Lack of Correlation of Serum Cytokine Levels with Bone Marrow Cytokine Production and Effects on Erythropoiesis

G.		Vreugdenhil ^a
M.		Jongen ^b
A.J.G.		Swaak ^b

^aDepartment of Internal Medicine, Sint Joseph Hospital, Veldhoven, ^bDepartment of Rheumatology, Dr. Daniel den Hoed Clinic, Rotterdam, The Netherlands

G. Vreugdenhil, Dept. Internal Medicine, Sint Joseph Hospital, PO Box 7777, NL-5500 MB Veldhoven (The Netherlands)

Dear Sir,

Although defective erythropoietin (EPO) production is the most important factor in the pathogenesis of anemia of chronic renal failure (ACRF), cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) may also be involved by suppressing erythropoiesis [1]. Buemi et al. [2] recently have studied whether a correlation exists between the cumulative dose of recombinant human EPO (rHuEPO) and serum levels of IL-1 and TNF- α , prior to treatment with rHuEPO. Such correlation was not observed. They also reported that serum TNF did not change during rHuEPO treatment, whereas a slight increase in serum IL-1 occurred. Their findings may suggest that these cytokines only play a minor role in the development of ACRF in these patients. Serum cytokine levels may reflect activity of the underlying renal disorder or stimulation of the mononuclear phagocyte system, exerted by dialysis treatment [3]. In inflammatory disorders, such as rheumatoid arthritis (RA), anemia of chronic disorders (ACD) is frequently present, and its pathogenesis is also explained by a relatively impaired EPO response to the anemia and suppressive effects of IL-1 and TNF on erythropoiesis [4]. As the degree of ACD is related to the activity of the underlying disorder, increased serum levels of TNF due to disease activity also correlate with the severity of anemia [5]. Serum levels, however, only constitute an indirect parameter of local production of cytokines, whereas their possible effects on erythropoiesis occur in the bone marrow compartment. We, therefore, have examined production of TNF as well as IL-6, a cytokine associated with the inflammatory response, in bone marrow cultures in 6 RA patients with and 5 without ACD and 5 controls. Hemoglobin (Hb), serological parameters of RA activity, ferritin and cytokines in serum and bone marrow cultures in groups 1 (RA and ACD), 2 (nonanemics) and 3 (controls)

Erythrocyte sedimentation rate; CRP

Data are expressed as median and range. ESR C-reactive protein.

* $p < 0.10$; ** $p < 0.05$, compared to controls.

controls bone marrow donors) and compared the marrow production with serum levels and degree of disease activity. IL-6 (determined by the B9 bioassay) and TNF (using an immunoradiometrical assay) were measured in serum and in supernatants of bone marrow cultures consisting of 1 million mononuclear cells obtained after centrifugation with the addition of fetal calf serum and burst-forming

unit erythrocyte mix incubated for 48 h at standard culture conditions [5].

Table 1 shows the results. No significant differences in bone marrow IL-6 and TNF production were found between the three subgroups, whereas serum cytokine levels were higher in RA patients as compared to controls. Bone marrow TNF and IL-6 production did not correlate with parameters of

disease activity or serum levels of TNF and IL-6. However, marrow TNF and IL-6 production were correlated (fig. 1).

The possible effects of cytokines on erythropoiesis occurring in bone marrow need not necessarily be reflected by their serum levels. For IL-1 and TNF, suppressive effects on in vitro erythropoiesis have been established [1, 4], but we do not know to what extent these effects are operating in vivo in ACRF and ACD. The absence of a correlation of bone marrow and serum IL-6 and TNF levels demonstrate that serum cytokine levels obtained from RA patients with ACD and probably patients with ACRF rather reflect the levels of activity of the underlying disorder than bone marrow cytokine levels affecting erythropoiesis. Thus, it cannot be ruled out that in the study by Buemi et al. [2], rHuEPO did affect bone marrow TNF or IL-1 production which was not expressed by changes in serum levels. Similarly, in RA patients treated with rHuEPO, hemoglobin levels increased, whereas serum IL-6 levels did not change [6].

Alternatively, it is possible that, both in ACRF and ACD, the suppressive effects of TNF and IL-1 are counteracted by supra-physiologic concentrations of EPO [7]. Possibly, longitudinal measurement of cytokines in serum and bone marrow, as well as in synovial fluid in RA patients with ACD and in dialysate in patients with ACRF, may elucidate whether rHuEPO affects local cytokine production and to what extent the increase in erythropoiesis is due to other effects than EPO itself.

1,000 2,000 TNF α (U/ml; 10⁶ cells)

Fig. 1. Correlation between bone marrow IL-6 and TNF production in ACD and non-anemics.

References

- Ogawa M: Effects of hemopoietic growth factors on stem cells in vitro. *Hematol Oncol Clin North Am* 1989;8:453-464. Buemi M, Allegra A, Aloisi C, Privitera M, Morabito N, Frisina N; Influence of the treatment with recombinant human erythropoietin on plasma concentration of interleukin-1-alpha and tumour necrosis factor-alpha in hemodialyzed subjects. *Nephron* 1993;64:333-334.
- Hoeffman-Cavaillon JM, Ciancioni C, Bado F, Delons S: In vivo induction of interleukin-1 during hemodialysis. *Kidney Int* 1989;35:1212-1218.
- Derienzo DP, Saleem A: Anemia of chronic disease: A review of pathogenesis. *Tex Med* 1990;86:80-83.
- Vreugdenhil G, Lowenberg B, Van Eijk HG, Swaak AJG: Tumor necrosis factor-alpha is associated with disease activity and the degree of anemia in patients with rheumatoid arthritis. *Eur J Clin Invest* 1992;22:488-493.
- Jongen M, Vreugdenhil G, Swaak AJG: Serial estimation of interleukin-6 during treatment with recombinant human erythropoietin in anemic rheumatoid arthritis patients. *J Rheumatol* 1993;20:1253-1254. Jongen M, Peeters R, Swaak AJG, Vreugdenhil G: Tumor necrosis factor-alpha reverses the effects of erythropoietin on in vitro erythropoiesis (abstract). *Eur J Clin Invest* 1993;23(suppl): A39.