

Daily Variations of Immunoreactive Serum Interleukin-6 Levels in Multiple Myeloma

To the Editor:

Interleukin-6 (IL-6) is a multifunctional cytokine postulated to play a central role as a growth factor for multiple myeloma (MM). However, very recently, an association of high levels of IL-6 secretion with low tumor burden and low proliferative fraction in a subset of MM patients has been proposed, suggesting that IL-6 is a major differentiation factor.¹ In MM, the serum levels of IL-6, as measured by bioassay, correlate with disease severity and survival.^{2,3} The level of serum immunoreactive IL-6, as measured by a sensitive enzyme-linked immunosorbent assay (ELISA), was recently found to be increased in 30% of MM patients at diagnosis and associated with survival, confirming the bioassay results.⁴ This study was extended by Pelliniemi et al,⁵ who observed increased levels of serum IL-6 in 42% of patients with newly diagnosed MM and convincingly proved that the serum concentration of immunoreactive IL-6 is a significant prognostic marker in MM, especially in stage II patients, when measured with a highly sensitive ELISA method. However, it should be noted that the serum levels of immunoreactive IL-6 have been also reported to be normal⁶ or even undetectable¹ in a large proportion of MM patients. Thus, the idea emerges that not only the precise biologic function but also the prognostic significance of IL-6 are still a matter of debate. In the present study, we emphasize that the discrepancies between the results of different studies designed to evaluate the prognostic value of serum IL-6 in MM might be not only due to technical reasons, ie, the lower sensitivity of some enzyme immunoassays, which Pelliniemi et al⁵ have overcome by applying a sensitive sandwich-type ELISA (Quantikine; R&D Systems, Minneapolis, MN). In fact, a possible alternative explanation was recently provided by Emile et al,⁷ who observed, for the first time, important daily variations in the serum IL-6 levels, as detected by ELISA, of four untreated newly diagnosed patients with MM, suggesting that the interpretation of IL-6 levels require repeated determinations on different serum samples collected at diagnosis over a period of several days. We decided to provide an independent confirmation of this finding by studying the behavior of serum IL-6 levels in a different clinical phase of MM, ie, the progression of the disease from a plateau phase. We applied the same highly sensitive ELISA method (Quantikine) used by Pelliniemi et al⁵ (range, <0.4 to 10.0 ng/L; median, 1.6 ng/L in 72 healthy controls) and measured the immunoreactive IL-6 concentrations (twice daily on 3 consecutive days) in the sera of four patients with MM and of two normal volunteers as controls. The four selected patients with MM (from 57 to 63 years of age) had been treated with conventional chemotherapy entering a plateau phase of variable duration (1 to 3 years), but, at the time of this study, they all showed signs of disease progression, including an increase in paraprotein or urinary light chain excretion

and an increase of serum β_2 microglobulin and C-reactive protein levels. We decided to measure serum levels of IL-6 to provide a confirmation of the clinical evolution of the patients' disease.⁸ However, when measurements were repeated twice daily and also over a period of 3 consecutive days, the serum levels of immunoreactive IL-6 resulted highly variable in two of the four patients examined (no. 2 and 3) and without any evident nycthemeral cycle (Table 1). Surprisingly, in patient no. 3, the IL-6 levels were extremely high at the first measurement in the morning but were undetectable when measured in the afternoon. In the other two patients (no. 1 and 4), IL-6 levels remained constantly high, although still showing significant variations. In contrast, in the sera from healthy volunteers, IL-6 concentration was consistently low, without any detectable variations. IL-6 is stable in human serum,⁹ and its irregular and even dramatic variations observed in the serum of our patients probably indicate daily fluctuations in production and/or catabolism of the cytokine. We are aware of the fact that IL-6 production may be enhanced during inflammation, infection, renal failure, and any conditions that might complicate the course of MM⁶ independently of the tumor mass and the growth kinetics of myeloma cells. However, our findings strongly suggest that a unique serum IL-6 measurement should be interpreted with caution and requires multiple confirmations over a period of several days, not only when evaluating the prognostic value of this cytokine at diagnosis⁷ but also when monitoring the clinical evolution of MM. In fact, during the natural history of the disease, escape from plateau phase is mediated by a variety of factors inherent to the malignant cells or factors reflecting host-tumor interactions.⁸ Among the bewildering array of host factors that contribute to the escape of a malignant plasma cell population from a quiescent phase, IL-6 excess and/or IL-6 receptor dysfunctions are likely to play a major role.⁸ Thus, the determination of serum IL-6 levels might help to document, in combination with other parameters, the progression of the disease. However, our data suggest that, even when applying a sensitive ELISA,⁵ standardization of IL-6 measurement is still needed before it can be routinely used as a reliable marker of disease activity in the clinical management of single MM patients. As shown by Pelliniemi et al⁵ as well, acute-phase proteins provide a good surrogate for IL-6 measurements in MM.⁵ Thus, evaluation of acute-phase protein levels should be invariably combined with the determination of serum IL-6 levels, not only in early stage MM⁷ but also in end stage MM.

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Table 1. Daily Variations of Immunoreactive Serum IL-6 Levels in Patients With MM

Patient No.	Day 1		Day 2		Day 3	
	9 AM	5 PM	9 AM	5 PM	9 AM	5 PM
1	100	35	70	107	73	90
2	7.4	107	102	8	7.5	105
3	104	9	100	0	6.4	3
4	80	106	100	55	101	71

Values are in nanograms of IL-6 per liter.

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Failure of Interleukin-1 and Granulocyte-Macrophage Colony-Stimulating Factor to Enhance Allogeneic Marrow Engraftment and Survival in Irradiated Dogs

To the Editor:

A publication by Blazar et al¹ in *Blood* described enhancement of engraftment of H-2 incompatible murine marrow by interleukin-1 α (IL-1 α) after 600 to 750 cGy of total body irradiation (TBI) delivered at 41 cGy/min. Equally impressively, mice receiving a suboptimal dose of IL-1 α along with granulocyte-macrophage colony-stimulating factor (GM-CSF) had significantly higher levels of donor alloengraftment (92%) than mice receiving either IL-1 α (57%) alone, GM-CSF (18%) alone, or no growth factor (8%). Their findings are of potential interest for clinical application of these growth factors in individuals exposed to radiation accidents. In that setting, marrow allografts would be more successful if they could be used along with those hematopoietic growth factors that have the potential to stimulate engraftment.

We have developed a canine model for radiation accidents using a barely supralethal dose of 450 cGy of TBI.^{2,3} At that dose, all dogs not receiving marrow rescue die. With a DLA-identical marrow infusion, virtually all dogs show initial allogeneic engraftment. Subsequently, grafts are rejected in 65% of cases, and most of these dogs die during the ensuing second period of pancytopenia. Overall, 60% of the dogs survive and, of these, a quarter show autologous recovery, whereas three quarters have persisting allografts. We investigated the usefulness of canine recombinant granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) in this model and found that overall survival of dogs was improved to 88%, including all dogs receiving G-CSF and SCF combined.² The survival advantage of G-CSF- and SCF-treated dogs was not due to an increase in allogeneic engraftment but rather to a decrease in infections resulting from higher granulocyte counts during the entire post-transplant course.

In the current study, we used DLA-identical grafts after 450 cGy of TBI delivered at 7 cGy/min from two opposing ⁶⁰Co sources to study the combination of recombinant human IL-1 α and recombinant canine GM-CSF.⁴ We asked whether the survival of dogs so treated

could be improved and allogeneic engraftment enhanced by the growth factor combination. IL-1 α was administered as a continuous subcutaneous infusion at 2.5 μ g/kg/day and GM-CSF at 30 μ g/kg/day subcutaneously in divided doses, both from day 0 to 11. The littermate donor-recipient pairs were selected on the basis of identity for the serologically detectable canine histocompatibility antigens DLA-A and -B and by identity for restriction fragment length polymorphism patterns for canine major histocompatibility complex class II genes.^{2,3} Recipients received 2.1 to 4.0 (median, 2.4) $\times 10^8$ nucleated marrow cells per kilogram of body weight by intravenous infusion within hours of TBI.^{2,3} Dogs did not receive postgrafting immunosuppression. Postgrafting care has been described.^{2,3}

Table 1 compares the results in the four current dogs with those in historical (n = 5) and concurrent (n = 12) control dogs not receiving growth factor after transplant.^{2,3} All four dogs showed initial evidence of allogeneic engraftment as demonstrated by promptly increasing peripheral blood granulocyte counts after the postirradiation nadir. After early recovery, granulocyte counts declined again, and marrow aplasia developed, findings that were consistent with acute graft rejection. Three of the four dogs were euthanized between days 17 and 22 because of uncontrollable intercurrent infections. They had no evidence of graft-versus-host disease. Their marrows were profoundly hypocellular. One of the four dogs showed ultimate hematopoietic recovery. This dog was euthanized on day 145, at the end of the study. Examination of the marrow and peripheral blood cells in this dog by (CA)_n dinucleotide markers⁵ showed a mixture of donor and host cells on day 20. A repeat examination on day 60 showed only cells of host type. Results in the current dogs were not statistically significantly different from those in controls. It could be argued that the number of dogs receiving transplants is too small to draw firm conclusions. However, given the uniform graft failure observed, even if 10 dogs received transplants under the current protocol, and the additional six all showed sustained allografts, there would still not have been statistically significant evidence for graft enhancement by IL-1 α /GM-CSF.

Table 1. Results in Dogs Receiving 450 cGy TBI and Marrow Grafts From DLA-Identical Littermates With or Without IL-1 α and GM-CSF

IL-1 α and GM-CSF	No. of Dogs					
	Studied	Graft Rejection	Early Death With Aplasia	Complete Allograft	Mixed Chimerism*	Autologous Recovery
Yes	4	4	3	0	0	1
No	17	11	7	3	3	4

* Mixed chimerism was assumed if either cytogenetics, dinucleotide (CA)_n repeat markers, or both showed mixtures of host and donor hematopoietic cells.