Interleukin-1 Production in Patients with Nonlymphocytic Leukemia and Myelodysplastic Syndromes

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A common feature of all cases of myeloid leukemia is a block in normal maturation of blast cells which may be associated with the disturbance in the biochemical pathways of receptor cross-modulation or with the control of expression of the genes encoding various differentiation factors [9].

In some cases the leukemic cells can produce colony-stimulating factor (CSF) [6]. It should be noted that leukemic cells from many patients do not proliferate in vitro in the presence of CSF, but those that do not grow in vitro obviously may proliferate in vivo [9]. These findings suggest that there may be some other hematopoietic growth factors which participate in the regulation of leukemic cell proliferation. One of them may be interleukin-1 (IL-1).

IL-1 does not induce normal hematopoietic cell colonies but has been reported to act synergistically with the colony-inducing cytokines (IL-3, CSFs) [8, 12]. On the other hand, it had been revealed that IL-1 also participates in the regulation of leukemic myelopoiesis. IL-1 can induce differentiation in certain murine myeloid leukemic clones (SL, mouse r1) so that this is mediated by the endogeneous production of the differentiation-inducing protein MGI2 and is also associated with the production of granulocyte-macrophage CSF (GM-CSF) [5].

The present work was carried out to determine IL-1 production in patients with myelodysplastic syndrome (MDS) and nonlymphocytic leukemia (NLL). MDS represents a diverse group of disorders with mild, relatively benign form and disorders with impressive symptoms and rapid progression to overt leukemia. In all instances MDS is a typical clonal disease in which the abnormal hematopoietic cells are derived from the mutated stem cell and the malignant clone progressively replaces normal hematopoiesis. It is especially interesting to examine the role of IL-1 in the transformation of MDS to leukemia.

Materials and Methods

Patients with acute NLL and MDS were examined. The diagnosis of acute myeloid leukemia (AML), acute myelomonocytic leukemia (AMML), or MDS was made using FAB criteria following conventional cytochemical staining. All blood samples were obtained prior to therapeutic manipulations or 2–3 weeks after the last steroid dose or chemotherapy.

Mononuclear cells were prepared by Ficoll-Pack sedimentation. Adherent cells were cultured for 24 h in a CO_2 incubator in Dulbecco's medium supplemented with 5% fetal calf serum with or without lipopolysaccharide (LPS) from *Bacillus prodigiosum*. Spontaneous and LPS-induced IL-1 production was determined by conventional murine thymocyte comitogenic assay [7]. IL-1 concentration was calculated using a standard recombinant human IL-1 B preparation and expressed in units per milli-

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Haematology and Blood Transfusion Vol. 35
Modern Trends in Human Leukemia IX
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meter and in units per million adherent cells.

Data were analyzed by nonparametric methods to avoid assumptions as to the distribution of the variables under study, using the Mann-Whitney test. χ^2 criteria were also used. Values are given as mean ± SEM.

Results

Spontaneous and Induced IL-1 Production in Acute Leukemia

Spontaneous IL-1 production was found in 5 of 14 examined patients with acute NLL (ANL) (Table 1). Various levels of spontaneous IL-1 production were also found in 3 of 18 healthy donors examined (30, 80 and 160 U/ml). Differences in spontaneous IL-1 production were found between the ANL patients and healthy donors (see Table 1). The induced IL-1 production in patients with ANL was 96.9 ± 29.5 U/ml and it did not differ from the rate in the control group. However, IL-1 production in patients with AML was similar to that in patients with AMML. Induced IL-1 production was not detectable in 5 of 14 patients with ANL but it was found in all healthy donors.

Figure 1 shows the relation between the patients' adherent cell counts and IL-1 production in U/ml culture media and IL-1 production per 10^6 adherent cells. It is clear that the decrease in IL-1 production in some patients was not caused by the decrease in adherent cell counts. In most patients there was a normal rate of IL-1 production and a rather high rate of production of IL-1 per cell. In two patients, the adequate IL-1 production was due to increased production of IL-1 per cell, accompanied by simultaneously decreased adherent cell count. In the other patient, IL-1 production was significantly higher, but it may be considered to be caused by increased adherent cell counts and by the cells enhanced ability to produce IL-1 in these patients. The data indicate that patients with acute leukemia represent a diverse group in relation to IL-1 production (Fig. 2).

Spontaneous and Induced IL-1 Production in Patients with MDS

Spontaneous IL-1 production was determined in 3 of 6 patients examined with refractory anemia (RA) without excess blasts (Table 1). In all patients with chronic myelomonocytic leukemia (CMML) spontaneous IL-1 production was found. Its level was significantly higher than in the control group. Induced IL-1 production in patients with RA did not differ from that in healthy donors (Table 2). Decreased induced IL-1 production were found in patients with RA with excess blasts (RAEB) and with RAEB in transformation (RAEBtr). Patients with CMML had greatly increased levels of both spontaneous and induced IL-1 production. The relation between the CMML patients' adherent cell counts

Table 1. Spontaneous IL-1 production (mean ± SEM) in patients with acute leukemia and MDS

Groups of patients	Number of patients	Patients with spontaneous IL-1 production	Spontaneous IL-1 production	
			(U/ml)	($U/10^6$ cells)
Healthy subjects	18	3	110 ± 33	1413 ± 420
ANL	14	4	2077 ± 1927	18270 ± 13843
RA	6	3	279 ± 148	2198 ± 1414
RAEB + RAEBtr	5	0	0	0
CMML	5	5	4232 ± 3872	ND

ND, no data.

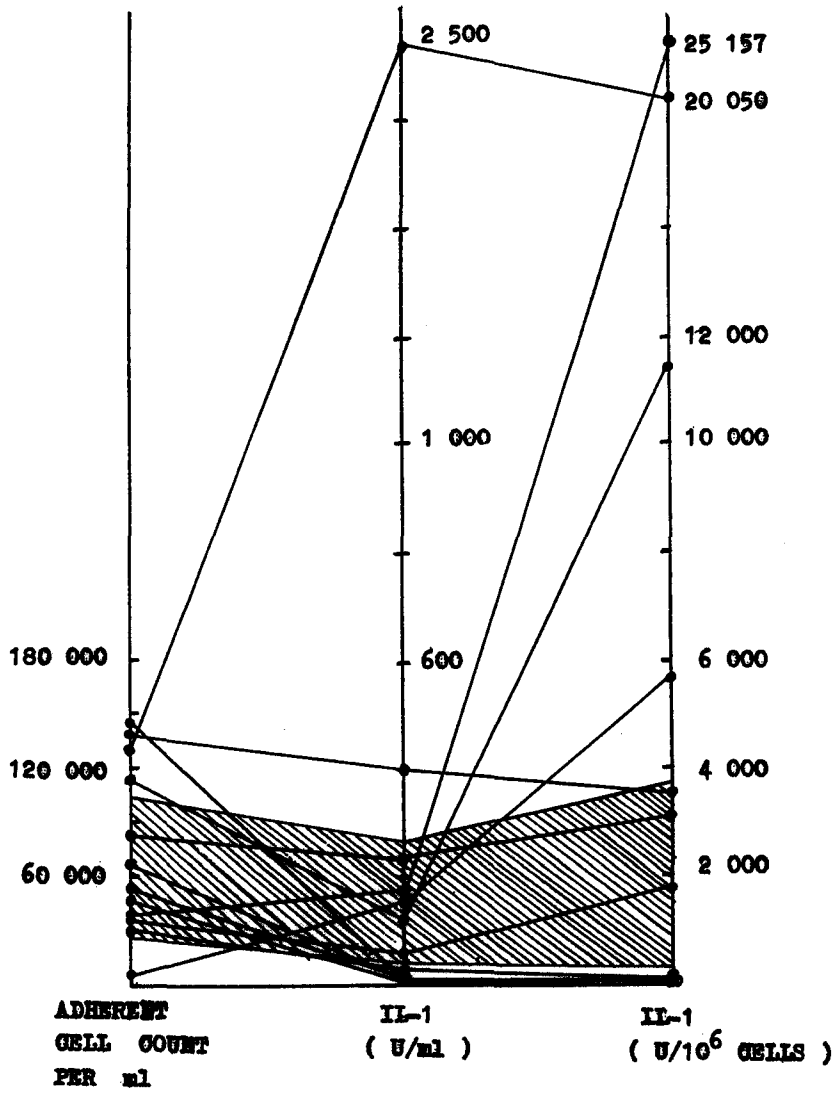

Fig. 1. Correlation between the adherent cell counts and IL-1 production in patients with acute leukemia

Table 2. Induced IL-1 production (mean ±SEM) in patients with acute leukemia and myelodysplastic syndromes

Groups of patients	Number of patients	Patients without detectable IL-1 production	LPS-induced IL-1 production		
			U/ml	U/10^6 cells	χ^2
Healthy subjects	15	0	123±30	1721±480	
ANL	14	5	102±33	4251±1908	8.28 ($\beta<0.057$
AML	9	4	79±33	4127±827	
AMML	5	1	154±86	4531±1701	
RA	6	1	277±92	2982±993	
RAEB+RAEBtr	5	3	40±25[a]	464±354	5.76 ($\beta<0.05$)
AML after myelodysplasia	5	2	118±66	5368±4022	
CMML	5	0	4756±3528	10915±4646	

[a] Analysis was performed by the nonparametric Mann-Whithney test, $\beta<0.05$.

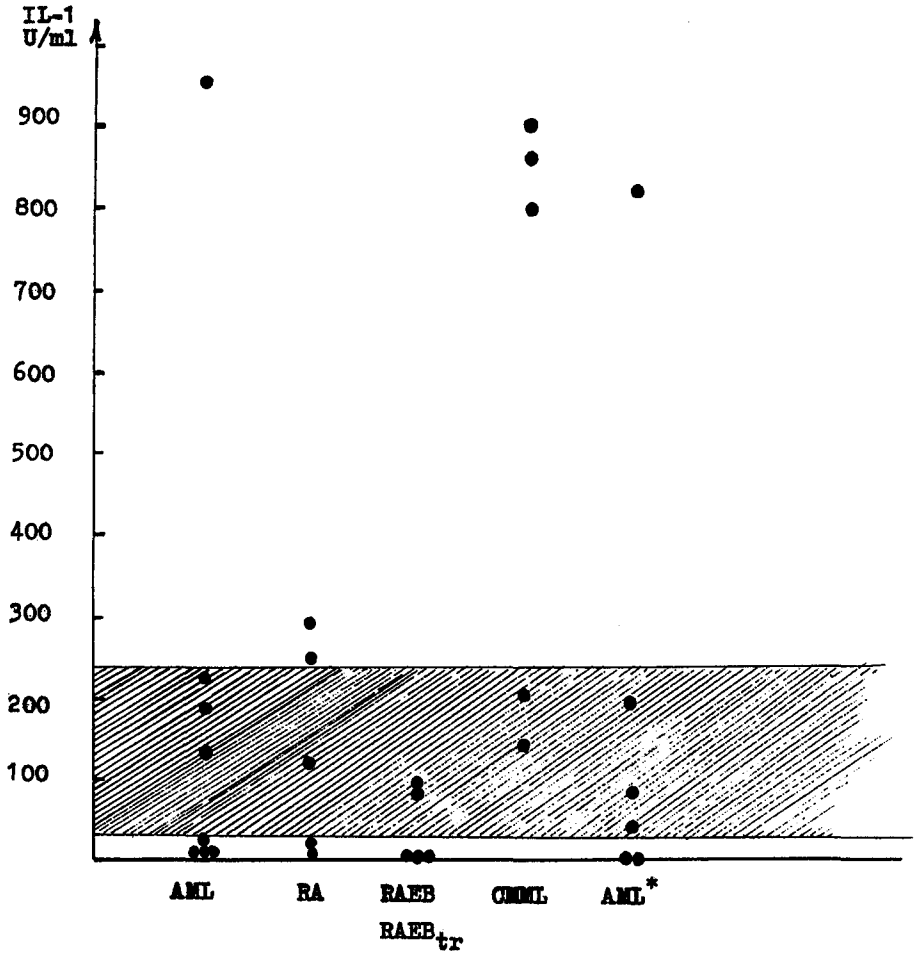

Fig. 2. Induced IL-1 production in patients with ANL and MDS. The *shaded area* represents the normal values (M + δ). In 27% of patients with MDS and 35% of patients with acute leukemia no IL-1 production was detectable.

and IL-1 production is presented in Fig. 3. Patients with CMML also represent a diverse group – 3 patients had a higher IL-1 production level than others. IL-1 production in patients with ANL differed significantly from that of CMML and CML patients. In some patients IL-1 production was not determined.

Survival was analyzed in patients with normal IL-1 production and those with decreased IL-1 production (Fig. 4). The mean value of IL-1 production in healthy donors was 122.7 ± 30.0 U/ml. A normal induced IL-1 production level was considered as one which was higher than the mean value in healthy donors minus SE, while a decreased level was considered to be lower than the mean value minus SE. No significant differences in survival of patients with normal or decreased IL-1 production levels were found.

Discussion

In this report we have demonstrated that IL-1 production by peripheral blood monocytes is not identical in patients with acute leukemia and in patients with MDS. In every group of patients there were significant differences in IL-1 production. The data imply that leukemia and MDS represent a diverse group of disorders with markedly different substrates of proliferation and different levels of cellular maturation. In all instances, MDS is a typical clonal disease in which the abnormal hematopoietic cells are derived from the same mutated stem cell but

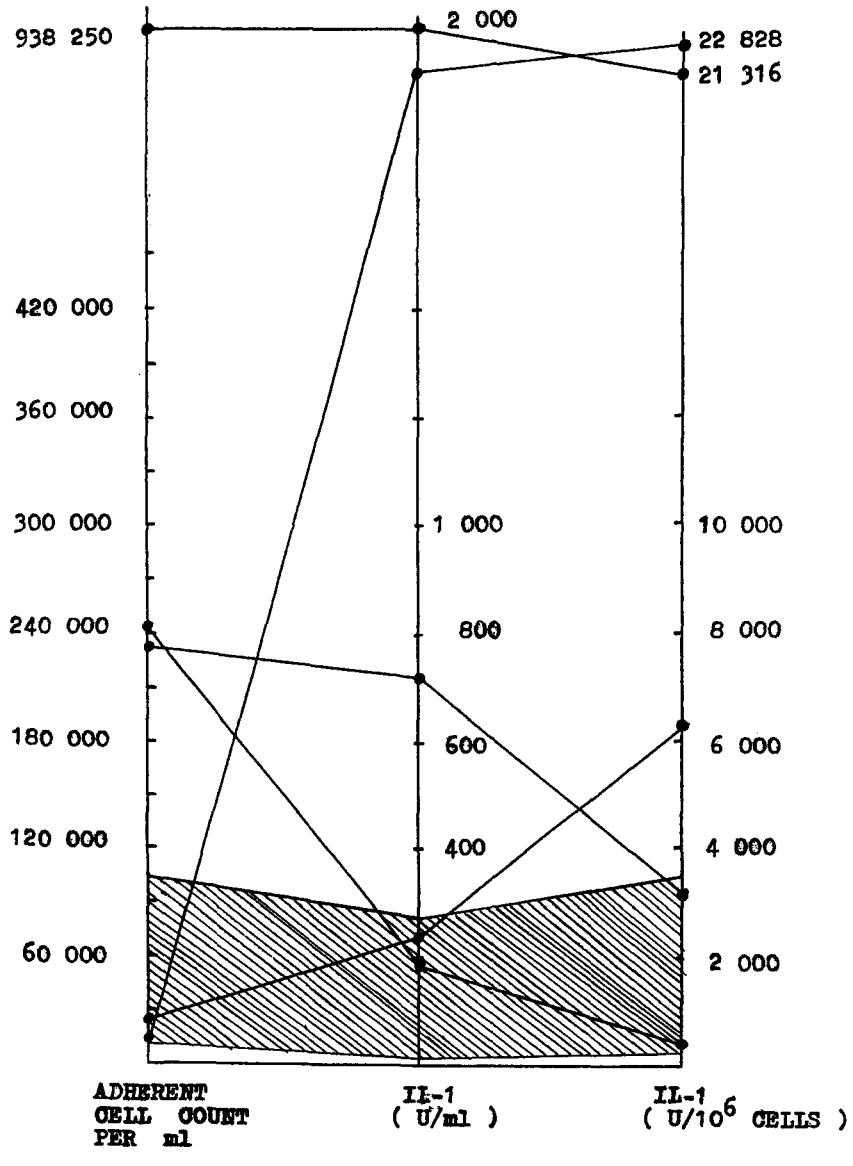

Fig. 3. Correlation between the adherent cell counts and IL-1 production in CMML patients

where clinical features and functional properties of abnormal cells may be different. Cells derived from the mutated clone often retain some form of maturation, leading to the peripheral appearence of abnormally differentiated cells with impaired function [9].

We have found two forms of disturbance of IL-1 production in patients with ANL and MDS. In the first case the IL-1 production was significantly increased; an especially high level was found in patients with CMML and in some patients with AMML. We suggest that the high IL-1 production was due to both an increase in monocytic cell counts in some patients and enhanced ability of individual cells to produce IL-1. Recently, it has been shown that monocytes of MDS patients may be abnormally differentiated cells [1]. Thus, the observed increase in IL-1 production in MDS patients may be due to enhanced IL-1 gene expression in these cells. The data obtained on the elevated induced IL-1 production in some patients agree with data from other authors. IL-1 may support proliferation of leukemia cells in patients with ANL [2–4, 10]. When freshly isolated adult T-cell leukemia (ATL) cells were cultured with recombinant or natural human IL-1a or IL-1b, the

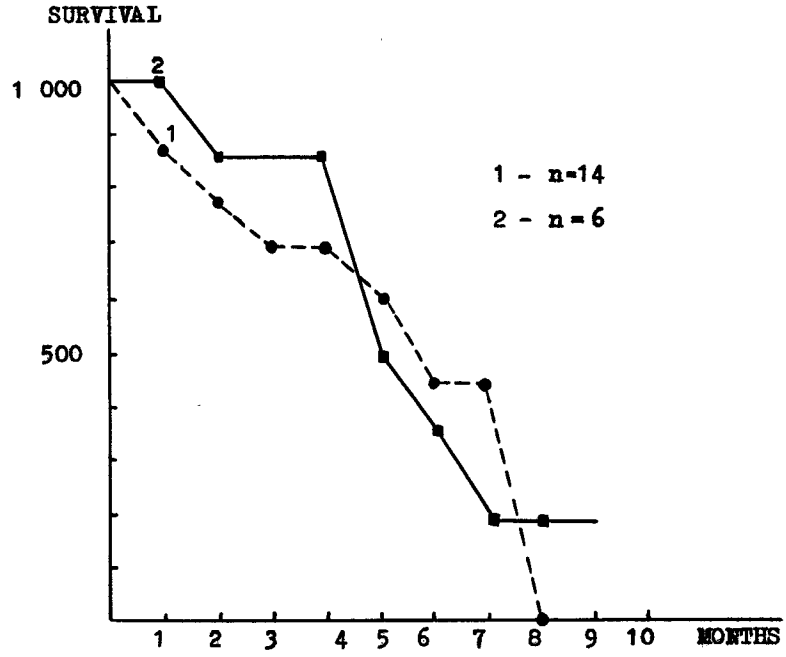

Fig. 4. Survival of patients with acute leukemia with various IL-1 production levels. *1* > M ± SE, *2* < M ± SE. No significant difference was revealed

growth of ATL cells was stimulated in a dose-dependent manner [11].

Regarding patients with MDS, a decreased induced IL-1 production was revealed in the group with RAEB plus RAEBtr, but in other groups there were no differences from the healthy donors. RAEB, RAEBtr, and overt leukemia may be considered as stages of one process, but the mechanism of transformation is not absolutely clear. It is possible that the decrease in IL-1 production in these patients may assist in the progression of disease. In the next stage of progression it is replaced by increased IL-1 production, which could support the leukemic proliferation.

On the other hand, in 27% of patients with MDS and in 35% of patients with ANL, no induced IL-1 production was detectable. What is the role of the lack of IL-1 production in the development of leukemia? This question remains unsolved, because even high IL-1 production does not guarantee the best survival.

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