Ann Hematol (1992) 64: 210–216

Annals of
Hematology

Original article

Differences of cellular composition and adhesion molecule expression in "leukemic" as compared with "normal" human long-term bone marrow cultures

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Received August 19, 1991 / Accepted March 3, 1992

Summary. Human long-term bone marrow cultures (HLTBMCs) were established with bone marrow samples collected from 15 patients with acute myeloid leukemia (AML) and compared with HLTBMCs from eight healthy volunteers. During 6 weeks of culture, the cellular composition of HLTBMCs was quantitatively studied. The cells of the HLTBMCs were divided into three main categories: fibroblasts, macrophages, and 'other cells' (endothelial cells, hematopoietic cells and undefined cells). HLTBMCs derived from healthy volunteers demonstrated a very consistent development. The number of fibroblasts increased during culture and the number of macrophages decreased, resulting in a steady state after 3 weeks of culture. In contrast, HLTBMCs derived from patients with AML showed a strikingly different pattern of irregular development and a steady state was not reached under our conditions. The APAAP technique was used to demonstrate expression of adhesion molecules. VLA2, VLA5, VLA6, LFA1, Mac1, p150/95, β_2-chain, HCAM, ICAM1, NCAM, and VCAM1 were more expressed on 'normal' as compared with 'leukemic' bone marrow stromal cells, although this reached significance only for β_2-chain and NCAM. VLA1, 3, and 4 were expressed in a higher percentage on 'leukemic' stroma (not significant). More expression was seen on 'normal' as opposed to 'leukemic' macrophages for the adhesion molecules tested, except for VLA5. The differences reached significance for the majority of molecules tested. It is concluded that striking differences exist in cellular composition and adhesion molecule expression between HLTBMCs from healthy individuals and those from patients with AML. This may have an impact on the pathogenesis of AML.

Key words: "Leukemic" HLTBMC's – Adhesion molecules

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Introduction

To study the hematopoietic microenvironment of bone marrow (BM) the model of the long-term bone marrow culture can be used [11, 12, 19, 32]. Human long-term bone marrow cultures (HLTBMCs) are characterized by a stromal layer composed of fibroblasts, macrophages, fat cells, and endothelial cells that produces an extracellular matrix and growth factors [1, 12, 14, 32].

A close interaction between stromal cells or extracellular matrix and stem cells has been demonstrated, and stromal cells play an important role in 'normal' and 'leukemic' hematopoiesis [13, 15, 16, 20, 26, 29, 33, 34]. Prolonged growth of 'normal' human bone marrow has been shown; granulopoiesis, erythropoiesis, and lymphopoiesis are maintained for several weeks in HLTBMCs [12, 19, 32]. However, in HLTBMCs cultured from acute myeloid leukemia (AML) BM aspirates, progenitors of abnormal (blast) colonies and clusters were not found after 4 weeks [7, 9, 20]. Murine LTBMCs derived from various hematopoietic organs had different capacities in CFU assays; these were related to differences in cellular composition [18]. Ultrastructural differences in bone marrow fibroblasts were found in HLTBMCs from both normal volunteers and leukemic patients [24].

Little is known about the composition of HLTBMCs derived from patients with AML and even less about their possible role in the pathogenesis of the disease. The mechanism by which immature hematopoietic cells are attached to the bone marrow and released into the blood stream is also not clearly understood [1, 25].

Adhesion of hematopoietic cells to their microenvironment is thought to be dependent on several components of the bone marrow compartment such as cells, extracellular matrix proteins, and growth factors [2–4]. Adhesion molecules probably play an important role in this interaction. They provide anchorage, allowing signals for growth and differentiation to be mediated [27, 31].

In contrast to normal immature cells, AML cells have a variable capacity to egress from the marrow into the peripheral blood [25, 35], which may be due to a deficiency

of adhesion molecules on AML cells or on bone marrow stromal cells, or both.

To further define the role of stroma in the pathogenesis of leukemia we studied both the cellular composition and the adhesion molecule expression of 'leukemic' versus 'normal' stromas.

Materials and methods

HLTBMCs

Bone marrow specimens from eight healthy volunteers and 16 untreated adult patients with AML were obtained by aspiration from the posterior iliac crest after informed consent. Mononuclear cells were separated by Ficoll-Hypaque density gradient centrifugation (Pharmacia), $d = 1.0771$ g/cm^3 and washed twice. Cells were cultured in six 25-ml plastic tissue culture flasks (Nunc), in a concentration of 1×10^6 cells per ml HLTBMC medium, and were inoculated in a total volume of 5 ml per flask [12, 19]. Weekly, half of the medium was refreshed. HLTBMC medium consisted of: 75% α-MEM, 12.5% fetal calf serum, 12.5% horse serum, 292 mg/ml glutamine (all from GIBCO), 10^{-6} *M* hydrocortisone sodium succinate (Upjohn), 10 mg/ml folic acid, 40 mg/l inositol, and 10 μl/ml penicillin-streptomycin (all from GIBCO) [9, 32].

Cytospin preparations and staining

Every week one stromal monolayer was trypsinized with 1 ml trypsin-EDTA (GIBCO), after it had been washed three times with HBSS (without phenol red, Ca and Mg) (GIBCO). Cytocentrifuge preparations (Cytospin 2, Shandon Elliot) were made and stained by the May-Grünwald-Giemsa method.

At least 500 cells were counted per sample preparation.

Since trypsinization may interfere with morphology, cells were further characterized with cytochemical stainings and monoclonal antibodies. It is known that trypsinization can lead to a loss of antigeneic capacity. Therefore, HLTBMCs were also cultured on glass coverslips (Nunc) and stained cytochemically and immunologically. These HLTBMCs were compared with trypsinized cells.

As has been described, fibroblasts and granulocytes can be stained with the alkaline phosphatase staining [4, 22], macrophages and some monocytes with acid phosphatase [2, 22], macrophages and monocytes with α-naphthyl acetate esterase [4, 22], inhibited by sodium fluoride in macrophages and monocytes [36], and myeloid cells with naphthol AS-D chloroacetate-esterase. For immunological phenotyping of the cells several defined monoclonal antibodies were used. For phenotyping fibroblasts the monoclonal antibodies TE 7 and anti-collagen III (SANBYO) were used [17, 28]. To stain macrophages we used CD 68 (EBM 11) (Dakopatts) [21] and CD 14 (3 C 10). For staining endothelial cells EN 4, anti-Factor VIII/VWF (Dakopatts), anti-laminin and anti-collagen IV (SANBYO) were used [2, 3, 22, 23, 28].

Alkaline phosphatase anti-alkaline phosphatase (APAAP)

In the sixth week stromal monolayers derived from eight healthy volunteers and 15 untreated patients with AML (seven FAB-type M2, four M4, two M5a, and two M5b) were trypsinized with trypsin-EDTA (GIBCO), after they had been washed three times with HBSS (without phenol red, Ca and Mg) (GIBCO).

Cytocentrifuge preparations (Cytospin 2, Shandon Elliot) were made and stained with the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique with slight modifications in the substrate preparation [8].

Cytospin preparations were fixed in buffered formal-acetone-methanol and rinsed in Tris buffered saline (TBS, Fluka) pH 7.6. Slides were incubated for 30 min with TBS containing 10% human decomplemented AB serum.

In the first step the cytospins were incubated for 60 min with monoclonal antibodies at room temperature. Aspecific monoclonal antibodies were used as controls. After rinsing with TBS in the second step, cytospin preparations were incubated for 30 min with rabbit anti-mouse immunoglobulins (Dakopatts) and for 30 min with APAAP complexes (Dakopatts). Slides were then rinsed in TBS and incubated for 20 min with the alkaline phosphatase substrate containing naphthol-AS-Bl-phosphate (Sigma), dimethylformamidum (Merck), 50 m*M* TBS pH 8.7, neo-fuchsin (Merck), sodium nitrite (Merck) and 1 m*M* levamisole (Sigma); slides were then counterstained with hematoxylin for 5 min. Five hundred stromal cells were analyzed and compared with May-Grünwald-Giemsa-stained slides to determine which stromal cells (fibroblasts, macrophages, or endothelial cells) were positively stained. Adhesion molecule expression was called positive if more than 20% of the cells were stained. In addition, the reactivity of macrophages was assessed as a percentage of macrophages stained positive.

Adhesion molecules

Monoclonal antibodies against VLA 1, 2, 3, 4, 5, 6, LFA 1, Mac 1, p 150/95, β_2-chain, HCAM, ICAM 1, NCAM, and VCAM 1 [5] were used (Table 1). Except for VLA6, which is a rat monoclonal antibody, all monoclonal antibodies are murine.

Statistics

In the morphological analysis percentages were compared between weekly specimens using the test of Friedman. Student's-*t* test was used to compare adhesion molecule expression on 'normal' and 'leukemic' bone marrow stromal cells and macrophages.

Results

Staining methods

Although the cell shape was changed by trypsinization, characteristics of nucleus, nucleoli, and cytoplasm were

Table 1. Monoclonal antibodies used

CD number	Antigen	Moab	Source
	VLA 1	TS 2/7	T Cell Science, Cambridge
CD 49 b	VLA 2	P 1 E 6	Telios Pharmaceuticals, San Diego
	VLA 3	P 1 B 5	Telios Pharmaceuticals, San Diego
CD 49 d	VLA 4	P 4 G 9	Telios Pharmaceuticals, San Diego
	VLA 5	P 1 D 6	Telios Pharmaceuticals, San Diego
CD 49 f	VLA 6	goH 3	NKI, Amsterdam, Dr. J. Hilgers
CD 11 a	LFA 1	NKI-L 15	NKI, Amsterdam, Dr. C. G. Figdor
CD 11 b	Mac 1	bear 1	NKI, Amsterdam, Dr. C. G. Figdor
CD 11 c	p 150/95	SHCL 3	NKI, Amsterdam, Dr. C. G. Figdor
CD 18	β_2-chain	CLB 54	NKI, Amsterdam, Dr. C. G. Figdor
CD 44	HCAM	gp 90/1	NKI, Amsterdam, Dr. C. G. Figdor
CD 54	ICAM 1	F 10.2	AZU, Utrecht, Dr. A. C. Bloem
CD 56	NCAM	123 C 3	NKI, Amsterdam, Dr. J. Hilgers
	VCAM 1	4 B 9	Seattle, Dr. J. M. Harlan

preserved in the May-Grünwald-Giemsa staining to allow recognition of different cell types (Fig. 1). Four main categories of cells were distinguished:

1. Fibroblasts with a spindle shaped nucleus, with two or three nucleoli and a basophilic cytoplasm
2. Macrophages with a round eccentric nucleus, without nucleoli and with an acidophilic cytoplasm
3. Endothelial cells with round heterochromatic nuclei, with a nucleolus and an acidophilic cytoplasm
4. Remaining cells, like hematopoietic cells and undefined cells (undefined cells are cells which do not belong to categories described before)

Groups 3 and 4 were called 'other cells' and taken together, because of the low number of these cells. Only two cytochemical staining methods were discriminitive: naphthol AS-D chloroacetate-esterase for myeloid cells and May-Grünwald-Giemsa staining (Table 2).

Acid phosphatase did stain macrophages and monocytes, but also fibroblasts. The alkaline phosphatase staining was not specific for fibroblasts and granulocytes, because it also stained macrophages.

Without sodium fluoride addition in the α-naphthyl acetate-esterase, fibroblasts, macrophages, and monocytes were stained. After adding sodium fluoride a few fibroblasts remained positive.

Some monoclonal antibodies appeared to have limited sensitivity or specificity, which may be due to trypsinization. Anti-collagen III and anti-laminin did not stain at all. Anti-collagen IV stained only a part of the endothelial cells. On the other hand, anti-Factor VIII/VWF stained endothelial cells, but also macrophages, which also can be demonstrated after GM-CSF is added to HLTBMCs [28]. CD 68 stained only 90% of the macrophages.

Finally, three monoclonal antibodies were found to be specific and sensitive: TE 7 for fibroblasts, CD 14 for macrophages, and EN 4 for endothelial cells (Table 2). The results of cell recognition with the May-Grünwald-Giemsa staining and these three monoclonal antibodies were comparable (Table 3).

No difference was demonstrated in cytochemical and monoclonal antibody staining patterns between stromal cells cultured on glass coverslips and those in cytospin preparations.

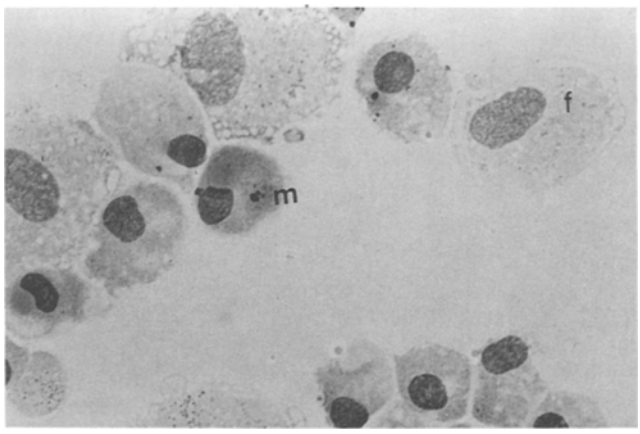


Fig. 1. Trypsinized stromal cells: fibroblasts (*f*) and macrophages (*m*)

Table 2. Results of cytochemical staining and immunoperoxidase staining with immunophenotyping antibodies on cultured bone marrow-derived cells

	F	M	E	H	U
Cytochemical					
Acid phosphatase	+	+	−	+	−
Alkaline phosphatase	+	+	−	+	−
α-Naphthyl acetate esterase	+	+	−	+	−
α-Naphthyl acetate esterase + sodium fluoride	±	−	−	−	−
Naphthol AS-D chloroacetate esterase	−	−	−	+	−
Monoclonal antibodies					
Anti-Factor VIII/VWF	−	±	+	−	−
Anti-laminin	−	−	−	−	−
Anti-collagen III	−	−	−	−	−
Anti-collagen IV	−	−	±	−	−
CD 68	−	±	−	−	−
TE 7	+	−	−	−	−
EN 4	−	−	+	−	−
CD 14	−	+	−	−	−

F, Fibroblasts; M, macrophages; E, endothelial cells; H, hematopoietic cells; U, undefined cells; ±, not all cells are stained

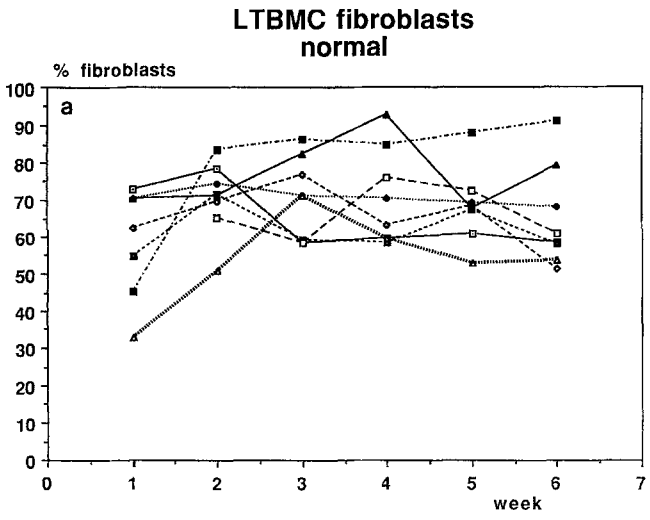
Cellular composition of normal HLTBMCs

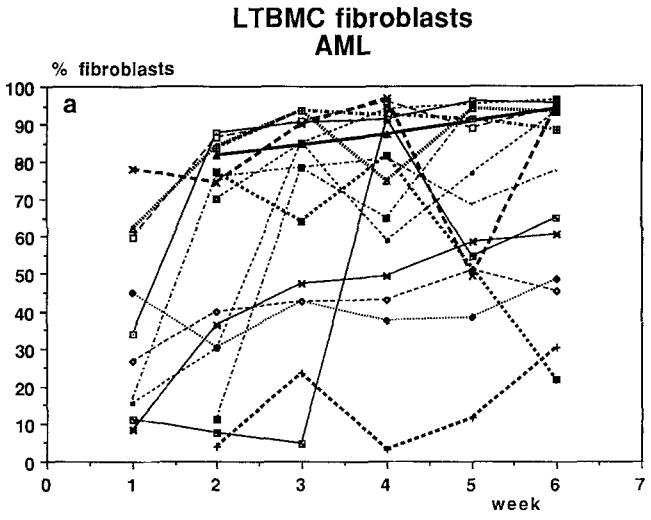
Cellular composition, as indicated in Figs. 2 and 3 and in Table 4, is based upon analysis of May-Grünwald-Giemsa-stained slides. The 'normal' stromal fibroblasts increased, from 58.3% ± 15.0% (mean ± standard deviation) to 70.4% ± 11.1% (Fig. 2a). In contrast, the macrophages decreased, from 28.9% ± 9.3% to 24.5% ± 8.9% (Fig. 2b). The 'other cells' demonstrated a decline from 12.7% ± 6.3% to 6.6% ± 3.7% (Fig. 2c).

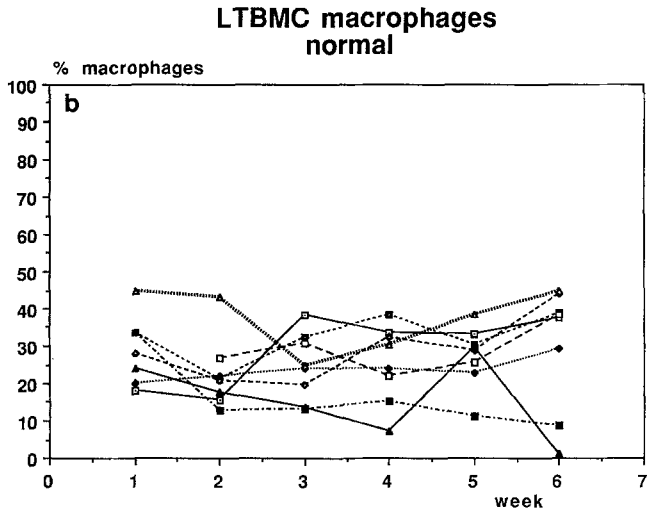
It must be emphasized that the changes in percentages of cells were not significant during culture, but, as is clear from Fig. 2a–c, all healthy samples showed a reproducible pattern. The three groups of cells showed a consistent development during the culture period. After 3 weeks

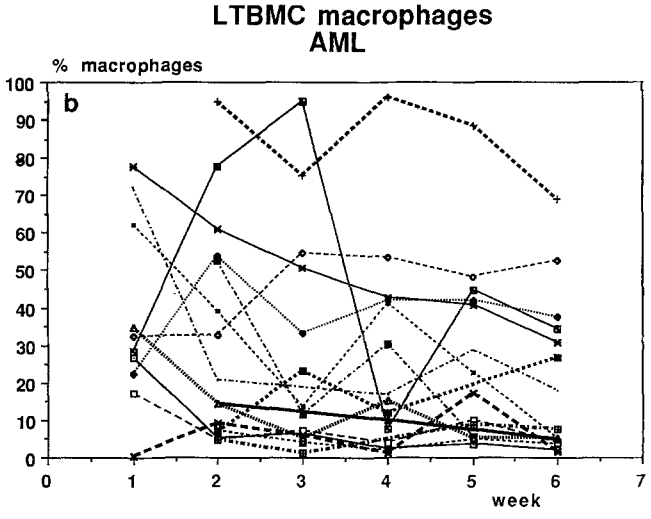
Table 3. Mean percentage of cell type based upon staining with May-Grünwald-Giemsa (MGG) as compared with staining with monoclonal antibodies (TE 7, CD 14 and EN 4) in HLTBMCs

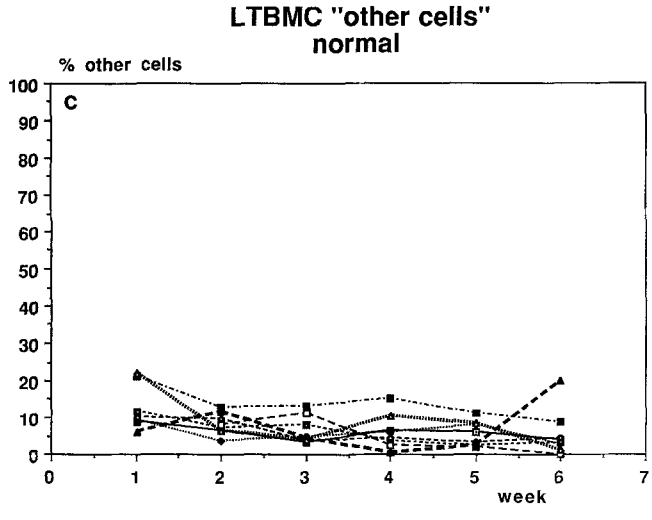
	MGG (%)	TE 7 (%)	CD 14 (%)	EN 4 (%)
'Normal' (1st week, $n = 4$)				
Fibroblasts	65.1	64.5	0	0
Macrophages	25.3	0	29.8	0
Endothelial cells	2.8	0	0	5.8
'Leukemic' (1st week, $n = 4$)				
Fibroblasts	32.1	36.6	0	0
Macrophages	46.6	0	49.3	0
Endothelial cells	2.3	0	0	7.4











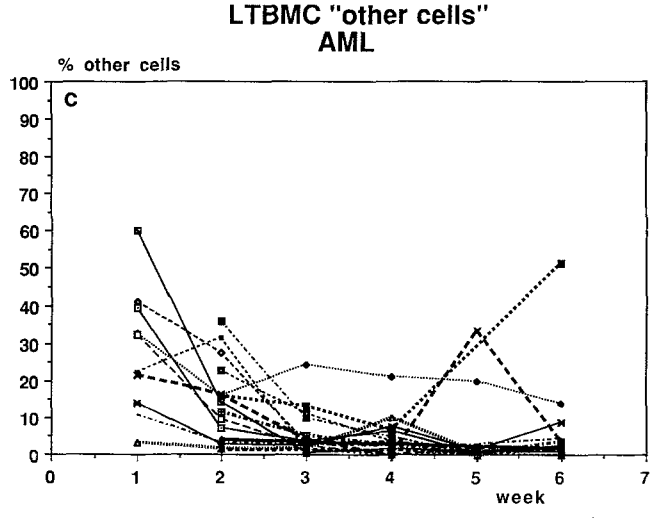


Fig. 2a–c. Each line represents the percentage of fibroblasts (**a**), macrophages (**b**), and other cells (**c**) in an HLTBMC of one healthy volunteer (n = 8), during 6 weeks of culture

Fig. 3a–c. Each line represents the percentage of fibroblasts (**a**), macrophages (**b**), and other cells (**c**) in an HLTBMC of one patient with AML (n = 15), during 6 weeks of culture

Table 4. Cellular composition of 'normal' versus 'leukemic' HLTBMCs (mean ± standard deviation), May-Grünwald-Giemsa staining

	'Normal' (%)	
Fibroblasts		
1st week	58.3 ± 15.0	(n = 7; 32.8 – 72.8)
6th week	70.4 ± 11.1	(n = 8; 58.2 – 86.6)
Macrophages		
1st week	28.9 ± 9.3	(n = 7; 18.2 – 45.0)
6th week	24.5 ± 8.9	(n = 8; 13.2 – 38.2)
'Other cells'		
1st week	12.7 ± 6.3	(n = 7; 6.0 – 22.2)
6th week	6.6 ± 4.7	(n = 8; 0.3 – 15.0)
	'Leukemic' (%)	
Fibroblasts		
1st week	35.7 ± 24.4	(n = 10; 8.4 – 78.0)
6th week	73.3 ± 26.1	(n = 15; 21.9 – 95.8)
Macrophages		
1st week	37.3 ± 25.1	(n = 10; 0.5 – 77.8)
6th week	20.1 ± 20.9	(n = 15; 18.0 – 68.8)
'Other cells'		
1st week	27.7 ± 16.9	(n = 10; 3.2 – 60.2)
6th week	6.5 ± 12.9	(n = 15; 0 – 51.2)

they reached a steady state. However, the absolute number of cells remained the same after 1 week of culture (i.e., 4×10^6).

HLTBMCs also consisted of fat cells, which were lost, however, during trypsinization. Seven of the eight BM samples produced a monolayer in the first week, the remaining sample during the second week.

Cellular composition of leukemic HLTBMCs

Ten monolayers were formed during the first week and five in the second week, while in one case it was not possible to establish a monolayer. When a monolayer was formed, no significant differences were found between cell numbers obtained from either 'normal' or 'leukemic' HLTBMCs. A total of 4×10^6 cells were harvested per flask. Although 'leukemic' stromal fibroblasts did not show a consistent development, they increased significantly during the 6 weeks from 35.7% ± 24.4% to 73.3% ± 26.1% ($p < 0.0003$; Fig. 3a). In the first week 37.3% ± 25.1% of the cells consisted of macrophages; these diminished in the sixth week to 20.1% ± 20.9% ($p < 0.0180$; Fig. 3b). Endothelial cells, hematopoietic cells, and undefined cells showed a significant decline ($p < 0.0147$) from 27.7% ± 16.9% to 6.5% ± 12.9% (Fig. 3c).

The number of 'other cells' appeared to be higher as opposed to those in 'normal' HLTBMCs in several cases, obviously due to the presence of cells of the leukemic population.

It is clear from Fig. 3a–c that the leukemic samples showed a much more irregular pattern of development, which also resulted in much higher standard deviations (Table 4).

The 'leukemic' stromal cells did not reach an equilibrium or a steady state even after 9 weeks of culture (data not shown). 'Leukemic' stromal cells also consisted partly of fat cells, which were lost during trypsinization.

We compared our data on the cultured acute myeloid leukemias with the FAB classification. Five M2, one M3, two M4, three M5a, and four M5b BM aspirates were cultured. No correlation of the cellular composition of 'leukemic' HLTBMCs with the FAB classification was demonstrated. There was no correlation between the initial number of blast cells and the cellular composition of leukemic cultures over time.

Adhesion molecule expression

The adhesion molecules, except for VLA1, 3, and 4, were expressed more on 'normal' than on 'leukemic' bone marrow stromal cells, although differences reached significance only for the β_2-chain ($p < 0.03$) and NCAM ($p < 0.009$; Table 5). VLA5 and HCAM were expressed on both 'normal' and 'leukemic' stromal cell populations in almost every sample (Table 5).

In 'normal' HLTBMCs expression of adhesion molecules was most strikingly demonstrated on the macrophage population (Fig. 4). The expression was less on macrophages in 'leukemic' HLTBMCs for all molecules tested excepting VLA5.

Differences between 'normal' and 'leukemic' macrophage adhesion molecule expression reached significance for the molecules tested excepting VLA2, 3, 4, and 5 (Fig. 4). When the analysis was restricted to macrophages in HLTBMCs from nonmonocytic leukemias (i.e., FAB-type M2, n = 7), the differences remained (data not shown).

Table 5. Adhesion molecule expression on 'healthy' (n = 8) and 'leukemic' (n = 15) stromal cells after 6 weeks of culture (a sample was scored positively when more than 20% of the cells were stained)

Molecule	'Normal' (n)	(%)	'Leukemic' (n)	(%)
VLA1	5/8	62.5	11/15	73.3
VLA2	5/8	62.5	7/15	46.6
VLA3	5/8	62.5	11/15	73.3
VLA4	4/8	50.0	8/15	53.3
VLA5	8/8	100	13/15	86.6
VLA6	4/8	50.0	3/15	20.0
LFA1	6/8	75.0	5/15	33.3
Mac1	7/8	87.5	11/15	73.3
p150/95	6/8	75.0	7/15	46.6
β_2-chain	7/8	87.5	6/15	37.5
HCAM	8/8	100	14/15	93.3
ICAM1	5/8	62.5	6/15	37.5
NCAM	7/8	87.5	4/15	26.6
VCAM1	7/8	87.5	7/15	46.6

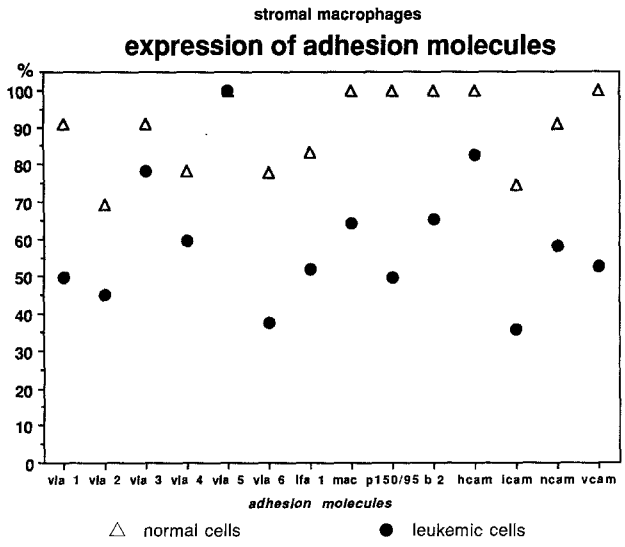


Fig. 4. Mean percentage of macrophages positive for adhesion molecules in HLTBMCs from healthy persons ($n = 8$) and AML patients ($n = 15$). Significance of differences: VLA1 $p < 0.001$, VLA2 $p < 0.08$, VLA3 $p < 0.16$, VLA4 $p < 0.15$, VLA5 $p < 1.00$, VLA6 $p < 0.004$, LFA1 $p < 0.01$, Mac1 $p < 0.002$, p150/95 $p < 0.00004$, β_2-chain $p < 0.0008$, HCAM $p < 0.02$, ICAM1 $p < 0.01$, NCAM $p < 0.01$, VCAM1 $p < 0.00004$

Discussion

In this study we demonstrated a remarkable difference in the development and cellular composition of HLTBMCs derived from healthy volunteers and those from patients suffering from AML. During 6 weeks of culture, 'normal' HLTBMCs showed an increase in fibroblasts, a decrease in macrophages, and a decrease in 'other cells', although the differences were not significant (Fig. 2, Table 4).

The decrease in 'other cells' is caused by death and also by loss of hematopoietic cells during refreshment of the culture medium. These 'normal' stromas showed a consistent development, reached a steady state, and showed a reproducible culture pattern. In contrast, 'leukemic' stromas did not reach a steady state, although they demonstrated an increase in fibroblasts and a decrease in macrophages and 'other cells' during 6 weeks of culture. These HLTBMCs showed a heterogeneous pattern in development (Fig. 3, Table 4). Even after 9 weeks of culture leukemic HLTBMCs did not reach an steady state (data not shown). Probably, the decrease of 'macrophages' is partly due to death of the leukemic monocytic population in the 'leukemic' HLTBMCs [7].

Obviously, leukemic cells interfere in the regulation of the cellular composition of 'leukemic' stromas. Although patients who achieve prolonged remission after successful chemotherapy may be cured, hematopoiesis remains abnormal for several years and possibly permanently. HLTBMCs of these patients show no confluency of stromal cells [6]. It may be argued that these abnormalities are due to cytostatic treatment. Therefore, we preferred to study HLTBMCs derived from marrow that was taken prior to cytostatic treatment. It is known that HLTBMCs derived from patients with untreated myelodysplastic syndromes did not show confluency either [10].

We also found differences in adhesion molecule expression between 'normal' and 'leukemic' HLTBMCs. Most adhesion molecules were more weakly expressed in 'leukemic' stromal cells, especially in the macrophage compartment of those cells. To be sure that these results were not based upon the analysis of 'stromal' cells that actually belonged to the residual leukemic cell population, we restricted our analysis to macrophages in HLTBMCs of leukemias without a monocytic component, i.e., FAB-type M2. This did not change our results. In contrast to our findings, other authors did not detect expression of VLA1 and VLA6 on stromal macrophages [30].

Less expression of adhesion molecules on leukemic stromal cells may be a key to understanding the release of AML cells from the bone marrow compartment into the peripheral blood. The variable egress of leukemic cells from the bone marrow may be influenced by cellular composition of the microenvironment and by adhesion molecule expression on either leukemic cells or 'leukemic' stroma, or both. As we have described previously, there seems to be a preferential egress into the peripheral blood of immature leukemic cells as opposed to cells that have matured to some degree [35]. This may relate to a deficient adhesion molecule expression on immature leukemic cells as compared with slightly matured cells. Otherwise, leukemic cells that are released from the bone marrow due to deficient adhesive properties of either these cells or their stroma are deprived of differentiation signals mediated by the microenvironment. The adhesive properties of leukemic cells are presently being studied in our department.

In conclusion, we demonstrated differences in cellular composition and adhesion molecule expression between HLTBMCs taken from healthy individuals and from patients with AML. As stromal cells play an important role in proliferation and differentiation of hematopoietic cells, the differences found may have an impact on the pathogenesis of acute myeloid leukemia.

Acknowledgements. We thank Dr. R. Schiro for kindly providing the monoclonal antibodies EN4 and TE7, Dr. H. J. Bos for CD14 (3C10), Dr. C. G. Figdor for LFA1 (NKI-L15), Mac1 (bear1), p150/95 (SHCL3), β_2-chain (CLB54), and HCAM (gp90/1), Dr. J. Hilgers for VLA6 (goH3), and NCAM (123C3), Dr. A. C. Bloem for ICAM1 (F10.2), Dr. J. M. Harlan for VCAM1 (4B9), and Dr. J. J. P. Nauta for the statistical analysis. We are grateful to Dr. A. Dräger for her critical reading of the manuscript.

References

1. Allen TD, Dexter TM (1984) The essential cells of the hemopoietic microenvironment. Exp Hematol 12: 517–521
2. Bainton DF (1985) Bone marrow stromal cells – fibroblasts and macrophages. In: Furth v R (ed) Mononuclear phagocytes: characteristics, physiology and function. Martinus Nijhoff, Dordrecht, The Netherlands, pp 125–135
3. Berneman ZN, Chen Z, Ramael M, Van Poucke K, Korthout M, Bockstaele DR, Peetermans M (1989) A quantitative and dynamic study of endothelial cells and megakaryocytes in human long-term bone marrow cultures. Leukemia 3: 61–67

4. Broudy VC, Zuckerman KS, Jetmalani S, Fitchen JH, Bagby Jr GC (1986) Monocytes stimulate fibroblastoid bone marrow cells to produce multilineage hematopoietic growth factors. Blood 68: 530–534
5. Carlos TM, Schwartz BR, Kovach NL, Yee E, Rosso M, Osborn L, Chi-Rosso G, Newman B, Lobb R, Harlan JM (1990) Vascular cell adhesion molecule-1 mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells. Blood 76: 965–970
6. Chang J, Geary CG, Testa NG (1990) Long-term bone marrow damage after chemotherapy for acute myeloid leukaemia does not improve with time. Br J Haematol 75: 68–72
7. Chang J, Morgenstern GR, Coutinho LH, Scarffe JH, Carr T, Deakin DP, Testa NG, Dexter TM (1989) The use of bone marrow cells grown in long-term culture for autologous bone marrow transplantation in acute myeloid leukemia: an update. Bone Marrow Transplant 4: 5–9
8. Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulasiz Z, Macdonald S, Pulford KAF, Stein H, Mason DY (1984) Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP) complexes. Histochem Cytochem 32: 219–229
9. Coulombel L, Eaves C, Kalousek D, Gupta C , Eaves A (1985) Long-term culture of cells from patients with acute myeloid leukemia. J Clin Invest 75: 961–969
10. Coutinho LH, Geary CG, Chang J, Harrison C, Testa NG (1990) Functional studies of bone marrow haemopoietic and stromal cells in the myelodysplastic syndrome (MDS). Br J Haematol 75: 16–25
11. Dexter TM, Allen TD, Lajtha LG (1977) Proliferation of haemopoietic stem cells in vitro. J Cell Physiol 91: 335–341
12. Gartner S, Kaplan HS (1980) Long-term culture of human bone marrow cells. Proc Natl Acad Sci USA 77: 4756–4759
13. Glück U, Zipori D, Wetzler M, Berrebi A, Shaklai M, Drezen O, Zaizov R, Luria D, Marcelle C, Stark B, Umiel T (1989) Long-term proliferation of human leukemia cells induced by mouse stroma. Exp Hematol 17: 398–404
14. Gordon MY (1988) Adhesive properties of haemopoietic stem cells. Br J Haematol 68: 149–151
15. Gordon MY (1988) The origin of stromal cells in patients treated by bone marrow transplantation. Bone Marrow Transplant 3: 247–251
16. Gordon MY, Goldman JM, Gordon-Smith EC (1983) Spatial and functional relationships between human hemopoietic and marrow stromal cells in vitro. Int J Cell Cloning 1: 429–439
17. Haynes BF, Scearce RM, Lobach DF, Hensly LL (1984) Phenotypic characterization and ontogeny of mesodermal-derived and endocrine epithelial components of the human thymic microenvironment. J Exp Med 159: 1149–1168
18. Heuvel RL van den, Schoeters GER, Vandenborght OLJ (1988) Haemopoiesis in long-term cultures of liver, spleen and bone marrow of pre- and postnatal mice: CFU-GM production. Br J Haematol 70: 273–277
19. Hocking WG, Golde DW (1980) Long-term human bone marrow cultures. Blood 56: 118–124
20. Iland HJ, Croaker GM, Repka E, Radloff TJ, Vincent PC (1987) Long-term bone marrow culture induces terminal differentiation of human myeloid leukemic cells. Exp Hematol 15: 1109–1114
21. Kelly PMA, Bliss E, Morton JA, Burns J, McGee JO'D (1988) Monoclonal antibody EBM 11: high cellular specificity for human macrophages. J Clin Pathol 41: 510–515
22. Lanotte M, Allen TD, Dexter TM (1981) Histochemical and ultrastructural characteristics of a cell line from human bone-marrow stroma. J Cell Sci 50: 281–297
23. Mukai K, Rosai J, Burgdorff WHC (1980) Localization of Factor VIII-related antigen in vascular endothelials cells using an immuno-peroxidase method. Am J Surg Pathol 4: 273–276
24. Payne CM, Greenberg BR, Cromey D, Woo L (1987) Morphological evidence of an altered bone marrow microenvironment in patients with acute nonlymphoblastic leukemia and myelodysplastic disorders. Exp Hematol 15: 143–153
25. Petrides PE, Dittmann KH (1990) How do normal and leukemic white blood cells egress from the bone marrow? Blut 61: 3–13
26. Roberts R, Gallagher J, Spooncer E, Allen TD, Bloomfield F, Dexter TM (1988) Heparan sulphate-bound growth factor: a mechanism for stromal cell-mediated haemopoiesis. Nature 332: 376–378
27. Ruoslathi E (1991) Integrins. J Clin Invest 87: 1–5
28. Schiro R, Couthinho LH, Testa NG, Dexter TM (1989) The effect of exogenous growth factors on stromal cells from human LTBMC. Tissue Antigens 3: 229
29. Schölzel C, Löwenberg B (1985) Stimulation of proliferation and differentiation of acute myeloid leukemia cells on a bone marrow stroma in culture. Exp Hematol 13: 664–669
30. Soligo D, Schiro R, Luksch R, Manara G, Quirici N, Parravicini C, Lambertenghi Deliliers G (1990) Expression of integrins in human bone marrow. Br J Haematol 76: 323–332
31. Springer TA, Lasky LA (1991) Sticky sugars for selectins. Nature 349: 196–197
32. Toogood IRG, Dexter TM, Allen TD, Suda T, Lajtha LG (1980) The development of a liquid culture system for the growth of human bone marrow. Leuk Res 4: 449–461
33. Tsai S, Patel V, Beaumont E, Lodish HF, Nathan DG, Sieff CA (1987) Differential binding of erythroid and myeloid progenitors to fibroblasts and fibronectin. Blood 69: 1587–1594
34. Umiel T, Friedman S, Zaizov R, Cohen IJ, Gozes Y, Epstein N, Kobiler D, Zipori D (1986) Long-term culture of infant leukemia cells: dependence upon stromal cells from the bone marrow and bilineage differentiation. Leuk Res 10: 1007–1013
35. Weide M van der, Langenhuijsen MMAC, Huijgens PC, Imandt LMFM, Waal FC de, Mol JJ, Rhenen DJ van, Kester APM (1987) Relation between leukaemic cell count and degree of maturation in acute myeloid leukaemia. Eur J Cancer Clin Oncol 23: 1125–1129
36. Yourno J, Mastropaolo W (1981) Nonspecific esterases of the formed elements: zymograms produced by pH 9.5 polyacrylamide gel electrophoresis. Blood 57: 939–945