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Natural Killer Activity in Preleukemic States

P. Obłąkowski [1]

A. Introduction

Natural killer cells (NKC) were first identified by their ability to kill without prior immunization certain tumor target cells grown in vitro [15, 16, 20]. In preliminary observations they were defined only in negative terms: that is, they were not thymus-derived (T) or bone marrow-derived (B)-lymphocytes, nor were they adherent or phagocytes, and they lacked demonstrable surface membrane immunoglobulin [17]. Most human blood NKC bear an Fcγ receptor [23]. As with rodents, a number of observations suggested that NKC were not necessarily divorced from the T-cell lineage. For example, they reacted with anti-T serums and anti-T monoclonal antibodies [5, 9, 11]. On the basis of the partial isolation of NKC on Percoll, they were characterized as identical to large granular lymphocytes (LGL) with cytoplasmic azurophilic granules [1, 21]. NKC and their regulation by interferons (IFN) and interleukin-2 (IL-2) are proposed to be one of the important factors in tumor immunosurveillance and tumor resistance [3, 4, 8, 14, 18, 22]. A significant reduction in NKC activity has been demonstrated in patients with various disorders such as the Chédiak-Higashi syndrome which are known for their high incidence of malignant diseases [6, 13]. It was of interest to examine NKC activity in preleukemic states and to determine its influence on the development of the disease.

[1] Department of Internal Medicine, Institute of Hematology, 00-957 Warsaw, Poland

B. Patients and Controls

Natural killer (NK) activity was determined in 20 patients ranging in age from 30 to 79 years (mean 59.9). There were six cases of acquired idiopathic sideroblastic anemia (AISA), nine cases of refractory anemia (RA), four cases of refractory anemia with an excess of blasts (RAEB), and one case of refractory anemia with an excess of blasts in transformation (RAEBt). The patients were classified as AISA, RA, RAEB, and RAEBt according to the FAB classification of myelodysplastic (preleukemic) syndromes [2] (Table 1). All patients had less than 10% of blasts in peripheral blood, and none received drugs which might influence NK cell activity. A control group constituted of 56 healthy blood donors.

Table 1. Diagnosis in 20 patients examined

	No. of patients	
	Female	Male
Acquired idiopathic sideroblastic anemia (AISA)	5	1
Refractory anemia (RA)	7	2
Refractory anemia with an excess of blasts (RAEB)	3	1
RAEB in transformation	0	1

C. Effector Cells

Effector cells were obtained by sedimentation of heparinized blood on "Lymphoprep". Mononuclear cells were washed and resuspended in MEM supplemented with 10% FCS.

D. Target Cells

Cells of the K562 line derived from a patient with blast crisis in CML were used as targets [7]. K562 cells were cultured in RPMI 1640 medium containing 10% FCS, gentamicin and L-glutamine, under standard conditions.

E. Cytotoxicity Assay

NK activity of mononuclear cells was measured in a 4-h cytotoxicity test with ^{51}Cr-labeled K562 cells as targets [16]. After 4-h incubation of effector cells together with targets in a 20:1 ratio, cells were centrifuged and supernatants were collected for determination of released ^{51}Cr in a gamma scintillation counter.

The percentage of cytolysis was calculated according to the formula:

$$\% \text{ CTX} = \frac{\text{experimental cpm} - \text{spontaneous cpm}}{\text{maximal cpm} - \text{spontaneous cpm}} \times 100$$

Maximal cpm was obtained by the incubation of target cells in the presence of 1% Triton X-100; spontaneous cpm was obtained by the incubation of target cells in MEM containing 10% FCS.

The results revealed strong suppression of NK activity in all of the preleukemic patients (11.5% ± 10.1%) and in each of the diagnosed syndromes, as compared with the control group (30.6% ± 11.5%; Table 2 and Fig. 1). The difference was statistically significant ($P < 0.01$) in the Wilcoxon–Mann-Whitney test. Similar changes in NK activity in preleukemia have been detected by others [12, 19]. There are reports that suppression of NK activity was not connected with dilution of effector cells by blasts or by a reduced frequency of LGL in mononuclear cells of examined patients. Takagi and co-workers suggested that suppression of NK activity in preleukemic patients was caused by the impaired IFN-linked regulatory system of NKC [19].

In this study, two of the 20 investigated patients developed leukemia 2–3 months after diagnosis. Both cases showed very low NK activity:

1. Patient M.S. (RAEB) %CTX = −4.2%
2. Patient W.M. (RAEBt) %CTX = −1.7%

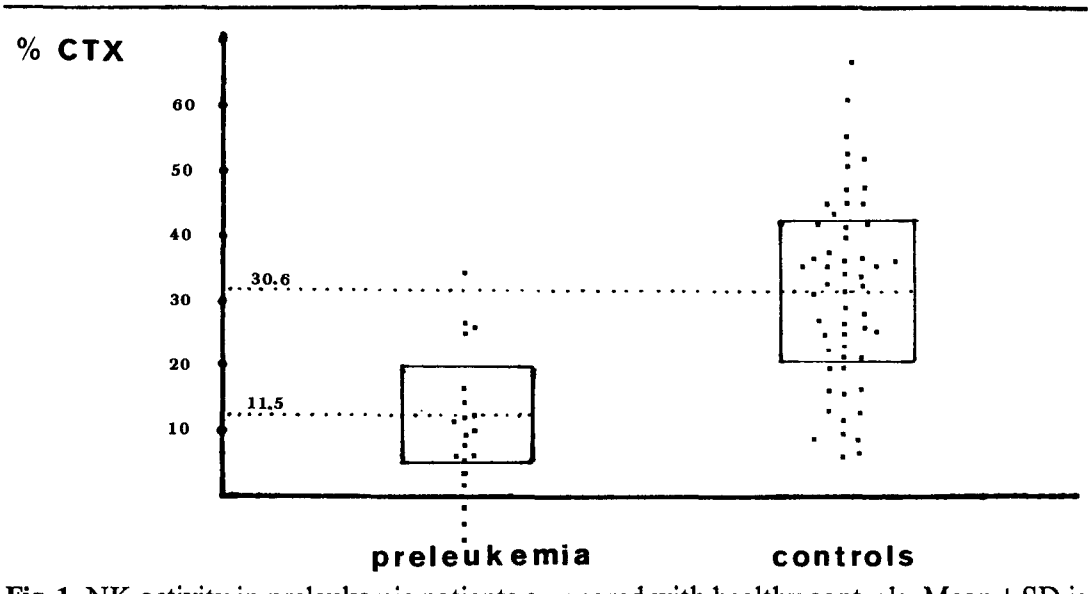

Fig. 1. NK activity in preleukemic patients compared with healthy controls. Mean ± SD is indicated for each group

Table 2. Percentage of NK activity in 20 patients according to preleukemic syndrome

Patient		AISA	RA	RAEB	RAEB t
Female	Male	Cytotoxicity (%)			
W. L.		8.0			
J. S.		25.9			
S. P.		27.6			
	S. G.	8.6			
K. K.		13.1			
M. W.		10.9			
I. D.			3.9		
	Z. N.		8.7		
K. L.			11.4		
T. S.			11.2		
A. G.			− 8.9		
M. K.			2.4		
J. F.			2.9		
	E. M.		25.7		
M. N.			30.2		
M. S.				− 4.2	
	T. H.			4.9	
H. K.				12.2	
F. D.				25.4	
	W. M.				−1.7
Mean		15.6	9.7	9.5	−1.7

These preliminary observations show that very low NK activity might be connected with a high risk for overt leukemia. Furthermore, the preleukemic patients were divided into two groups on the basis of their risk for leukemic transformation. The "high-risk" group, consisting of patients with RA, RAEB, and RAEBt, showed a mean NK activity of 5.8%. The "low-risk " group, consisting of patients diagnosed as having AISA, which has a better prognosis for long survival, showed a higher mean percentage of NK activity (15.6%), but the difference was not statistically significant (Table 2).

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