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LOW SERUM LEVELS OF TUMOR NECROSIS FACTOR AND INTERLEUKIN-1 β IN MYELODYSPLASTIC SYNDROMES RESPONSIVE TO RECOMBINANT ERYTHROPOIETIN

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ABSTRACT

Background. **Tumor necrosis factor (TNF) and interleukin-1 β (IL-1) are two cytokines with erythropoietic inhibitory activity which may be involved in the pathogenesis of some types of anemia that may respond to recombinant erythropoietin (r-EPO). The aim of the present study was to evaluate whether TNF and IL-1 serum levels are related to clinical response in patients with myelodysplastic syndromes (MDS) receiving r-EPO. TNF and IL-1 serum levels were measured by means of immunoenzymatic assays in 26 patients affected by MDS and treated with r-EPO administered subcutaneously at dosages up to 1050 U/kg a week, for at least two months. Four patients (15%) showed a significant response, with an increase of hemoglobin > 2 g/dL and complete suspension of transfusions. Higher mean serum levels of both TNF (54.2±93 vs 4.2±7.9 pg/mL, p < 0.001) and IL-1 (114±58.5 vs 36.1±21.7 pg/mL, p < 0.001) were measured in MDS patients than in a group of 42 normal controls. However, responders showed significantly lower mean levels of TNF (8.2±9.6 vs 58.5±65.2 pg/mL, p < 0.05) and IL-1 (30±24.8 vs 127.8±51.4 pg/mL, p < 0.001) than those of non responders. In terms of absolute values, all responders evidenced undetectable or normal levels of both cytokines. No relationship was found between TNF or IL-1 and values of hemoglobin, serum erythropoietin, ferritin, soluble transferrin receptor or transfusional requirements. MDS patients who respond to r-EPO have lower serum levels of TNF and IL-1 than those who do not respond.**

Key words: myelodysplastic syndromes, tumor necrosis factor, interleukin-1, erythropoietin

Tumor necrosis factor α (TNF) and interleukin-1 β (IL-1) are two pleiotropic cytokines which exert, among other functions, important inhibitory activity on erythropoiesis.[1-4] In fact, both TNF and IL-1 blunt the endogenous production of erythropoietin (EPO) and have an antiproliferative effect on the erythroid progenitors BFU-E and CFU-E. In particular, the inhibitory effects of IL-1 β are mediated by interferon-γ.[1]

It has been suggested that TNF and IL-1 might play a role in the pathogenesis of some forms of anemia which respond to recombinant EPO (r-EPO), such as the anemia of chronic disorders or that of cancer patients,[2] where elevated levels of these molecules may be produced. Interestingly, high levels of these *inflammatory* cytokines have also been found recently in the serum of patients with myelodysplastic syndromes (MDS),[5,6] a group of clonal hematological disorders in which r-EPO may be useful in reducing or even abolishing transfusional requirements, albeit in a limited percentage of patients.[1,7-9]

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These considerations prompted us to evaluate the possible relationship between serum levels of TNF and/or IL-1 and response to r-EPO in patients affected by MDS.

Patients and methods

Baseline levels of TNF and IL-1 were measured by means of two commercially available immunoenzymatic assays (TNF EASIA, Medgenix Diagnostics, Brussels, Belgium and BIOKINE IL-1 Beta Test kit, T-cell Diagnostics, Medical Systems, Genoa, Italy) on stored frozen serum samples from 26 transfusion-dependent patients, aged 18 to 75 years, affected by MDS: 17 refractory anemias, 5 of whom with blast excess, and 9 sideroblastic anemias according to FAB criteria. All these patients had failed to improve their hemoglobin levels with conventional approaches and started treatment with r-EPO (Eprex, Cilag; Eritrogen, Boehringer-Mannheim; Epoxitin, Janssen) at dosages up to 1050 U/Kg a week, subcutaneously, for at least two months. Transfusional requirements and initial hemoglobin, serum ferritin levels, endogeneous EPO and soluble transferrin receptor (STR) were correlated to pretreatment TNF and IL-1 values by means of Pearson's coefficient. Student's t-test was used to determine statistical differences between responders and non-responders, as well as between MDS patients and a group of 42 normal controls.

Results

Four patients (15%), two with refractory anemia and two with sideroblastic anemia, achieved a complete response as defined by an increase of hemoglobin superior to 2 g/dL and suspension of packed red cell transfusions. Figure 1 illustrates the values of TNF and IL-1 observed in individual patients and controls. Serum TNF and IL-1 levels were found to be significantly higher in MDS patients than in normal controls (Table 1). However, the four patients who had a complete response showed significantly lower TNF and IL-1 serum levels than those observed in the remaining non-responsive patients (Figure 1, Table 1).

Interestingly, TNF and IL-1 were both found to be unmeasurable or within the normal range in all responders; this occurred in only 2/22 cases among unresponsive patients, some of whom showed normal levels of one of these cytokines, but not of both. In MDS patients pre-treatment TNF and IL-1 levels did not correlate with each other or with hemoglobin, transfusion requirements, serum EPO, ferritin, or erythroid marrow activity, as measured by serum levels of circulating STR (data not shown). In addition to low serum levels of TNF and IL-1, responders were also characterized by a recent diagnosis, a normal karyotype and circulating levels of EPO and STR inadequate for the degree of anemia according to calculation of the observed/predicted (O/P) ratio.[1]

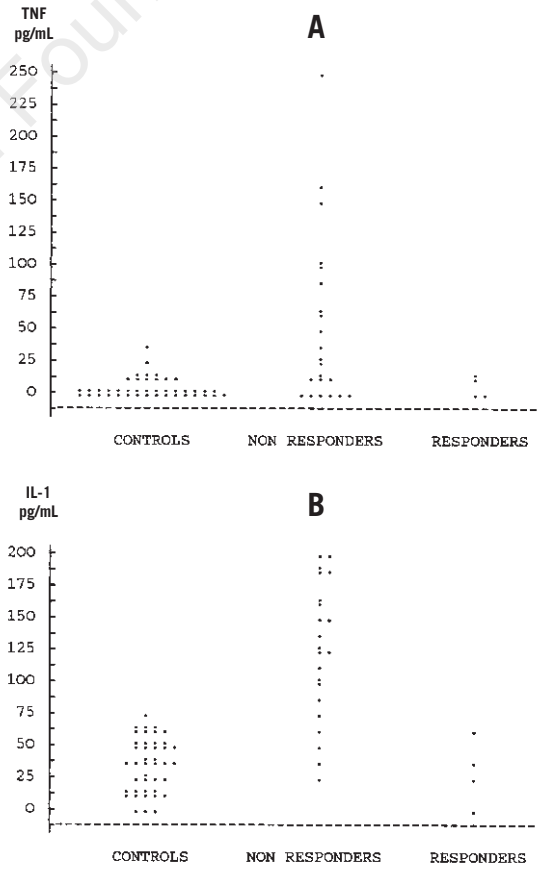

Figure 1. Serum TNF (A) and IL-1 (B) levels measured in controls and in MDS patients (subdivided into responders and non-responders) before treatment with r-EPO.

Table 1. Mean (±SD) serum TNF and IL-1 levels measured in MDS patients (with a comparison between responders and non-responders to r-EPO) and controls. Values are expressed as pg/mL.

	patients (26)	*controls (42)*	*responders (4)*	*non-responders (22)*
TNF	54.2±93	4.2±7.9	8.2±9.6	58.5±65.2
	($p < 0.001$)		($p < 0.05$)	
IL-1	114±58.5	36.1±21.7	30±24.8	127.8±51.4
	($p < 0.001$)		($p < 0.001$)	

Discussion

r-EPO is active in about 20-25% of patients with MDS.[1, 7-9] Due to the small proportion of responders and the high cost of the treatment, it is crucial to identify those subjects who are the best potential candidates for receiving r-EPO. To this end, some clinical and laboratory parameters have been proposed, such as serum EPO level, the time elapsed from diagnosis, FAB subtype, the number of red cell transfusions received, marrow erythroid cellularity, karyotype characteristics or the presence of normal circulating erythroid progenitors. None of these, however, has been definitively confirmed as a reliably predictive marker for response to r-EPO in MDS patients.

Our preliminary and intriguing results do not clarify wehere TNF and IL-1 play a pivotal role in the pathogenesis of anemia in MDS. In particular, it must still be elucidated whether these cytokines are involved in the *inflammatory* phenomena of MDS anemia which have been described as potential inhibitors of response to r-EPO in other types of anemias.[10]

As mentioned above, TNF and IL-1 exert a negative regulation on the production of endogenous EPO, as well as on the proliferative activity of erythroid progenitors. Since we did not find any correlation between TNF or IL-1 and serum EPO or STR, one of the possible explanations for the lack of response to r-EPO in MDS patients with high serum levels of TNF or IL-1 could be related to inhibitory activity on the proliferative stimulus provided by r-EPO at the early stages of erythropoiesis. This leads to the question of whether higher doses of r-EPO could be useful in MDS patients with elevated serum TNF or IL-1 levels, in order to overcome the possible negative effects mediated by these cytokines.

On the other hand, different mechanisms probably concur in the so-called anemias of chronic disorders, in which *inflammatory* cytokines such as TNF, IL-1 and γ-interferon are likely to be involved (as revealed by their frequently increased serum levels), but where a good response to r-EPO is also often seen. In these conditions the presence of only normal, non-clonal erythropoiesis, with its high potential responsiveness to r-EPO, might represent a relevant difference with respect to MDS. Nevertheless, despite the fact that several biological aspects still appear to be unclear, our findings suggest that reduced serum TNF and IL-1 levels might be useful in helping to identify those MDS patients who will respond to conventional doses of r-EPO. With a view to possibly employing this test for the selection of such patients, this statement warrants confirmation in larger case series.

References

1. Cazzola M. Erythropoietin: biology and clinical applications. Forum 1993; 4:344-61.
2. Erslev AJ. Erythropoietin. N Engl J Med 1991; 324:1339-44.
3. Jelkmann W, Wolff M, Fandrey J. Modulation of the production of erythropoietin by cytokines: in vitro studies and their clinical implications. Contrib Nephrol 1990; 87:68-74.
4. Faquin WC, Scheider TJ, Goldberg MA. Effect of inflammatory cytokines on hypoxia-induced erythropoietin production. Blood 1992; 79:1987-94.
5. Zoumbos N, Symeonidis A, Kourakli A, et al. Increased levels of soluble interleukin-2 receptors and tumor necrosis factor in serum of patients with myelodysplastic syndromes. Blood 1991; 77:413-4.
6. Verhoef GEG, De Schouwer P, Ceuppens JL, Van Damme A, Grossens W, Boogaerts MA. Measurement of serum cytokine levels in patients with myelodysplastic syndromes. Leukemia 1992; 6:1268-72.
7. Musto P, Catalano L, Andriani A, et al. Recombinant erythropoietin for the treatment of anemia in myelodysplastic syndromes: personal data and review of the literature. In: Cazzola M, Barosi G, Ascari E, eds. Erythropoietin Symposium 1992. Pavia: Ferrata Storti Foundation, 1992: 75-84.
8. Depaoli L, Levis A, Isabella N, et al. Serum erythropoietin

levels and marrow erythroid infiltration predict response to recombinant human erythropoietin in myelodysplastic syndromes. Haematologica 1993; 78:118-22.
9. Aloe-Spiriti MA, Petti MC, Latagliata R, et al. Recombinant human erythropoietin in the treatment of myelodysplastic syndromes. An interim report. Haematologica 1993; 78:123-6.
10. Beguin Y, Loo M, R'Zik S, et al. Early prediction of response to recombinant human erythropoietin in patients with the anemia of renal failure by serum transferrin receptor and fibrinogen. Blood 1993; 82:2010-6.