Increased Levels of Endothelin-1 in Plasma of Sickle Cell Anemia Patients

To the Editor:

Interactions between circulating blood cells and the vascular endothelium are tightly regulated to maintain the integrity of a functional circulatory system. Endothelial cells, which are bipolar, provide the vascular system with a nonthrombogenic surface on the luminal side and perform a number of specialized metabolic and transport functions while in contact with the subendothelial matrix on the basal side. Injury to the vascular endothelium can expose the thrombogenic subendothelium and upset the delicate balance between blood flow and hemostasis. In sickle cell (SS) disease, injury to the vascular endothelium has been shown to result from increased adherence of SS red blood cells (RBCs) to endothelial cells and by vaso-occlusion of abnormally shaped SS RBCs. Vaso-occlusion by SS RBCs can produce prolonged hypoxia to local areas of the microvasculature resulting in endothelial cell damage. In addition, vaso-occlusion can contribute to the adherence of activated polymorphonuclear neutrophils, which can further damage endothelial cells by release of reactive oxygen metabolites.

One endothelial-cell-derived component extremely sensitive to cell injury is the vasoconstrictor peptide, endothelin-1 (ET-1), which has been found to be increased in the plasma of patients with diabetes, uremia, myocardial infarction, cardiogenic shock, and in patients after hemodialysis. Damage to endothelial cells in SS disease may be another system in which plasma ET-1 is increased. If this were the case, the local vasoconstriction produced by ET-1 could decrease the diameter of some blood vessels and cause slower microvasculature transit times hypothesized to be necessary for cell sickling in vivo. If ET-1 were elevated in SS disease, it would not only be a marker for endothelial cell damage but could also be a factor in exacerbating a vaso-occlusive event.

We measured the plasma levels of immunoreactive ET-1 in patients with SS disease in both steady state and crisis, and in normal age- and race-matched controls (AA) using an enzyme-linked immunosorbent assay (ELISA) method (Amersham Pharmacia Biotech, Arlington Heights, IL). Thirty-seven homozygous SS patients, (13 in crisis and 24 in steady state) from the Bronx Comprehensive Sickle Cell Center and 10 hematologically normal (AA) controls participated in the study. Individuals with hypertension or renal disease were excluded from the study. Plasma from 1 mL of heparinized blood was acidified with 0.25 mL 0.1% trifluoroacetic acid; the eluant lyophilized; and the remnants resuspended in 2 mL of 80% methanol, 0.1% trifluoroacetic acid; and loaded onto Sep-Pak C18 columns (Waters Associates, Milford, MA). ET-1 was eluted from the columns with 2 mL 2 N HCl and loaded onto Sep-Pak C18 columns (Waters Associates, Milford, MA). ET-1 was eluted from the columns with 2 mL 2 N HCl and loaded onto Sep-Pak C18 columns (Waters Associates, Milford, MA). ET-1 was eluted from the columns with 2 mL 2 N HCl and loaded onto Sep-Pak C18 columns (Waters Associates, Milford, MA). ET-1 was eluted from the columns with 2 mL 2 N HCl and loaded onto Sep-Pak C18 columns (Waters Associates, Milford, MA). ET-1 was eluted from the columns with 2 mL 2 N HCl and loaded onto Sep-Pak C18 columns (Waters Associates, Milford, MA). ET-1 was eluted from the columns with 2 mL 2 N HCl and loaded onto Sep-Pak C18 columns (Waters Associates, Milford, MA).

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was significantly higher than the median ET-1 plasma level of 0.58 pg/mL (n = 10) for AA control subjects (P < .0001). Because the distribution of data points was not symmetrical, we used a nonparametric analysis, the Kruskal-Wallis One-Way Analysis of Variance on Ranks. There was no significant difference in median plasma ET-1 levels in SS patients in steady state (18.79 pg/mL) and those in crisis (26.16 pg/mL, n = 13) (Fig 1). Asterisks indicate that the median plasma ET-1 levels for SS steady state and crisis were significantly different than the AA control median plasma ET-1 level at P < .0001.

Our data indicate that ET-1 is elevated in SS disease; however, the mechanism by which this occurs has not been determined. Direct evidence for damage to the endothelium in SS disease comes from studies by Sowemino-Coker et al, who measured circulating endothelial cells in plasma from SS patients and found increased circulating endothelial cells during crisis. An alternative mechanism to account for increased plasma levels of ET-1 in SS disease is the upregulation of specific endothelial cell genes by circulating plasma factors and/or hypoxic conditions. In a recent report, Phelan et al found that SS RBCs that have previously undergone sickling induce a fourfold to eightfold increase in the transcription of the ET-1 gene as well as a fourfold to sixfold increase in ET-1 peptide production. The induction by sickled SS RBCs is specific for ET-1 and unsickled SS RBCs had no effect. Now that animal models for SS disease have been developed, it should be possible to determine which mechanism is responsible for increased plasma ET-1. Specific inhibitors of endothelin converting enzyme (ECE) that prevent the conversion of proET-1 to ET-1 are available; increased synthesis of ET-1 would be blocked by these ECE inhibitors. The physiological significance of increased ET-1 in SS disease is also an open question. So many factors contribute to the pathophysiology of SS disease (eg, tissue hypoxia, vaso-occlusion, increased blood flow) that it is difficult to assess the contribution of one specific factor. A mouse model for SS disease where one of the autocrine functions of ET-1, such as endothelial cell proliferation, can be assessed will be an invaluable system in which to study the role of ET-1 in SS disease.

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REFERENCES
Activating Mutations of the Transmembrane Domain of MPL In Vitro and In Vivo: Incorrect Sequence of MPL-K, an Alternative Spliced Form of MPL

To the Editor:

Recently, many gene alterations have been identified as causes of leukemia, most of which are gross rearrangements of transcription factors, receptors, and kinases derived from chromosomal translocations. In addition, mutations of tyrosine kinase receptors such as c-kit and FLT-3 have been reported in mastocytosis and myeloid leukemia, respectively. In particular, it is noticeable that duplication of the juxtamembrane region of FLT-3 is observed in 20% of patient leukemic cells. However, no cytokine receptors (type I cytokine receptor family) have been reported to be involved in human leukemia except that truncation of the C-terminal domain of the granulocyte colony-stimulating factor (G-CSF) receptor caused by various point mutations is implicated in a fraction of leukemic disorders. Most of these leukemias are secondary acute myeloid leukemias (AMLs) developed from Kostmann syndrome, and the significance of the mutations in leukemogenesis is still controversial. 3, 4

MPL, thrombopoietin (TPO) receptor, is the only hemopoietin receptor (type I cytokine receptor family) identified as an oncogene. 5, 6 Thus, MPL was originally identified as a truncated form v-mpl that is an oncogene of a murine retrovirus MPLV, which causes myeloproliferative disorders in mice, and was later recognized as a receptor for TPO. Using a combined strategy including polymerase chain reaction (PCR)-driven random mutagenesis and retrovirus-mediated high-efficiency gene transfer, we have recently identified a constitutive active form of MPL. 7 This point mutation causes a single amino acid substitution from Ser498 to Asn498 in its transmembrane domain. Expression of the mutant MPL in a mouse interleukin-3 (IL-3)–dependent pro-B cell line Ba/F3 resulted in constitutive activation of both the Ras-Raf-MAPK and the JAK-STAT pathways and IL-3–independent growth. Moreover, when the Ba/F3 transfectants expressing the mutant MPL were injected into syngeneic mice after sublethal irradiation, they developed severe infiltration of the Ba/F3 transfectants in liver and spleen, suggesting that the mutant form of MPL is highly oncogenic in vivo. We were interested in whether similar mutations can be found in patients’ leukemic cells, and examined the sequence of the transmembrane portion of MPL in 43 patients, including 2 patients with essential thrombocythosis (ET), 6 with AML-M1, 6 with AML-M2, 1 with AML-M3, 3 with AML-M4, 2 with AML-M5, 9 with AML-M6, 12 with AML-M7 (megakaryoblastic leukemia), and 2 with myelodysplastic syndromes (MDS).

To sequence the corresponding part in the patients’ sample and to avoid the contamination of the plasmid harboring the mutant MPL, a DNA fragment spanning the transmembrane portion of MPL (exon 9) and a part of intron 10 of the human MPL gene 8 was amplified from genomic DNA by PCR using a 5’ transmembrane primer (ATCTCTTCTGGTGACC) and a primer in the 10th intron (AGATCTTGGGTCACACAGAG) (Fig 1). The function of MPL-K product was not known. 6 During the course of our screening for MPL mutations in leukemic patients, we happened to find a sequence error in the sequence of intron 10 that had been published as a part of the MPL-K transcript (Fig 1). Thus, the sequence CG (1616-1619) was GGGC (1616-1619) in all patients tested as well as in a normal control, which will result in frame-shift and earlier termination in the MPL-K product (Fig 2). The predicted length of the intracellular domain of MPL-K should be 36 instead of 66 amino acids. To confirm this, it is required to molecularly clone cDNA for MPL-K and confirm the sequence of the corresponding part.


Fig 1. PCR primers to amplify the transmembrane region of MPL from genomic DNA. TM (shadowed box), transmembrane region; arrows, PCR primers.
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