We describe a significant increase in the DNA binding activity of nuclear factor-κB (NFκB) in bone marrow cells from patients with myelodysplastic syndrome (MDS) compared with normal donors. Furthermore, MDS samples with increased NFκB activity consistently showed higher mRNA levels of NFκB-regulated cytokines.

Increased intramedullary apoptotic death of hematopoietic cells is believed to contribute to the ineffective hematopoiesis in myelodysplastic syndromes (MDS), a myeloid hematopathy with a tendency to evolve to acute leukemia. However, the underlying molecular mechanisms that are responsible for this alteration remain elusive.

A deregulated expression of Bcl-2 and Bcl-xL has been associated with disease progression in MDS. As these antiapoptotic genes are transcriptionally regulated by Stat3 and Stat5 in different cell systems, we first analyzed the DNA binding activity of these Stat proteins in bone marrow from 14 patients with MDS and 6 normal donors. However, as shown in Figure 1, there were no significant differences in Stat activation. Another transcription factor associated with Bcl-2 family members (Bcl-xL, Bcl-2, A1) is NFκB. We, therefore, analyzed the activity of NFκB present in 17 MDS bone marrow samples (refractory anemia, n=5; refractory anemia with ring sideroblasts, n=5; refractory anemia with excess blasts, n=3; refractory anemia with excess blasts in transformation, n=2; chronic myelomonocytic leukemia, n=2), and found that at least six of them, including 4 refractory anemia (samples #3, 11, 14, and 17), 1 refractory anemia with ring sideroblasts (sample #4), and 1 refractory anemia with excess blasts (sample #7) showed a significant increase in the signal given by the NFκB-DNA complex, as assessed by electrophoretic mobility shift assay (EMSA) using a NFκB radiolabeled probe. (C) MDS patient number 11 was preincubated with antibodies specific for p50, p65, p52, c-Rel, and RelB. 18S rRNA was used as a loading control.

Figure 2. Expression of NFκB target genes in MDS bone marrow cells. (A) Total RNA from MDS samples 2 (low NFκB activity) and 11 (high NFκB activity), and from a normal donor (C5) was obtained and analyzed for the expression of a number of NFκB target genes by semiquantitative RT-PCR. (B) Bone marrow cells from MDS sample 11 were cultured for 24 h with the inhibitor, and then total RNA was extracted and analyzed for the expression of FasL and TNFα by semiquantitative RT-PCR. Amplified products were electrophoresed onto a 2% agarose gel and stained with ethidium bromide. 18S rRNA was used as an amplification control. Each amplification was repeated at least three times, and similar results were obtained.

Figure 1. DNA binding activity of Stat3, Stat5 and NFκB in MDS. Mononuclear cells were obtained from bone marrow cells of MDS patients and normal donors (controls). Formation of protein-DNA complexes was determined by EMSA using radiolabeled probes that contained consensus sites for Stat3, Stat5, and NFκB. Nuclear extracts from sample 11 were preincubated with antibodies specific for p50, p65, p52, c-Rel, and RelB.
trophoretic mobility shift assay (EMSA), compared to that found in normal marrow (Figure 1). To identify the NFκB family members that bind to the DNA target site, supershift experiments were performed using antibodies specific for p50, p65, p52, c- Rel, and RelB. Figure 1 reveals the presence of p50-p50 homodimers and p50-p65 heterodimers. Although NFκB has been mostly associated with inhibition of apoptosis, it also induces the expression of FasL and tumor necrosis factor (TNF)α, hence giving NFκB a pro-apoptotic role. We, therefore, analyzed the mRNA levels of these apoptogenic cytokines, as well as the expression of other NFκB-regulated genes in MDS samples with elevated NFκB activity and compared them with MDS and normal marrow samples with low NFκB activity. Figure 2A shows a representative experiment, in which MDS samples 2 (low NFκB activity) and 11 (high NFκB activity), and a normal marrow sample of controls) were analyzed by RT-PCR. Interestingly, TNFα mRNA levels were clearly upregulated in the high NFκB activity sample, and the expression of FasL was gradually increased in accordance with the level of NFκB activity. Consistent with these data is the observation that TNFα and FasL mRNA are upregulated in patients with MDS, and that in vitro blockade of these cytokines increases the number of hematopoietic colonies. Other NFκB-regulated genes such as TRAIL, and anti-apoptotic members of the Bcl-2 family (Bcl-2, Bcl-xL, A1) and inhibitor of apoptosis (IAP1, XIAP) were discussed as potential therapeutic targets.

In conclusion, our study reveals the alteration of a transcriptional pathway that may contribute to intramedullary apoptosis in MDS, and opens the possibility of developing therapeutic strategies aimed at avoiding the expression of NFκB-dependent apoptogenic cytokines.

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References


Erythropoietin does not modify the prothrombotic effect induced by uremic media on endothelial cells

Recombinant human erythropoietin (rHuEPO) administration has been associated with an increased risk of hypertension and thrombosis in uremic patients. rHuEPO and uremic media independently alter endothelial function both in vitro and in vivo. We investigated the effect of rHuEPO on endothelial cells cultured in an uremic environment. Results indicate that rHuEPO does not exert an additional activating effect to that caused by the uremic media alone.

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Uremic patients suffer from complex hemostatic disorders, with the coexistence of a bleeding tendency and an increased risk of thrombotic complications and cardiovascular events. There is increasing evidence of endothelial dysfunction in uremic patients, characterized by in vivo increases in plasma levels of endothelial cell damage markers, impaired endothelium dependent vasodilatation, and in vitro increases in the thrombogenic properties of the endothelial cell extracellular matrices (ECM). Treatment with HUPEO corrects the defect in primary hemostasis frequently observed in uremia, both through an increase in the number of red cells and by improving platelet dysfunction. However, there is some concern about the potential deleterious effects of HUPEO treatment on blood pressure, on the incidence of thrombotic events, and on the high cardiovascular risk reported in uremic patients.

Endothelial cells (EC) cultured in the presence of uremic sera (uremic EC) were exposed to HUPEO (1U), at final concentrations of 0, 3, 10, 50 and 100U/mL, to evaluate changes in tyrosine phosphorylation of proteins in control EC were almost undetectable, increased significantly when cells were grown in the presence of uremic sera, and did not change after incubation with increasing concentrations of rHuEPO (Figure 1). Platelet adhesion on ECM-coated coverslips, measured as the