To the Editor:

Deletion of a single G nucleotide (ΔG) in exon 3 of the thrombopoietin (TPO) gene was found to cosegregate with thrombocythosis and elevated TPO serum levels in a Japanese kindred with thrombocythemia.1 The ΔG mutation affects the 5′-untranslated region (5′-UTR) of the TPO mRNA. Cells transfected with a cdNA carrying this mutation produced more TPO protein than controls transfected with the normal TPO cdNA, strongly suggesting that the ΔG mutation is responsible for thrombocythemia in this family.1 However, the molecular mechanism of how this mutation causes TPO overproduction was not elucidated, and in particular, no evidence for increased RNA stability or more efficient translation of the mutant TPO mRNAs was found.1

We have previously shown that the 5′-UTR of TPO mRNA exerts a strong inhibitory effect on translation.2 This inhibition is mediated by factor, and macrophage colony-stimulating factor, which, in turn, can affect the healing process.

Therefore, in the search for alternative remedies for HU-related skin ulcers, our preliminary experience would suggest that topical GM-CSF therapy may be helpful in the management of these common skin lesions when discontinuation of HU treatment is not advisable.

REFERENCES

A Single-Base Deletion in the Thrombopoietin (TPO) Gene Causes Familial Essential Thrombocythemia Through a Mechanism of More Efficient Translation of TPO mRNA

To the Editor:

37 months), we noticed the appearance of painful perimalleolar skin ulcers. Ulcers appeared with an erythematous border, whereas the epidermis was replaced by a fibrinous exudate and the dermis scattered by necrotic areas. Skin biopsies of the ulcerated lesions showed in all cases an histologic picture compatible with small vessel vasculitis. Circulating immune complexes were not detectable and doppler-fluximetry was always found in normal range.

Because GM-CSF has been reported to be effective in either preventing and reducing drug-induced mucositis or decreasing the healing period in cut and burn wounds,8 we tried a topical GM-CSF treatment after obtaining patients’ informed consent. Briefly, GM-CSF (Mielogen 150; Schering Plough, Milan, Italy) was diluted in sterile water for injection up to 5 µg/mL, and every 1 mL was dispensed in a sterile syringe and stored at 4°C until a maximum of 15 days. Skin ulcers were then douched twice a day, dried up to 20 minutes, and dressed. All patients received GM-CSF treatment for 2 weeks, with the exception of 1 patient (no. 2) who needed 2 further weeks of therapy. Topical GM-CSF was able to heal the cutaneous lesions and none of the patients required discontinuation of HU therapy.

It is noteworthy that 1 patient (no. 1) also experienced local treatment with granulocyte colony-stimulating factor (G-CSF) without any lesion improvement, whereas topical GM-CSF administration healed it. However, 2 months later, this patient entered CML blastoid transformation and developed a second skin malleolar ulcer that this time showed only a partial response to an additional GM-CSF treatment.

The appearance of HU-related skin ulcers represents a serious clinical problem for CML patients in long-term continuous treatment. In the past, we have observed several CML patients with HU-related cutaneous lesions who have been unsuccessfully treated with a variety of approaches such as topical antibiotics, subcutaneous calcium-heparin injections, and hemorheologic drugs. Furthermore, the incidence of HU-related cutaneous lesions was not lowered by reducing the schedule of HU administration, suggesting a close correlation with HU cumulative dose. Long-term cytoreductive therapy could play a pivotal role in affecting endothelial cells’ function, therefore leading to vascular suffering and tissue-organ damage. In this context, we have also found abnormally high vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1) serum levels in these 4 CML patients.9

The mechanisms through which GM-CSF acts are not yet clarified. It might be hypothesized that its promoting activity on both recruitment and proliferation of monocytes and macrophages could modulate the in situ production of cytokines such as interleukin-1, tumor necrosis factor, and macrophage colony-stimulating factor, which, in turn, can affect the healing process.

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Fig 1. Analysis of the translational efficiency of normal and mutant TPO transcripts in reticulocyte lysates. (A) Exon composition and ORFs of the normal and mutant TPO mRNAs originating from promoter 2 (P2). Exons are drawn as numbered boxes and the TPO protein coding region is shaded. The position of the single G nucleotide deletion is indicated (ΔG). The patterns of uORFs are drawn separately for normal and mutant TPO mRNA. The uAUG codons (D) are placed in the 3 possible reading frames (Roman numbers) and numbered in the order in which they appear in the full-length P1 transcript. The resulting uORFs are shown as horizontal lines and the position of stop codons is indicated by short vertical lines. Thick solid lines with arrowheads represent the normal and ΔG-mutant TPO-ORFs, with the corresponding amino acid sequences indicated below. Initiator methionines are highlighted by black boxes. (B) In vitro transcription-translation analysis of mRNAs originating from P2. Equal amounts of in vitro-transcribed TPO mRNA variants (lower panel) were translated in vitro in reticulocyte lysate in the presence of 35S-methionine (upper panel). D UTR, mRNA with deletion of the entire 5′-UTR; n, normal mRNA; ΔG, ΔG-mutant mRNA. The protein bands in the upper panel were AUG8, the normal TPO protein initiated at the physiological start site; AUG7, mutant TPO protein originating from AUG7; asterisk, cryptic non-AUG initiation in exon 3. (C) TPO secretion by COS cells transfected with either the normal (n) or ΔG-mutant (ΔG) TPO cDNA. TPO protein concentration in COS cell supernatants was determined by ELISA (■) and by bioassay (□). Error bars represent the standard deviations. CO, supernatant from nontransfected COS cells. The abundance of transfected TPO mRNA was assessed by Northern blot. To confirm equal transfection efficiencies, mRNA for vector-derived neomycin resistance gene (neo) was determined by reprobing of the Northern blot and equal RNA loading was verified by visualizing the 18S ribosomal RNA with ethidium bromide staining (lower panels).
Circulating Apoptotic Endothelial Cells

To the Editor:

In their article published in the May 1, 1999 issue of Blood, Mutin et al. find no circulating endothelial cells in their control group of patients and are unable to demonstrate activated or apoptotic endothelial cells. These results disagree with previously published data on the characteristics of circulating nucleated endothelial cells in normal healthy persons. There are several possible reasons for the failure to identify apoptotic endothelial cells in this study. (1) Neither the antibody nor the methods used by the authors have ever been validated for their ability to reliably identify and retrieve apoptotic endothelial cells from peripheral blood. (2) The morphologic criteria used by the investigators for identification of endothelial cells after staining with acridin orange exclude cells with characteristics of apoptotic endothelial cells after such staining. (3) They use a kit for detection of apoptosis that fails to identify cells in early stages of apoptosis. (4) All patients are on aspirin, heparin, and a β-blocker. Aspirin prevents activation of endothelial cells in vitro and reduces endothelial detachment and numbers of circulating endothelial cells in vivo. It also stimulates ferritin production in endothelial cells and iron chelation was shown to prevent apoptosis of these cells. Heparin reduces endothelial cell detachment and numbers of circulating endothelial cells in vivo. It also activates the constitutive nitric oxide synthase in endothelial cells, an effect that prevents apoptosis in these cells. β-Blockers may reduce the influence of endogenous adrenergic stimulants that are known to increase the number of circulating endothelial cells. They also prevent the formation of angiotensin II and the latter has a known ability to induce apoptosis in endothelial cells.

The use of poorly validated methods for detection and isolation of apoptotic cells, the exclusion of cells in early and late stages of apoptosis, and administration of antiapoptotic factors may have significantly influenced the results as reported by Mutin et al.

Thomir Štefanc
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A Single-Base Deletion in the Thrombopoietin (TPO) Gene Causes Familial Essential Thrombocythemia Through a Mechanism of More Efficient Translation of TPO mRNA

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