Lack of Alterations in the Cytoplasmic Domains of the Granulocyte Colony-Stimulating Factor Receptors in Eight Cases of Severe Congenital Neutropenia

To the Editor:

Kostmann’s syndrome (KS) is characterized by severe congenital neutropenia associated with a maturation arrest at the promyelocytic stage with normal maturation of erythroid and megakaryocytic lineages. Patients with KS have frequent episodes of severe bacterial infections starting in the first month of life and generally die in the first or second decade of life. The etiology of KS is not known, although the ability of recombinant human granulocyte colony-stimulating factor (G-CSF) to dramatically effect long-term restoration of granulocyte production has led to the hypothesis that the defect may reside in either G-CSF, in G-CSF production, or in the functionality of the receptor for G-CSF. Previous studies have failed to show alterations in G-CSF or its production in the ability of G-CSF to associate with its receptor on cells of the granulocyte lineage. Therefore, recent studies have focused on the possibility that KS involves alterations in the cytoplasmic domain of the G-CSF receptor that contains distinct domains that are required for mitogenesis or to support granulocytic differentiation. In support of this hypothesis, a recent study identified a somatic point mutation in one allele of the receptor for G-CSF in one of six patients with KS. The mutation results in a cytoplasmic truncation that was associated with a defect in transducing a maturation signal. However, in another study of six patients, no mutations were detected by single-strand conformational polymorphism (SSCP) analysis.

To determine the frequency with which mutations in the cytoplasmic domain of the G-CSF receptor might contribute to KS, we examined the receptors from eight patients that were referred to St Jude Children’s Research Hospital (Memphis, TN) for evaluation and management of severe congenital neutropenia. Five of the eight patients responded well to G-CSF, whereas two patients experienced severe cycling of granulocyte counts. Another patient was refractory to therapy with G-CSF as has been described in detail. Polymerase chain reactions (PCRs) were used to amplify the genomic region containing the exons 16, 17, and 17', which encode the receptor cytoplasmic domain. Reverse transcriptase PCR (RT-PCR) was used to obtain amplified fragments corresponding to the receptor cytoplasmic domain of the expressed gene from six patients. As illustrated in Fig 1, PCR of genomic DNA gave the expected product of 1.2 kb. RT-PCR gave the expected product (0.65 kb) for cDNAs that would not contain the exon 17' sequence, indicating that this previously described splice variant is not expressed in these patients or the normal individuals we have examined (data not shown). Two individual PCR products from eight patients and six individual RT-PCR products from eight patients were subsequently sequenced. None of the sequences contained mutations, supporting the thesis that mutations of the G-CSF receptor are not a common cause of congenital neutropenia.

Genomic DNA was prepared by standard procedures. One nanogram of genomic DNA was used in PCR reactions containing 2 mmol/L deoxynucleotide triphosphate, PCR buffer with MgCl, and 2.5 U Taq polymerase. Primers consisted of an oligonucleotide (5'-CCCTGTCGACCTTGACCCCAGAGGGGT-3') from the intron-exon 16 boundary and an oligonucleotide (5'-ATCTCGAGTCAACTACTGAAGTTATAAGGAAA-3') from the 3' end of exon 17. The conditions consisted of 1 minute at 92°C, 1 minute at 50°C and 2 minutes at 72°C for 30 cycles and one additional cycle with 15 minutes at 72°C. One microgram of total RNA was used for cDNA synthesis and a 10 microliter aliquot was used for RT-PCR with an oligonucleotide primer (5'-CCCTGTCGACCTTGACCCCAGAGGGGT-3') from the 5' end of the transmembrane domain and primer from the 3' end of exon 17 in reactions as described above with the exception that the annealing temperature was 62°C. The PCR and RT-PCR products were cloned into T vectors and analyzed by Southern blot hybridization under standard conditions. The filters were hybridized with an oligonucleotide probe from exon 16.

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REFERENCES


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