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Nonspecific Cross-Reacting Antigen as a Marker of Myelocytic Leukemias in Individual Stages of Myelocytic Cell Differentiation *

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The aim of the study was to establish:

1. The distribution of nonspecific cross-reacting antigen (NCA) and carcinoembryonic antigen (CEA) in cells of different types of myelocytic leukemias
2. The presence of NCA in individual stages of granulocyte differentiation
3. NCA and CEA serum levels

A total of 17 acute myelocytic leukemia (AML) cases were studied, classified according to the proposals of the FAB cooperative group, and 14 cases of chronic granulocytic leukemia (CGL) – 5 of these were in myeloblastic crisis (CGL-BC) and formed a separate group. The control studies were performed on cells of 6 ALL patients and on granulocytes of 6 normal donors.

To separate the myelocyte cells into fractions containing granulocytes in individual stages of maturation, discontinuous density gradient centrifugation (Ficoll–Hypaque 1.05–1.105 g/ml) was applied. Dextran isolates were prepared in only some AML cases. The cells of each density layer were checked for NCA and CEA by immunofluorescence (IF) and were also stained with Wright–Giemsa to determine differential morphology.

The anti-CEA and anti-NCA sera used in IF were additionally absorbed on columns prepared by coupling purified CEA or NCA to CNBr-activated sepharose 4B to remove anti-CEA or anti-NCA activity, respectively. The results of NCA content in different types of AML are summarized in Table 1. The number of NCA-positive cells increased from individual blasts of AML with features of maturation (Fig. 1). Monoblastic leukemias were usually NCA negative and, in one erythroleukemia case, the percentage of fluorescent cells was similar to the amount of mature granulocytes. The analysis for NCA in patients with CGL-BC and CGL isolated by density gradient centrifugation showed that expression of this antigen increased as more mature cells in denser layers were obtained (Table 2). The comparison of fluorescence with phase-contrast pictures showed that some blasts from CGL-BC and individual blasts detectable in the chronic phase of CGL showed distinct cytoplasmic NCA-dependent staining. Many pathologic myelocytes and metamyelocytes were NCA positive (40%–70%), but their amount and fluorescence intensity varied from one case to another (Fig. 2). The fluorescence of mature neutrophils, focused mainly in fractions 1.09–1.105 g/ml, was observed in 80%–90% of cells. Lymphoblasts of ALL patients and healthy donors' lymphocytes were always negative. Anti-CEA serum stained neither AML, ALL, nor any fraction of CGL-BC and CGL cells.

Serum NCA levels in patients with AML and ALL were very low or undetectable (Table 1). In CGL-BC and CGL patients, circulating NCA was always elevated to a mean value of 100 and 140 ng/ml, respectively. Serum CEA was within the normal range (0–7 ng/ml) in all patients studied.

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Table 1. NCA content in peripheral blood cells and serum of AML patients

Leukemias	Number of cases	Wright-Giemsa morphology (%)[b]				IF NCA-positive cells (%)	NAC serum level (ng/ml)
		Blasts	PMN	Mon	Lym		
AML classification[a]							
M0 (without maturation)	2	81	2		17	0.5	5.0
M1 (weak maturation)	2	88	4		8	6.0	3.0
M2 (distinct maturation)	4	81	9		10	13.0	0.0
M4 (myelomonocytic)	3	87	9		4	5.0	0.0
M5 (monoblastic)	5	77	6	6	11	4.0	7.0
M6 (erythroleukemia)	1	19	81			70.0	30.0
ALL	6	74	6		20	0.0	7.5

[a] According to FAB cooperative group

[b] Blasts = myeloblasts; PMN = polymorphonuclear neutrophils; Mon = monocytes; Lym = lymphocytes

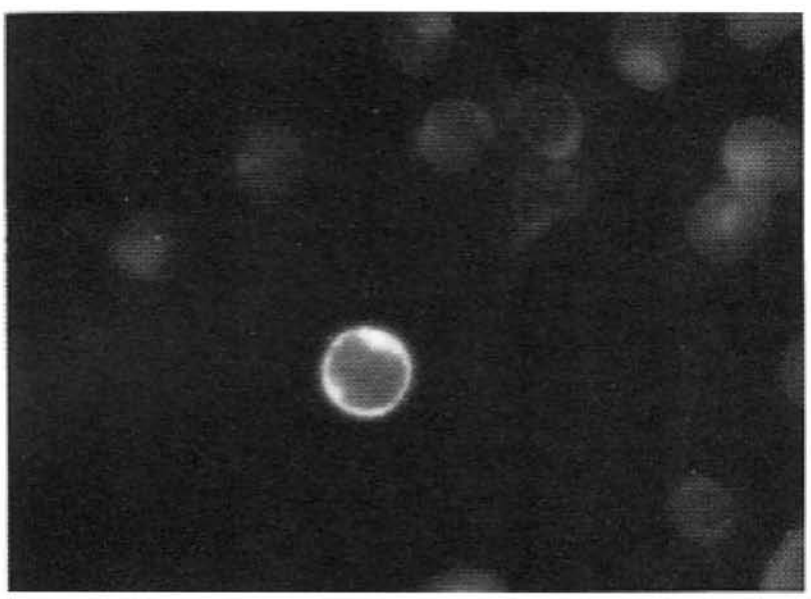

Fig. 1. AML (M2) cells treated with anti-NCA serum. Cytoplasmic fluorescence of an individual blast

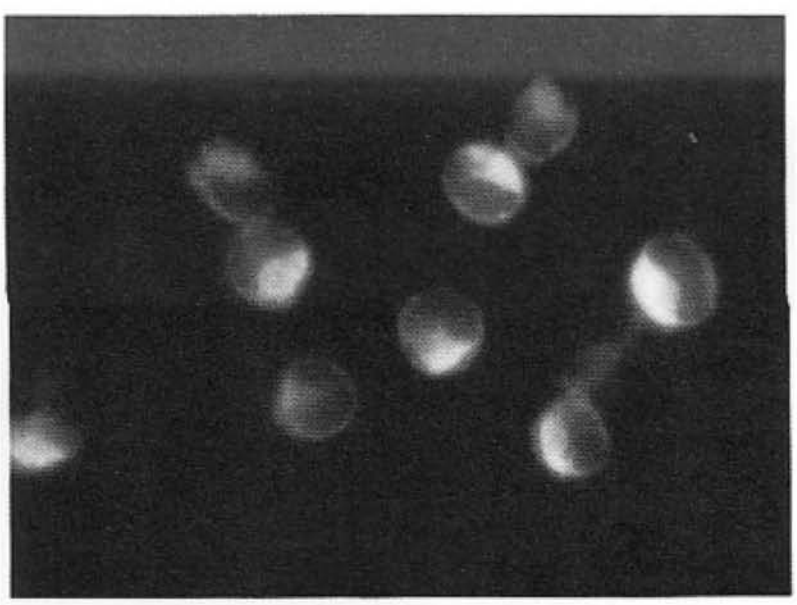

Fig. 2. CGL cells isolated in 1.07 g/ml fraction treated with anti-NCA serum. Cytoplasmic fluorescence of majority myelocytes and metamyelocytes

Table 2. NCA content in peripheral blood cells separated by density gradient centrifugation in CGL, CGL-BC patients, and normal donors

Material	Number of cases	Density layer (g/ml)	Wright-Giemsa morphology (mean %)					IF test NCA-positive cells (%)	NCA serum level (ng/ml)
			Blasts Pro	Myel[a] Mta	Band PMN	Eos Bas	Lym		
CGL	9	Dextran	6.7	36.5	50.0	2.6	4.2	79.8	142.0
		1.06–1.07	3.6	49.8	37.4	4.8	4.4	57.4	(20.0–410.0)
		1.08–1.09	1.2	25.0	67.9	5.2	0.7	92.5	
		1.105		21.5	70.3	8.2		92.8	
CGL-BC	5	Dextran	22.3	36.7	41.0			64.8	100.0
		1.05	62.0	30.0	8.0			18.3	
		1.06–1.07	21.0	38.3	37.0	3.0	0.7	53.6	
		1.08–1.09	9.0	21.7	65.3	3.5	0.5	86.1	
		1.105	0.7	14.6	82.0	2.7		83.7	
Normal granulocytes	6	1.105			92.0	3.0	5.0	90.0	30.0

[a] Myel = myelocytes; Band = band forms; Eos = eosinophils; Pro = promyelocytes; Mta = metamyelocytes; Bas = basophils. For other abbreviations see the legend to Table 1

Parallel studies by IF and immunodiffusion (ID) showed the immunologic relationship of the NCA extracted from CGL cells and purified from normal lung tissue.

Our results may be summarized as follows:

1. AML blasts without the ability to mature (M0) and monoblasts did not synthesize NCA.
2. Individual AML blasts with features of maturation (M1, M2) and some myeloblasts of CGL-BC showed limited ability to express cytoplasmic NCA.
3. The number of NCA-containing cells increased as the more mature granulocyte fractions were isolated on Ficoll –Hypaque density gradients.
4. Myelocytic NCA is immunologically related to NCA isolated from normal lung tissue.
5. CEA is undetectable in myelocytic cell series.