Morphologic Characteristics of Human Blood Cells in Semi-Solid Marrow Cultures

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Blood cells proliferated and differentiated in vitro should be classified according to the classical hematologic nomenclature only after unequivocal identification by classical hematologic techniques. This has been done by Maxinow [6] and others at the beginning of this century, more than 50 years ago, when they established their in vitro methods of bone marrow in semi-solid media. In contrast, modern experimental hematologists sometimes use the nearly 100 years old classification of human blood cells which is rather uncritical to characterize blood cells grown in vitro.

Materials and Methods

8870 colonies of bone marrow cultures from 301 patients have been investigated cytologically (Pappenheim staining), cytochemically (peroxidase, acid phosphatase, α-N-esterase) or immunocytochemically (FITC conjugated anti human immunoglobulins).

Agar cultures were prepared using the double layer agar technique of Pike and Robinson [12]. Plasma clot cultures were prepared according to Axelrad [13] and Tepperman [14], modified by Hellwege [5]. Instead of erythropoietin placenta conditioned medium [4] was used to stimulate the granulopoietic cell differentiation and proliferation.

Results

1. Agar gel culture (Table 1): We mainly found colonies with macrophages, always strongly acid phosphatase positive, and monocytes, weakly peroxidase positive. In addition, strongly peroxidase positive pure eosinophilic colonies and mixed colonies of monocytes and eosinophils were observed. Besides this, we found plasma cells in colonies from leukemic patients and two normal persons, and blast cells only in leukemic patients. Using

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Table 1. Cytological classification of colonies (%) grown in soft agar with leukocyte feeder layer

	number of patients	number of colonies analyzed	mono-cytes[a]	macro-phages	eosino-phils[b]	plasma cells[c]	blasts
normal and leukemic patients in remission	184	1978	77.5	8	6.5	7	1[d]
ALL untreated	21	164	81	10	4	4	1
AML untreated	12	74	19	—	32	41	8
	217	2216					

[a] mostly monocytes and macrophages
[b] 50% pure eosinophils, 50% mixed colonies with eosinophils
[c] mixed colonies with plasma cells (demonstrated by immunofluorescence)
[d] blast cell colonies from leukemic patients in remission

immunofluorescence techniques, we were able to show that plasma cells in vitro produce immunoglobulins [1,2,11].

2. *Plasma clot culture* (Table 2): In contrast to agar gel culture, we only found very few macrophage colonies, but mainly monocytoid (Fig. 1b) and eosinophile (Fig. 1a) cells in plasma clot cultures. The monocytoid cells were peroxidase positive (Fig. 1b, c, d) and therefore belong to the myeloid cell lines. However, they could not clearly be identified as neutrophils since the stainings were rather diffuses and not granular (Fig. 1b). In contrast to bone marrow cultures from normal persons we found a higher amount of peroxidase negative colonies (Table 3, Fig. 1e) in leukemic patients. In addition, we found further evidence for abnormal differentiation as a rather weak peroxidase reaction and an apparent vacuolization of the cytoplasm (Fig. 1f). Quantity and size of colonies were also diminished in leukemic patients. The percentage of cluster in relation to colonies became normal about two years after having finished therapy (Table 3).

Table 2. Cytological classification of colonies (%) grown in plasma clots with placenta conditioned medium

	number of patients	number of colonies analyzed	pure eosinophile colonies	colonies of peroxidase positive monocytoid cells[a]	peroxidase negative colonies	mixed colonies of eosinophils and monocytes
normal bone marrow	22	2612	31.4	65.0	0.8	2.8
ALL in therapy	33	1914	38.0	45.8	15.0	1.2
ALL in remission and without therapy	29	2182	28.0	63.0	8.3	0.7
	84	6708				

[a] very rarely macrophages

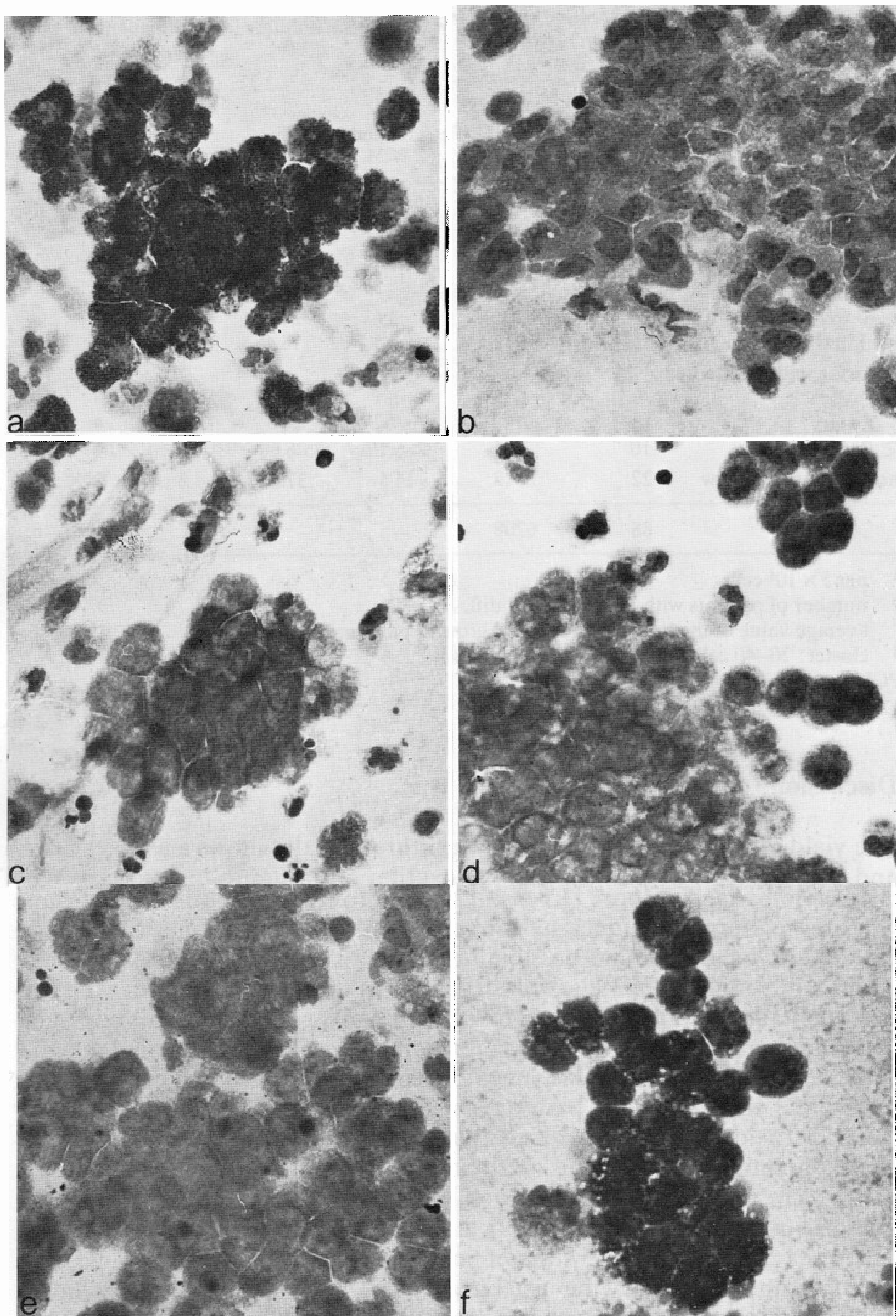

Fig. 1. Colonies of plasma clot cultures from human bone marrow **a)** peroxidase positive eosinophile colony **b)** peroxidase positive monocytoide colony **c)** small peroxidase positive colony (cluster) **d)** part of a large peroxidase positive colony **e)** peroxidase negative colony **f)** eosinophils with vacuoles **e + f** = colonies of bone marrow cultures from leukemic patients

Table 3. Quantitative and qualitative criteria in plasma clot cultures from all patients in different stages of the disease

	number of patients	number of colonies analyzed	colonies[a,c] without PCM	with PCM	[c,d] cluster colony	unnormal cell diff.[b]
relapse without therapy	4	10	0	<1		
relapse with therapy						
≲ 9 weeks	8	301	1.1	13.2	4.8	5
remission with therapy						
9–40 weeks	10	701	4.6	24.6	4.0	8
40–130 weeks	15	912	4.5	18.4	4.3	6
remission without therapy						
≲ 1 year	9	630	8.1	18.3	4.7	4
1–2 years	10	751	7.2	24.6	2.7	4
≳ 2 years	10	801	6.1	26.6	1.3	4
normal bone marrow	22	2612	14.5	37.0	1.8	0
	88	6708				

[a] per 5×10^4 cells
[b] number of patients with unnormal cell diff.
[c] average value of the patients' individual growth patterns
[d] cluster: 20–40 cells to avoid background faults
PCM = placenta conditioned medium

Discussion

The yield of colonies in bone marrow cultures usually shows a wide variation and therefore is no reliable diagnostic criterion in the follow up control of leukemic patients. Moore [7,8,9,10] has improved the significance of bone marrow culture techniques by introducing the cluster-colony relationship in AML, which we found possible higher in risk patients. The plasma clot technique for granulopoietic cells described in this paper allows an even better qualitative interpretation of growth criteria. We describe in this paper additional qualitative growth criteria for bone marrow cultures from leukemic patients. These are peroxidase negative colonies (Fig. 1e) and cells showing unnormal differentiation patterns as vacuolization (Fig. 1f) and a rather weak peroxidase reaction. These criteria are of additional value for the diagnostic identification of bone marrow from normal and leukemic patients. They were found in bone marrow cultures from patients in remission even after as many as six years without therapy. It is reasonable to assume that there is a pathological alteration of bone marrow stem cells in these patients which is not reflected by the usual myelogram and colony number counting in bone marrow culture. Further follow-up studies will have to show whether these additional criteria might become significant with regard to diagnostics of preleukemic states and therapy of leukemic patients and whether the plasma clot method will prove to be more efficient than other in vitro techniques.

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