duction. We present here experimental evidence in support of this model.

To compare the translational efficiencies of normal and mutant TPO mRNAs, the ΔG mutation was introduced into the 5′-UTR of TPO cDNA by recombinant polymerase chain reaction (PCR) and subcloned into the pcDNA3 vector, as previously described.2 Mutant and control TPO mRNAs were transcribed in vitro using T7 polymerase and translated in reticulocyte lysate in the presence of 35S-methionine (Fig 1B). Translation of normal TPO mRNA was strongly repressed, as previously described.2 In contrast, the ΔG-mutant mRNA was translated with high efficiency, producing amounts of TPO protein comparable to an artificial construct with a deletion of all but the last 7 nucleotides of the 5′-UTR (ΔUTR). Consistent with presence of an extended N-terminus resulting from translation initiation at AUG7, the translation product of the ΔG-mutant migrated higher than the normal TPO protein on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Previous mutational analysis demonstrated that AUG7 is used very efficiently, whereas AUG5 and AUG6 are weak inhibitors of translation.3 Thus, the ΔG-mutation improves the efficiency of TPO translation in reticulocyte lysates by eliminating the inhibitory effect normally exerted by uORF7. The ΔG mutation also improved translation of TPO transcripts originating from promoter 1 (P1) and of a rare P1-variant that lacks exon 2 (not shown).

To demonstrate that the addition of 23 amino acids to the TPO signal peptide resulting from the ΔG frameshift in uORF7 does not interfere with secretion of a biologically active TPO protein, we transfected COS cells with TPO cDNA expression constructs and measured TPO protein concentrations in tissue culture supernatants (Fig 1C). Cells transfected with a construct carrying the ΔG mutation secreted 7-fold more TPO protein, as determined by enzyme-linked immunosorbent assay (ELISA), than cells transfected with the corresponding normal construct (solid bars). A similar increase was detected using a TPO bioassay (open bars), demonstrating that the extended signal peptide can ensure secretion of biologically active TPO.

Our results show that derepression of translation is responsible for overproduction of TPO protein in individuals carrying the ΔG mutation in this Japanese HT family. A different TPO gene mutation, which also results in derepression of TPO mRNA translation, was previously described as the cause of HT in a Dutch family.3 These data suggest that increased efficiency of TPO mRNA translation might be a common mechanism in the pathogenesis of HT and illustrate the importance of translational repression for normal platelet homeostasis.

REFERENCES

To the Editor:

In their article published in the May 1, 1999 issue of Blood, Mutin et al1 found 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.3 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 orange4 exclude cells with characteristics of apoptotic endothelial cells after such staining.5,6 (3) They use a kit for detection of apoptosis7 that fails to identify cells in early stages of apoptosis.8 (4) All patients are on aspirin, heparin, and a β-blocker.1 Aspirin prevents activation of endothelial cells in vitro8 and reduces endothelial detachment9 and numbers of circulating endothelial cells in vivo.10 It also stimulates ferritin production in endothelial cells,9 and iron chelation was shown to prevent apoptosis of these cells.10 Heparin reduces endothelial cell detachment9 and numbers of circulating endothelial cells in vivo.10 It also activates the constitutive nitric oxide synthase in endothelial cells,14 an effect that prevents apoptosis in these cells.15 β-Blockers may reduce the influence of endogenous adrenergic stimulants that are known to increase the number of circulating endothelial cells.16 They also prevent the formation of angiotensin II,17 and the latter has a known ability to induce apoptosis in endothelial cells.18

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.1

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Fulminant Intravascular Disseminating Malignant Melanoma Mimicking Acute Leukemia

To the Editor:

A 48-year-old male patient with a previous history of 4 primary melanomas was admitted 1 year after the last melanoma excision because of recent onset of fatigue, weakness, weight loss, shortness of breath, and limb pain. The initial hematological laboratory evaluation disclosed moderate anemia, thrombocytopenia, and a marked leukocytosis of $22.5 \times 10^3$ cells/$\mu$L, with 28% atypical plasmacytoid cells, and acute leukemia was suspected. Repeated Wright-Giemsa stains of peripheral blood smear confirmed the presence of 30% to 40% abnormal, large pleomorphic cells, with abundant cytoplasm, eccentric nuclei, and marked anisokaryosis (Fig 1A). These cells were negative for myeloid-, T-, B-, and natural killer (NK)-cell markers. Surprisingly, they were identified as circulating amelanotic melanoma cells by their reactivity with S100 protein-, vimentin-, and melanoma-specific HMB-45 monoclonal antibodies. Serum levels of the melanoma tumor markers S100 and MIA protein were markedly elevated, whereas polymerase chain reaction (PCR) for tyrosinase mRNA was negative. Bone marrow biopsy showed an almost complete replacement of hematopoiesis by HMB-45–positive melanoma cells. Chest x-ray and abdominal ultrasound showed no further evidence of macroscopic disease. The condition of the patient rapidly deteriorated and, despite supportive measures, he died on the ninth hospital day. Postmortem examination showed the presence of HMB-45–positive tumor cell aggregates trapped in the microcirculation of the liver (Fig 1B), the lungs, the kidneys, and the heart, with concomitant diffuse infiltrates in the perivascular space. The spleen also showed diffuse infiltration by tumor cells. The immediate cause of death was plugging of the pulmonary vasculature resulting in congestive right heart failure.

Bone marrow infiltration of human melanoma is found in up to 7% of in vivo staging procedures and in up to 45% of autopsy cases.\textsuperscript{1}
Circulating Apoptotic Endothelial Cells

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