA GGA GTA GGT GAC AGA GGA GAC, 5'-GGG CCG AAG GTA TGT GGA GGG TAG (bp 1563–2104); I, 5'-CCA TGC CCC CTC CTC TTT TTT CTC, 5'-ACT GCC TCC TTT CCC TGG GTG AA (bp 2594–3171); K, 5'-AAC TTT TTT CTC TTT GGG GTA GGT GGA, 5'-GGG CTG GCC TGC TCT TAC (bp 1163–1837). Homozygosity was determined by hybridization of the PCR product fragment C to a [32P]dATP-endlabeled internal oligonucleotide (H. downstream; sequence above). Labeled oligo was mixed with hybridization buffer (75 mM NaCl, 5 mM EDTA) in a ratio of 1:10, and 10 μl was added to 30 μl PCR product. Hybridization was cycled in a thermal cycler: 95°C for 5 min and 55°C for 10 min. Reactions were immediately placed on ice. Products were resolved on 4–20% Tris/borate/EDTA polyacrylamide gel (Novex) at 250 V.

RNA Blotting Assay. 10 μg of total RNA isolated from the patient's bone marrow and 0.25, 0.5, and 1 μg of control polyadenylated (pA)-mRNA was electrophoretically separated, blotted, hybridized, and washed as described (27). The membrane was stripped by boiling and stored at −20°C.

Immunoblotting. Protein quantitation was performed using a BCA Protein Assay kit (Pierce Chemical Co.) according to the manufacturer's instructions. 10–100 μg protein extracts were

Figure 1. (A) Sequence data from PCR products of normal control genomic DNA (top) and SGD patient DNA (bottom). Sequencing was performed on three separate PCR s from the SGD patient and three normal controls. Color coding of nucleotides on sequence scan is red, T; green, A; black, G; blue, C. Underlined nucleotides, 5-bp deletion. Arrowhead, location of deletion in the SGD patient sequence. Schematic drawing of C/EBPε locus shows three exons, two alternative promoters, Pα and Pβ; translational start codons; and bZIP region. (B) Schematic drawing of the three human C/EBPε protein isoforms. Second drawing (from top) shows the C/EBPε–SGD isoform with predicted missense region and premature termination codon occurring after the arrowhead. (C) PCR products after liquid hybridization of DNA region containing 5-bp deletion. Lanes 1 and 2, normal controls. Lane 4, patient DNA. Lane 3, PCR products from normal control DNA mixed equimolar with patient DNA. Arrowheads indicate normal allele (top) and SGD allele (bottom). Over 30 normal controls were tested.
electrophoretically separated, transferred to nitrocellulose, and incubated with primary antibody as described (29). Primary antibody was generated in rabbits by Research Genetics, Inc., using a synthetic peptide encoded in exon 2 of C/EBPε, downstream of the SGD deletion (DPRAVAVKEEP RPGEGR). The membranes were washed, incubated with anti-rabbit horseradish peroxidase conjugate antibody (ECL Western blot kit; Amersham Pharmacia Biotech, Inc.), and developed according to the manufacturer’s instructions. Membranes were stripped and reblotted with anti-mouse human β-actin antibody (Boehringer Mannheim) to control for protein loading.

In Vitro Mutagenesis. The patient’s mutation was introduced into the pCMV-C/EBPε32 expression vector using a Stratagene QuickChange site-directed mutagenesis kit per manufacturer’s instructions using a complementary oligonucleotide (PAGE purified; purchased from Genosys Biotech) containing the deletion (5′-CCA CT A CT T GCC GCC CTC GGC CCT TTG CCT ACC). Presence of the mutation and maintenance of the vector sequences was verified by sequencing and restriction enzyme digestion, respectively.

Transient Transfections. HeLa cells were maintained in DMEM (BioWhittaker) supplemented with 10% heat-inactivated FBS and penicillin/streptomycin at 37°C and 5% CO2. Cells were plated in 6-well plates and transfected within 24 h, at 30–50% confluency. Transfections, using the Mammalian Transfection System (Stratagene), were performed using 5 μg reporter plasmid (G-CSF receptor promoter-luc); 1, 2, or 5 μg inducer plasmid (pCMV-C/EBPα, pCMV-C/EBPε32 isoform, pCMV-C/EBPε27 isoform, or pCMV-C/EBPε32-SGD, described above); and 0.5 μg pCMVβ, as described (23, 31). The DNA content of transfections was normalized, and transfection was performed according to the manufacturer’s instructions, with 300 μl transfection solution applied to the cells. Samples were harvested 24 h after transfection.

Luciferase and β-galactosidase activities were measured using a Dual-Light kit (Tropix, Inc.), according to the manufacturer’s protocol, on a Turner 20/20 Luminometer. Samples were read for 15 s after a 3-s delay.

Results and Discussion

Sequencing of PCR products from genomic DNA detected a 5-bp deletion, TGACC, in exon 2 of the patient’s C/EBPε sequence. Fig. 1 A shows sequence data from one normal control (top sequence) and the SGD patient (bottom sequence). The mutation predicts a frameshift and a premature termination of the encoded C/EBPε32 isoform (Fig. 1 B). The missense code after the frameshift results in the loss of the critical DNA binding domain and leucine zipper region required for C/EBP dimerization and function. C/EBPε transcripts encoding the shorter 27- and 14-kD isoforms are predicted to be unaffected, based upon the splice donor and acceptor and translational start sites (23).

Homozygosity of the deletion was determined by PCR amplification of the affected region and resolution of the DNA fragments on a 4–20% polyacrylamide gel (Fig. 1 C). DNA from one normal control and the SGD patient were mixed before amplification and electrophoresis (lane 3), showing bands from both affected and normal alleles. In comparison, PCR products from the SGD patient (lane 4) and normal controls (lanes 1 and 2) show only one fragment, indicating homozygosity for their respective alleles.

RNA blot analysis of the SGD patient’s bone marrow total RNA showed decreased amounts of C/EBPε transcripts in comparison with control human bone marrow pA-RNA (Fig. 2 A). Hybridization with a [32P]dCTP-labeled actin probe (provided by L. Perera, National Cancer Institute, NIH) showed that 10 μg of SGD patient bone marrow total RNA was equivalent to 1 μg of normal bone marrow pA-mRNA and verified the stability and quality of the patient’s RNA preparation. Specific loss of C/EBPε transcripts in the SGD patient is likely due to mRNA instability secondary to the frameshift and the premature termination codon, as seen in other similar gene mutations (32, 33). Residual C/EBPε message is likely comprised by C/EBPε14 and C/EBPε27 transcripts, which are unaffected by the 5-bp deletion and similar in size to the C/EBPε32 transcript. Transcripts of C/EBPα were present in normal amounts. C/EBPα has a more proximal role in the myelopoietic pathway and specifically induces expression of C/EBPε (31, 34, 35). As expected, message for lactoferrin was not detected in the SGD patient’s bone marrow RNA.

As predicted from the C/EBPε transcript maps (Fig. 1 B), immunoblotting detected C/EBPε27 and C/EBPε14 isoforms, but not C/EBPε32, in neutrophils from the SGD patient (Fig. 2 B). All three isoforms were seen in the normal control. The antibody used is specific for a peptide sequence immediately downstream of the 5-bp mutation and should not bind the C/EBPε32-SGD protein.

Transient transfection assays in HeLa cells using the G-CSF receptor promoter driving the luciferase gene (31), compared the transactivation potentials of the inducer genes C/EBPα, C/EBPε32, C/EBPε14, and C/EBPε32-SGD.
C/EBP\textsubscript{e}32 has been shown to transactivate the G-CSF receptor promoter, whereas the C/EBP\textsubscript{e}14 isoform lacks transactivating function (23). Transient transfection of these plasmid constructs showed a significant loss of transactivation with the C/EBP\textsubscript{e}32-SGD isoform (P < 0.02, Mann-Whitney U test). The demonstrated in vitro data, as well as the in vivo SGD phenotype, mark the full length, 32-kD isoform as the major transactivator encoded in the C/EBP\textsubscript{e} locus.

The temporal link between granule protein production and myeloid lineage differentiation is well described: primary granule proteins are synthesized in myeloblasts and promyelocytes, secondary granules are produced in myelocytes and metamyelocytes, and tertiary granule proteins are generated in band and segmented neutrophils (36).

Previous work suggested that C/EBP\textsubscript{e} functions at the terminal stages of myeloid differentiation (23, 26). However, the total absence of patient neutrophil secondary granules and the selective loss of primary granule defensins marks an early myelopoietic block at the promyelocyte transition (Fig. 4). Further evidence for this conclusion comes from in vitro differentiation experiments using C/EBP\textsubscript{e}-deficient stem cells, which do not proceed beyond the promyelocyte stage (26). Other functional defects seen in mouse and human C/EBP\textsubscript{e}-deficient neutrophils, such as loss of tertiary granule gelatinase (27) and abnormalities in chemotaxis and cytokine expression (6, 27), may occur secondary to the block at the promyelocyte or later stage.

Functional analysis of the previously developed C/EBP\textsubscript{e} knockout mouse model (26, 27) was critical for the inter-
pretation of the C/EBPε mutation in SGD. The apparent multiplicity of C/EBPε target genes at different cell stages suggests that C/EBPε transactivates a set of early cell stage-specific genes, inducing normal promyelocyte differentiation and granule development. Additional evidence supporting these conclusions comes from recent observations suggesting that C/EBPε is induced by and transduces the G-CSF signal in neutrophils early in myelopoiesis (37). Absence of secondary granules, defensins, eosinophil cationic protein, eosinophil-derived neurotoxin (12), and platelet α-granule high-molecular-mass von Willebrand factor (14) in SGD demonstrates a critical role for C/EBPε in the development of granules and their contents in multiple myeloid lineages.

We are grateful to Dr. Helene Rosenberg for providing SGD patient bone marrow RNA and Dr. Mitchell Horwitz for providing normal peripheral blood CD34+ selected cells and expertise.

Address correspondence to John I. Gallin, Bldg. 10, Rm. 2C146, 10 Center Dr. M Sc 1504, Bethesda, MD 20892-1504. Phone: 301-496-4114; Fax: 301-402-0244; E-mail: jgallin@cc.nih.gov

Received for publication 9 March 1999 and in revised form 1 April 1999.

References

1. Spitznagel, J.K., M.R. Cooper, A.E. McColl, L.R. DeChatelet, and I.R.H. W evil. 1972. Selective deficiency of granules associated with lysozyme and lactoferrin in human polymorphs with reduced microbicidal capacity. J. Clin. Invest. 51:93a (Abstr.).

2. Strauss, R.G., K.E. Bove, J.F. Jones, A.M. Mauer, and V.A. Fulginiti. 1974. An anomaly of neutrophil morphology with impaired function. N. Engl. J. Med. 290:478–484.

3. Parmley, R.T., M. Ogawa, C.P. Darby, and S.S. Spicer. 1985. Anomalous neutrophil granule distribution in a patient with lactoferrin deficiency. N. Engl. J. Med. 313:1317–1329.

4. Ambruso, D.R., M. Sasada, H. Nishiyama, A. Kubo, A. Komiyama, and T. Akabane. 1979. Abnormal neutrophil maturation in a patient with recurrent bacterial infections. Blood. 53:1515–1533.

5. Breton-Gorius, J., C.L. Mason, D. Bruiot, J.L. Vilde, and C. Griscelli. 1980. Lactoferrin deficiency as a consequence of a lack of specific granule in neutrophils from a patient with recurrent infections. A. M. J. Pathol. 99:413–419.

6. Galin, J.I., J. Fletcher, B.E. Seligmann, S. Hoffstein, K. Cehrs, and N. Mounessa. 1982. Human neutrophil-specific granule deficiency: a model to assess the role of neutrophil-specific granules in the evolution of the inflammatory response. Blood. 59:1317–1329.

7. Boxer, L.A., T.D. Coates, R.A. Haak, J.B. Wolbach, S. Hoffstein, and R.L. Baehner. 1982. Lactoferrin deficiency associated with altered granulocyte function. N. Engl. J. Med. 307:404–410.

8. Ambruso, D.R., M. Sasada, H. Nishiyama, A. Kubo, A. Komiyama, and R.H. Allen. 1984. Defective bactericidal activity and absence of specific granules in neutrophils from a patient with recurrent bacterial infections. J. Clin. Immunol. 4:23–30.

9. Borregaard, N., L.A. Boxer, J.E. Smolen, and A.I. Tauber. 1985. Anomalous neutrophil granule distribution in a patient with lactoferrin deficiency. A. M. J. Hematol. 18:255–260.

10. Ganz, T., J.A. Metcalf, J.I. Gallin, L.A. Boxer, and R.I. Lehrer. 1988. Microbicidal/cytotoxic proteins of neutrophils are deficient in two disorders: Chediak-Higashi Syndrome and “specific” granule deficiency. J. Clin. Invest. 82:552–556.

11. Tamura, A., K. Agematsu, T. Mori, H. Kawai, T. Kuratsuji, M. Shimane, K. Tani, S. Asano, and A. Komiyama. 1994. A marked decrease in defensin mRNA in the only case of congenital neutrophil-specific granule deficiency reported in Japan. Int. J. Hematol. 59:137–142.

12. Rosenberg, H.F., and J.I. Gallin. 1993. Neutrophil-specific granule deficiency includes eosinophils. Blood. 82:268–273.

13. Sakura, T., H. Uramaki, T. Matsuhashi, J. Tamura, M. Sawamura, and T. Suchiya. 1993. Ultrastructure of neutrophil phagosome of autologous platelet in vivo in specific granule deficiency. A. M. J. Hematol. 43:149–150.

14. Parker, R.I., L.P. McKeown, J.I. Gallin, and H.R. Gránick. 1992. Absence of the largest platelet-von Willebrand multimers in a patient with lactoferrin deficiency and a bleeding tendency. Thromb. Hemost. 67:320–324.

15. Lomas, K.J., J.I. Gallin, D. Otrosen, G.D. Raphael, M.A. Kaliner, E.J. Benz, L.A. Boxer, and H.L. Malech. 1989. Selective defect in myeloid cell lactoferrin gene expression in neutrophil-specific granule deficiency. J. Clin. Invest. 83:514–519.

16. Raphael, G.D., J.L. Davis, P.C. Fox, H.L. Malech, J.I. Gallin, J.N. Baraniuk, and M.A. Kaliner. 1989. Glandular secretion of lactoferrin in a patient with neutrophil lactoferrin deficiency. A. M. J. Allergy Clin. Immunol. 84:914–919.

17. Lekstrom-Himes, J.A., and K.G. Xanthopoulos. 1998. Biological role of the CCAAT/enhancer-binding protein family of transcription factors. J. Biol. Chem. 273:28545–28548.

18. Williams, S.C., C.A. Cantwell, and P.F. Johnson. 1991. A family of C/EBP-related proteins capable of forming covalently linked leucine zipper dimers in vitro. Genes Dev. 5:1553–1567.

19. Antonsson, P., B. Stellan, R. Yamanaka, and K.G. Xanthopoulos. 1996. A novel human CCAAT/enhancer binding protein gene, C/EBPε, is expressed in cells of lymphoid and myeloid lineages and is localized on chromosome 14q11.2 close to the T-cell receptor α locus. Genomics. 35:30–38.

20. Chevakov, A.M., I. Grillier, E. Chevakovka, D. Chin, J. Slater, and H.P. Koeffler. 1997. Cloning of the novel human myeloid-cell-specific C/EBPε transcription factor. M. O. Cell. Biol. 17:1375–1386.

21. Kolke, M., A.M. Chevakov, S. Takeuchi, T. Tsuchiya, R. Yang, T. Nakamaki, N. Sutorouka, and H.P. Koeffler. 1997. C/EBPε: chromosomal mapping and mutational analysis of the gene in leukemia and preleukemia. Leuk. Res. 21:833–839.

22. Williams, S.C., Y. Du, R.C. Schwartz, S.R. Weiler, M. Ortiz, J.R. Keller, and P.F. Johnson. 1998. C/EBPε is a myeloid-specific activator of cytokine, chemokine and macrophage-colony-stimulating factor receptor genes. J. Biol. Chem. 22:13493–13501.
23. Yamanaka, R., G.-D. Kim, H.S. Rodomska, J. Lekstrom-Himes, L.T. Smith, P. Antonson, D.G. Tenen, and K.G. Xanthopoulos. 1997. CCAAT/enhancer binding protein e is preferentially up-regulated during granulocytic differentiation and its functional versatility is determined by alternative use of promoters and differential splicing. Proc. Natl. Acad. Sci. U.S.A. 94:6462-6467.

24. Ossipow, V., P. Descombes, and U. Schibler. 1993. CCAAT/enhancer-binding protein mRNA is translated into multiple proteins with different transcription activation potentials. Proc. Natl. Acad. Sci. U.S.A. 90:8219-8223.

25. Descombes, P., and U. Schibler. 1991. A liver-enriched transcriptional activator protein, LAP, and a transcriptional inhibitory protein, LIP, are translated from the same mRNA. Cell. 67:569-579.

26. Yamanaka, R., C. Barlow, J. Lekstrom-Himes, L. Castilia, P. Liu, M. Eckhaus, T. Decker, A. Wynshaw-Boris, and K.G. Xanthopoulos. 1997. Impaired granulopoiesis, myelodysplasia, and early lethality in C/EBPα-deficient mice. Proc. Natl. Acad. Sci. U.S.A. 94:13187-13192.

27. Lekstrom-Himes, J.A., and K.G. Xanthopoulos. 1999. C/EBPα is critical for effective neutrophil-mediated response to inflammatory challenge. Blood. 93:3096-3105.

28. Kuhns, D.B., H.A. Young, E.K. Gallin, and J.I. Gallin. 1998. Ca²⁺-dependent production and release of IL-8 in human neutrophils. J. Immunol. 161:4332-4339.

29. Dorman, S.E., and S.M. Holland. 1998. Mutation in the signal-transducing chain of the interferon γ receptor and susceptibility to mycobacterial infection. J. Clin. Investig. 101:2364-2369.

30. Laird, P.W., A. Zijderveld, K. Linders, M.A. Rudnicki, R. Jaenisch, and A. Berns. 1991. Simplified mammalian DNA isolation procedure. Nucl. Acid Res. 19:4293.

31. Smith, L.T., S. Hohaus, D.A. Gonzalez, S.E. Dziennis, and D.G. Tenen. 1996. PU.1 (Spi-1) and C/EBPα regulate the granulocyte colony-stimulating factor receptor promoter in myeloid cells. Blood. 88:1234-1247.

32. Hovnanian, A., A. Rochat, C. Bodemer, E. Petit, C.A. Rivers, C. Prost, S. Fraitag, A.M. Christiano, J. Uitto, M. Lathrop, et al. 1997. Characterization of 18 new mutations in COL7A1 in recessive dystrophic epidermolysis bullosa provides evidence for distinct molecular mechanisms underlying defective anchoring fibril formation. Am. J. Hum. Genet. 61:599-610.

33. Christiano, A.M., S. Amano, L.F. Eichenfield, R.E. Burgson, and J. Uitto. 1997. Premature termination codon mutations in the type VII collagen gene in recessive dystrophic epidermolysis bullosa result in nonsense-mediated mRNA decay and absence of functional protein. J. Invest. Dermatol. 109:390-394.

34. Zhang, D.-E., P. Zhang, N.-D. Wang, C.J. Hetherington, G.J. Darlington, and D.G. Tenen. 1997. Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein alpha-deficient mice. Proc. Natl. Acad. Sci. U.S.A. 94:569-574.

35. Rodomska, H.S., C.S. Huettner, P. Zhang, T. Cheng, D.T. Scadden, and D.G. Tenen. 1998. CCAAT enhancer binding protein alpha is a regulatory switch sufficient for induction of granulocytic development from bipotential myeloid progenitors. Mol. Cell. Biol. 18:4301-4314.

36. Borregaard, N., M. Sehested, B.S. Nielsen, H. Sengelov, and L. Kjeldsen. 1995. Biosynthesis of granule proteins in normal human bone marrow cells. Gelatinases are a marker of terminal neutrophil differentiation. Blood. 85:812-817.

37. Nakajima, H., J.L. Cleveland, S. Nagata, and J.N. Ihle. 1998. Granulocyte colony-stimulating factor regulates myeloid differentiation through CCAAT enhancer binding protein e. Blood. 92:712a.