Ann Hematol (1999) 78:449–455

ORIGINAL ARTICLE

H.M. Schmetzer · H.H. Gerhartz · W. Wilmanns

GM-CSF stimulates proliferation of clonal leukemic bone marrow cells in acute myeloid leukemia (AML) in vitro

Received: August 17, 1998 / Accepted: April 28, 1999

Abstract Granulocyte-macrophage colony-stimulating factor (GM-CSF) is known to stimulate granulocytes, monocytes, and macrophages. We studied the effect of GM-CSF on (clonal) bone marrow (BM) cells obtained from AML patients after 7 days of culture in vitro: BM samples were obtained from 19 AML patients at diagnosis (DIA), from two patients with persisting disease (PERS), from eight patients in complete remission (CR), and from 12 healthy donors. Flow-cytometric comparison of differentiated, CD 15-positive cells or of CD34-positive blast cells before and after cultivation showed that the proportion of CD15-positive cells was increased in nine of 12 healthy BM samples, in 14 of 19 cases at DIA, in one of three cases during PERS, and in five of six cases in CR of AML. The proportion of CD34-positive cells was increased in one of 12 healthy BM samples, in seven of 19 cases at DIA, in one of two cases during PERS, and in three of seven cases in CR of AML. Southern blot analysis (SBA) performed in six cases during the course of AML, before and after cell culture, showed that clonal DNA increased after GM-CSF treatment in three of five cases studied at DIA, in six of nine cases studied in CR, in the one case studied at PERS, and in the one studied at relapse (REL). In one case of trisomy 8 at DIA a normal karyotype was demonstrated in CR. However, after 7 days of cultivation of the cells in GM-CSF the trisomy 8 was detected in two of 17 metaphases isolated from colony-cells from methylcellulose cultures. Our data show that a 7-day treatment of BM cells with GM-CSF induced a differentiation of healthy and leukemic BM cells in the great majority of cases. An enrichment of CD34-positive cells was not achieved in healthy BM samples. However, in 70% of the cases in CR and in 30% of the cases at DIA of AML, clonal CD34-positive cells were enriched. This means that GM-CSF stimulates ('primes') leukemic cell growth in vitro.

Key words Southern blot · Clonality · AML · Cytokines · Residual disease · Flow cytometry

H.M. Schmetzer (✉) · W. Wilmanns
Klinikum Großhadern, Third Department of Medicine, University of Munich, Marchioninistrasse 15, D-81377 Munich, Germany
Tel.: 0049-89-7095-4974/4977,
Fax: 0049-89-7095-4978

H.H. Gerhartz
Klinikum Kalkweg, Department of Medicine, Duisburg, Germany

This work was supported by the Wilhelm-Sander-Foundation.

Introduction

AML results in the accumulation of leukemic blasts through clonal proliferation from one abnormal progenitor cell; the blasts are usually morphologically and biologically homogeneous [11]. Leukemic cell populations can be identified by flow cytometry using a panel of antibodies [27]. Most AML cases show a CD34-positive blast phenotype. Clonal cell populations can be identified by cytogenetics [29], polymerase chain reaction (PCR) [5] or by Southern blot analyses (SBA): Clonal BM cells rearranged in T-cell-receptor, immunoglobulin, GM-CSF, G-CSF, IL-3, or M-bcr genes can be detected in about 50% of AML cases [5, 7, 12, 16, 28, 34]. These rearrangements can be used to study the presence of clonal, gene-rearranged cells in the course of the disease [5, 13, 31].

About 70% of AML patients in CR normally relapse within 2 years; thus, residual leukemic cells must survive [4]. In patients who do not relapse mechanisms must exist which inhibit or even eradicate these leukemic cells. Several cytotoxic mechanisms are known, mediated by natural killer cells, for example, which can suppress leukemic growth in vivo [25, 40]. Besides cellular mechanisms, several soluble factors seem to play a role in the suppression of leukemic cells. Up to now animals have been treated in some trials with several cytokines such as IL-2 or interferon (IFN) α in order to find cytotoxic factors or to test the efficacy of factors in res-

toring normal bone marrow (BM) cells [2, 17, 21]. Moreover, IL-3, GM-CSF, and G-CSF can regulate the proliferation of leukemic cells [35, 39]. About 70% of AML cases produce cytokines such as IL-1β, IL-3, IL-6, erythropoietin or GM-CSF in an autocrine manner [3, 8, 26]. The GM-CSF gene is often constitutively expressed in AML, which is supposed to induce an autocrine stimulation of leukemic cells. GM-CSF is a very effective growth factor; it induces proliferation of healthy and leukemic hematopoietic progenitor cells, with only normal BM cells being able to differentiate [10]. Based on these data, we investigated the influence of GM-CSF on the differentiation and proliferation of healthy and leukemic (clonal) BM cells in vitro. The influence of GM-CSF on the proliferation and differentiation of CD15-positive granulocytes and CD34-positive blast cells is demonstrated. Proof of persisting clonal cells was established through SBA in cases with a gene rearrangement at DIA or by cytogenetics in a case of trisomy 8 at DIA.

Patients and methods

Patients

We examined cultured and uncultured BM samples from 19 patients at diagnosis (DIA) of AML according to cytological and cytochemical criteria, from eight patients in complete remission (CR), and from two with persisting disease (PERS). All patients were previously untreated and entered the study at the time of initial therapy. Patients were treated according to approved therapy standards. CR was determined to be achieved when the BM was normocellular, containing $<5\%$ blasts, and when neutrophil granulocytes in peripheral blood (PB) had recovered to 1500/μl and platelets to 100,000/μl according to the Cancer and Leukemia Groups (CALGB) criteria [4]. BM cells obtained from 12 healthy donors were studied as a control.

Cell preparation

BM cells were obtained by aspiration from the posterior iliac crest after the patients had given their informed consent, and were collected in preservative-free heparin. Mononuclear cells (MNC) were obtained from BM or PB cells by Ficoll density gradient centrifugation and then washed in Hank's balanced salt solution.

Culture of BM cells with GM-CSF

BM-MNC of AML patients obtained at different times in the course of their disease and from healthy BM donors as a control were cultured for 7 days at 37 °C and 5% CO_2 in a humidified atmosphere in Iscove's modified medium and 20% fetal calf serum (FCS) in the presence of 500 units/μl GM-CSF. Some samples were cultured in medium containing 0.8% methylcellulose in order to isolate single colonies.

Surface marker analysis

Flow-cytometric analysis was performed on uncultured and cultured BM-MNC in order to estimate the percentage of CD34- or CD15-positive cells [27]. Antibodies conjugated with fluorescent dyes (phycoerythrin or FITC) were used. Analysis was performed on a flow cytometer (Cytoron Absolute, Ortho Diagnostic Systems) and data were evaluated using special software from the same company. The percentual increase or decrease of cells positive for the antibodies after GM-CSF treatment compared with the results before treatment was evaluated.

Southern blot analysis (SBA)

SBA was performed on cultured or uncultured BM cells in order to detect clonal markers or to evaluate the proportions of clonal, gene-rearranged cells in the course of the disease after culturing of BM cells in GM-CSF-containing media. DNA was prepared according to standard procedures [19], then cut by restriction enzymes. The resulting fragments were electrophoretically separated on a 0.6% agarose gel, blotted onto nitrocellulose, and hybridized to several digoxigenated probes: Ig-JH [37], TcR-β [42],or GM-CSF [41]. The reaction was developed by 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt (BCIP) and nitroblue tetrazolium chloride (NBT). Bands in addition to the germline band were interpreted as being 'clonal bands' due to clonal leukemic cells. In order to estimate the proportion of clonal gene-rearranged cells the extinction of the rearranged bands was compared with that of the germline bands. The percentual increase or decrease of clonal cells after cytokine treatment compared with the results before treatment was evaluated by comparison of the optical densities of the rearranged bands related to the total amount of DNA. Densitometric evaluation was carried out on a Polaroid system; calculations were done with specially adapted computer software (Cybertech).

Cytogenetics

Karyotypic analysis of uncultured bone marrow cells at presentation was performed according to standard techniques [30, 38]. Chromosome preparations of single colonies picked from methylcellulose cultures were accomplished according to described methods [9, 24] with slight modifications.

Results

Influence of GM-CSF on the proliferation and differentiation of BM cells

BM samples from 19 AML patients at DIA, from two patients during PERS, and from eight patients in CR of AML, as well as from 12 normal BM donors, were cultured in a GM-CSF-containing suspension culture in order to study the effect of GM-CSF on the proliferation and differentiation of BM cells (Table 1). Flow-cytometric comparison of differentiated CD15-positive cells or of CD34-positive blast cells showed that the proportion of CD15-positive cells was increased in nine of 12 healthy BM samples, whereas the proportion of CD34-positive cells was more or less the same. In 14 of 19 cases at DIA of AML, the proportion of CD15-positive cells was increased by cultivation of BM cells in GM-CSF. In seven of 19 cases CD34-positive cells were also enriched, in nine cases the proportion of CD34-positive cells was decreased, and in three cases it was more or less the same. In one of two cases studied during PERS the CD34-positive cell proportion was increased, while in the remaining case it was decreased. The proportion of CD15-positive cells was increased in

Table 1 Influence of GM-CSF on the proliferation and differentiation of mononuclear BM cells after 7 days culture in vitro. Flow cytometry was used to evaluate percentages of CD 15- and CD34-positive cells before and after cultivation (*u* uncultured BM-MNC, *c* BM-MNC after 7-day culture with GM-CSF, *n.d.* not determined/not done, *CR* complete remission, *PERS* persisting leukemia)

	AML at diagnosis ($n=19$)					AML during course of disease ($n=10$)					Normal BM ($n=12$)		
Patient no.	FAB type	Cells	CD15[a]	CD34[a]	Patient no.	Stage	Cells	CD15[a]	CD34[a]	Patient no.	Cells	CD15[a]	CD34[a]
1	M4	u	7	10	20	CR	u	40	n.d.	30	u	20	3
		c	30	7			c	95	5		c	70	7
2	n.d.	u	5	60	21	CR	u	25	5	31	u	75	5
		c	55	20			c	78	15		c	80	0
3	n.d.	u	7	25	22	CR	u	20	15	32	u	60	3
		c	10	5			c	88	17		c	80	4
4	M4	u	5	5	23	CR	u	40	12	33	u	49	0
		c	60	13			c	7	13		c	80	3
5	M5	u	3	20	24	CR	u	70	13	34	u	70	10
		c	0	0			c	n.d.	2		c	70	5
6	M2	u	5	10	25	CR	u	70	5	35	u	24	2
		c	7	0			c	n.d.	25		c	78	3
7	M2	u	5	7	26	CR	u	14	0	36	u	50	5
		c	40	30			c	65	2		c	50	0
8	M2	u	5	40	27	CR	u	16	0	37	u	45	5
		c	44	28			c	80	40		c	90	7
9	M4	u	13	20	28	PERS	u	20	60	38	u	15	5
		c	45	30			c	30	4		c	60	0
10	$M4_{Eo}$	u	20	55	29	PERS	u	13	20	39	u	50	5
		c	40	44			c	15	25		c	71	25
11	M5	u	50	40						40	u	80	0
		c	45	55							c	75	5
12	n.d.	u	65	70						41	u	80	0
		c	23	13							c	70	4
13	M2	u	4	65									
		c	23	26									
14	M5	u	15	15									
		c	40	45									
15	M3	u	5	0									
		c	60	0									
16	n.d.	u	21	1									
		c	43	20									
17	M5	u	35	40									
		c	22	33									
18	M4	u	7	15									
		c	20	15									
19	M2	u	12	7									
		c	15	20									

[a] Percent of BM-MNC reacting with an antibody

one of the two cases and remained more or less the same in the other. In three of seven cases studied in CR the CD34-positive cell proportion was increased; it was decreased in one case and remained more or less the same in three cases. The proportion of CD15-positive cells was increased in five cases and decreased in one case studied. Our data show that GM-CSF leads to an increase of differentiated CD15-positive cells, but not of blast cells, in healthy BM samples. In contrast, an increase of both CD15-positive differentiated cells and of blast cells was seen in a large portion of the AML cases, both at DIA and in the course of the disease.

Influence of GM-CSF on clonal, gene-rearranged cells in AML

Clonal, gene-rearranged cells can be detected in about 50% of AML cases at DIA and in many cases during the course of the disease [5, 7, 12, 13, 16, 28, 34]. There was some question as to whether CD34-positive cell populations in AML BM samples that had been stimulated by GM-CSF (Table 1) were healthy, undifferentiated cells or clonal, leukemic cells. Therefore, we performed SBA before and after the GM-CSF treatment in all cases where a clonal marker was detectable in untreated BM-MNC. In Table 2 the percentual increase or decrease of clonal DNA as compared with untreated cells is shown. The percentages of gene-rearranged

Table 2 Influence of GM-CSF on clonal, uncultured AML BM cells at DIA or during the course of the disease. BM samples of AML patients in different stages of the disease were cultured in GM-CSF containing media for 7 days. DNA from these samples was prepared and hybridized to different gene probes. The percentage of clonal DNA was estimated densitometrically (*u* DNA prepared from uncultured cells, *c* DNA prepared from cells cultured for 7 days in GM-CSF)

Patient no.:	Hematological status	Percentage of clonal BM-DNA		Percentual increase or decrease of clonal BM-DNA[a]	Gene probe used
		u	c		
1:	DIA AML	54	70	+ 30	Ig-JH
	CR	47	70	+ 49	
	REL	52	75	+ 44	
2:	DIA AML	95	82	− 14	GM-CSF
	PERS	37	87	+135	
	CR	22	90	+309	
3:	CR	8	14	+ 75	TcR-β
4:	DIA AML	51	57	+ 12	Ig-JH
	CR2	36	42	+ 17	
5:	DIA AML	35	59	+ 69	Ig-JH
	CR	40	5	− 18	
	CR	14	41	+193	
	CR	2	32	+937	
6:	DIA AML	76	64	− 16	Ig-JH
	CR	82	71	− 13	
	CR	61	57	− 7	

[a] As compared with uncultured cells

clonal cells were estimated after densitometric evaluation of rearranged bands as compared with the germline bands before and after cultivation. A slight reduction of clonal, gene-rearranged cells was detected in only two of four BM samples at DIA of AML after cultivation with GM-CSF, in two of two samples studied at REL or PERS, and in five of eight samples studied in CR. Figure 1 shows two examples of SBA in the course of AML, case 1 presenting with a gene rearrangement of the GM-CSF gene, case 2 with an Ig-JH gene rearrangement. These examples demonstrate that clonal DNA persists in the course of AML. Moreover, it is shown that incubation of cells with GM-CSF in vitro leads to an increase of the clonal DNA. One AML case presented with an aberrant karyotype at diagnosis: a trisomy of the chromosome 8 in six of 17 metaphases. In CR a normal, female karyotype was seen in metaphases obtained from uncultured BM cells. However, two of 17 metaphases – obtained after a 7-day stimulation of BM cells in methylcellulose culture with GM-CSF with consecutive isolation of single cell clones – again showed the trisomy 8. This means that clonal cells are stimulated by GM-CSF, which leads to an increase of clonal DNA measurable by densitometry, or to the redetection of cytogenetically aberrant cells. No clonal gene rearrangements were detected in three of the normal BM samples.

In summary, our data show that GM-CSF induced a differentiation of hematopoietic cells to CD15-positive cells in healthy BM samples, in most of the samples in CR of AML, and in about 75% of the AML cases at diagnosis. An enrichment of undifferentiated CD34-positive cells was not regularly obtained in normal BM by GM-CSF-culture; however, it was achieved in 70% of the cases in CR and in 30% of the cases studied at DIA. In cases with a clonal marker it was shown that the percentage of clonal DNA or clonal, cytogenetically aberrant metaphases was increased in 12 of 16 samples studied after GM-CSF culture, particularly in cases in CR.

Discussion

Southern blot analysis enables the detection of clonal, gene-rearranged cells at diagnosis and during the course of AML

AML is a stem cell disease that develops clonally but is heterogeneous with respect to cell differentiation [11]. SBA is a good tool for detecting clonal, gene-rearranged cell populations at DIA and in the course of AML: Gene rearrangements in Ig-, TcR-, or cytokine-rearranged genes (GM-CSF, G-CSF, IL-3) can be seen [5, 7, 12, 16, 28, 34]. Compared with cytogenetics, SBA has the advantage of being a proliferation-independent method; it therefore allows the detection of clonal gene rearrangements in dividing and nondividing cells. In this context, we used SBA to identify gene rearrangements at DIA of AML and to evaluate the persistence of clonal cells in vivo and after in vitro treatment of BM cells. SBA has already been demonstrated by other groups and us [5, 7, 12, 13, 16, 28, 32–34] to be a good method for studying the persistence of leukemic cells or residual leukemia. Moreover, the detection of several gene rearrangements at DIA of AML might point to

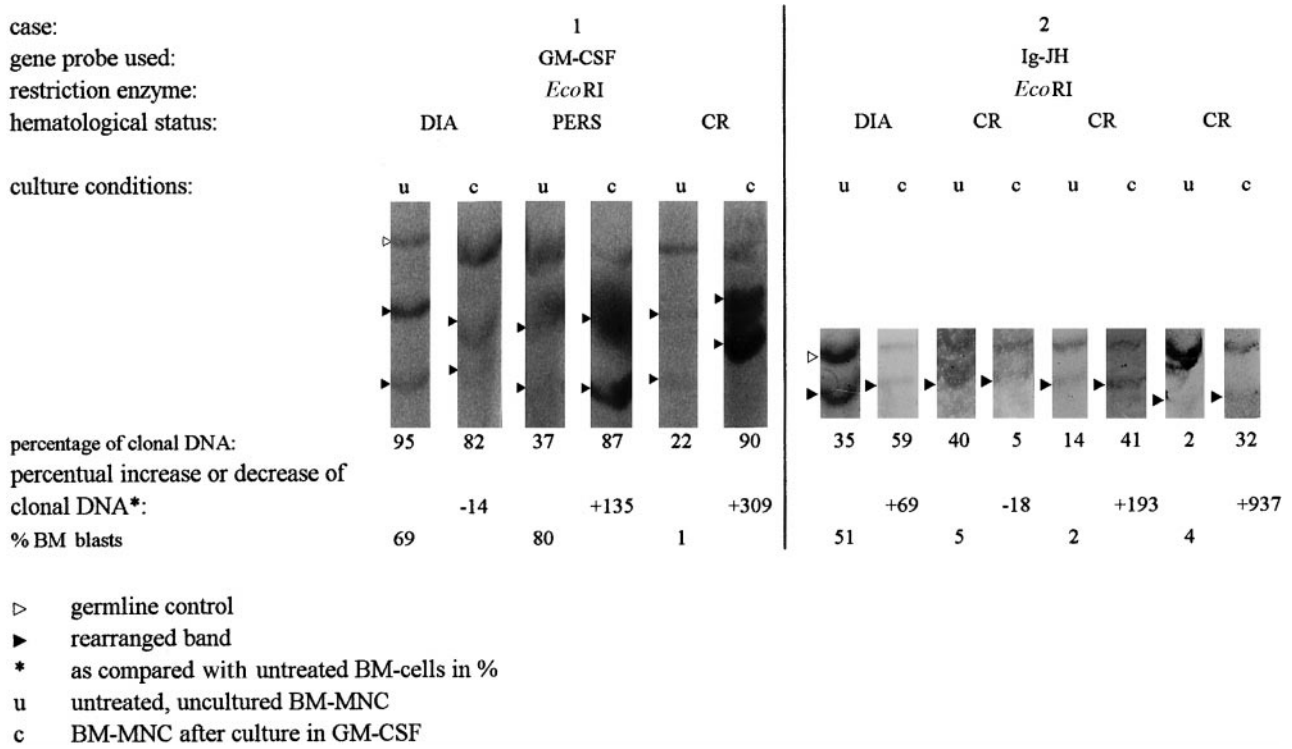

Fig. 1 Southern blot analysis of BM DNA obtained at different stages of AML, with or without culture in GM-CSF. DNA was isolated and hybridized to a digoxigenated GM-CSF gene probe (*1*) or to an Ig-JH gene probe (*2*). The percentual increase or decrease of clonal DNA is shown

the coexistence of genetically different leukemic clones [32]. SBA is also useful for studying clonality at DIA of AML and for detecting clonal, gene-rearranged cells in CR or at REL.

GM-CSF stimulates clonal, immature cells in AML and induces differentiation of healthy BM cells in vitro

Cytokines are known to regulate the proliferation and differentiation of hematopoietic cells, to influence the antitumor reactivity of immunocompetent cells, and to be responsible for the restoration of functional hematopoiesis [20, 35]. Therefore, recombinant human cytokines may provide a potentially interesting new approach to the treatment of AML. GM-CSF, IL-3, and G-CSF are known to regulate the proliferation of healthy and leukemic BM cells [10].

Our data show that a 7-day culture of healthy BM cells with GM-CSF increases the proportion of CD15-positive cells but (with one exception) not that of CD34-positive BM cells. This indicates that GM-CSF is an inducer of granulopoiesis, but not of undifferentiated blast cells in the healthy BM. This effect has already been shown by others [10, 22]. If cell differentiation could be stimulated by GM-CSF without simultaneous stimulation of blast cells, GM-CSF could be administered to AML patients after chemotherapy in order to restore functional hematopoiesis within a short time. Incubation of BM samples obtained from AML patients at DIA showed that an induction of cell differentiation was achieved in 14 of 19 cases; however, a simultaneous stimulation of undifferentiated cells was induced in seven of 19 cases. In the further course of the disease as well, 7-day culture of BM cells with GM-CSF led to an increase of CD15-, but also of CD34-positive cells in the majority of the cases tested. This implies that GM-CSF does not selectively induce granulopoiesis, but also has an effect on undifferentiated cells. It has already been reported that GM-CSF also affects CD34-positive cells [6, 10, 23]. It was not clear whether healthy CD34-positive cells or leukemic blast cells were stimulated. Therefore, we studied the clonality of the cells obtained in parallel in order to detect and quantify clonal markers (a) at diagnosis and in the course of AML and (b) before and after GM-CSF treatment. Cytogenetic analysis detected clonal, cytogenetically aberrant cells in colonies obtained after GM-CSF stimulation in a single AML case in CR. Moreover, results obtained by SBA showed that clonal DNA from BM cells at different stages of AML increased after GM-CSF culture in most of the cases. Thus the leukemic origin of CD34-positive cells was proven in those cases where a clonal marker was available prior to GM-CSF treatment. Preliminary experiments with GM-CSF-, IL-3-, or placenta-conditioned medium as a colony-stimulating factor in a colony assay in agar showed that these factors can be used for the cultivation of both healthy

and leukemic clones in AML patients, whereas no colonies were grown, and therefore no proliferation/differentiation was achieved, without added cytokines (data not shown) [14, 15, 31]. These colonies expressed CD15 antigens as well as blast antigens such as CD34 [13, 15, 31]. These results demonstrate that AML cells can partially differentiate in vitro in the presence of GM-CSF. However, leukemic markers are maintained under the differentiating effect.

In conclusion, our data imply that GM-CSF does not effect a reduction of leukemic cells in vitro, but rather a stimulation in most cases. Patients receiving GM-CSF after chemotherapy showed a significantly lower remission rate ($p = 0.008$), with more persistent leukemia and no therapeutic benefit [4]. In another trial, no benefit concerning survival or remission was seen in patients who had been given GM-CSF after chemotherapy as compared with the group of patients who had received only chemotherapy; moreover, no clinical benefit was detected for patients who had received GM-CSF after completion of chemotherapy [18]. These results may point to a 'leukemia-stimulating' effect of GM-CSF also in vivo. Our results imply that the use of GM-CSF could be of therapeutic benefit for patients with AML cells by enhancing the susceptibility of the malignant cells to chemotherapeutic drugs. Preliminary in vivo data show that this effect was obtained by recruiting malignant cells to the S-phase of the cell cycle [36], or by enhancing the susceptibility of malignant cells to natural killer cell lysis by stimulating adhesion molecule expression [1]. In conclusion, our results confirm that GM-CSF can be used to 'prime' leukemic cells. We think that GM-CSF might be administered prior to courses of chemotherapy in vivo in order to achieve a more effective eradication of malignant cells; after chemotherapy GM-CSF could possibly induce a stimulation of leukemic proliferation. New combinations of cytokines have to be tested in order to find combinations which might reduce leukemic cells and simultaneously stimulate healthy BM cells.

Acknowledgements The authors thank Mrs. G. Mellert, Mrs. K. Hecht, Mrs. C. Maerkl, and Mrs. M. Hadwiger for their skillful technical assistance. Moreover, we thank Dr. Hullin and Mrs. Arcifa, MED 1, Klinikum Großhadern, for providing the densitometric apparatus.

References

1. Bendall L, Kortlepel K, Bradstock KF (1995) Natural killer cells adhere to bone marrow fibroblasts and inhibit adhesion of acute myeloid leukemia cells. Leukemia 9:999–1005
2. Brugger W, Möcklin W, Heimfeld S (1993) Ex vivo expansion of enriched peripheral blood CD34+ progenitor cells by stem cell factor, interleukin 1β (IL-1β), IL-6, IL-3, interferon γ and erythropoietin. Blood 81:2579–2586
3. Bruserud O, Tore-Gjertsen B, Terje-Brustugun O (1995) Effects of interleukin 10 on blast cells derived from patients with acute myelogenous leukemia. Leukemia 9:1910–1920
4. Büchner T (1997) Treatment of adult acute leukemia. Curr Opin Oncol 9:18–25
5. Campana D, Ching-Hon P (1995) Detection of minimal residual disease in acute leukemia: methodologic advances and clinical significance. Blood 85:1416–1434
6. Carlo-Stella C, Mangoni L, Almici C, Frassoni F, Fiers W, Rizzoli V (1990) Growth of CD34+ acute myeloblastic leukemia colony-forming cells in response to recombinant hematopoietic growth factor. Leukemia 4:561–566
7. Cheng GYM, Kelleher CA, Miyauchi J, Wang C, Wong G, Clark SC, McCulloch EA, Minden MD (1988) Structure and expression of genes of GM-CSF and G-CSF in blast cells from patients with acute myeloblastic leukemia. Blood 71:204–208
8. Dokter WH, Tuyt L, Sierdsma SJ (1995) The spontaneous expression of interleukin-1 beta and interleukin-6 is associated with spontaneous expression of AP-1 and NF-kappa B transcription factor in acute myeloblastic leukemia cells. Leukemia 9:425–432
9. Dube JD, Eaves CJ, Kalousek DK, Eaves AC (1981) A method for obtaining high-quality chromosome preparations from single hemopoietic colonies on a routine basis. Cancer Genet Cytogenet 4:157–168
10. Faussat AM, Marie JP, Delmer A (1993) In vitro effect of GM-CSF on proliferation and maturation of leukemic cells from patients with acute myelogenous leukemia. Leuk Lymphoma 11:99–104
11. Fialkow PJ, Singer JW, Adamson JW, Berkow RL, Friedman JM, Jacobson RJ, Moohr JW (1979) Acute non-lymphocytic leukemia expression in cells restricted to granulocytic and monocytic differentiation. N Engl J Med 301:1–7
12. Fiedler W, Suciu E, Wittlief C, Ostertag W, Hossfeld DK (1990) Mechanisms of growth factor expression in acute myeloid leukemia (AML). Leukemia 4:459–461
13. Gerhartz HH, Schmetzer HM (1990) Detection of minimal residual disease in acute myeloid leukemia. Leukemia 4:508–516
14. Gerhartz HH, Schmetzer H (1991) Minimal residual disease in acute leukemia. Eur J Cancer 27:809–810
15. Gerhartz HH, Schmetzer H, Mayer F (1994) Immunological detection and surveillance of residual acute myeloid leukemia (AML) during remission. In: Bergmann L, Mitrou PS (eds) Cytokines in cancer therapy. Karger, Frankfurt, pp 347–359
16. Greenberg JM, Quertermous T, Seidman JG, Kersey JH (1986) Human T-cell gamma-chain gene rearrangements in acute lymphoid and nonlymphoid leukemia: comparison with the T-cell receptor β-chain gene. J Immunol 137:2043–2049
17. Higuchi C, Thompson J, Petersin F (1991) Toxicity and immunomodulatory effects of interleukin-2 after autologous bone marrow transplantation for hematologic malignancies. Blood 77:2561–2566
18. Löwenberg B, Suciu S, Archimbaud E, Ossenkoppele G, Verhoef GEG (1997) Use of recombinant granulocyte-macrophage colony-stimulating factor during and after remission induction chemotherapy in patients aged 61 years and older with acute myeloid leukemia (AML): final report of AML-11, a phase III randomized study of the Leukemia Cooperative Group of the European Organisation for the Research and Treatment of Cancer (EORTC-LCG) and the Dutch Belgian Hemato-Oncology Cooperative Group (HOVON). Blood 90:2952–2961
19. Maniatis T, Fritsch E, Sambrook J (1982) Molecular cloning, a laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor, NY
20. McCarthy P (1994) Down-regulation of cytokine action. Baillieres Clin Haematol 7:153–173
21. McNiece IK, Langley KE, Zsebo KM (1991) Recombinant human stem cell factor synergises with GM-CSF, G-CSF, IL-3 and Epo to stimulate human progenitor cells of the myeloid and erythroid lineages. Exp Hematol 19:226–231
22. Metcalf D, Begley CG, Johnson GR (1986) Biologic properties in vitro of a recombinant human granulocyte-macrophage colony-stimulating factor. Blood 67:37–45

23. Pietsch T, Kyas W, Steffens U (1992) Effects of human stem cell factor (c-kit ligand) on proliferation of myeloid leukemia cells: heterogeneity in response and synergy with other hematopoietic growth factors. Blood 80:1199–1206
24. Rajendra BR, Sciorra LJ, Lee M (1980) A new and simple technique for chromosomal preparations from peripheral blood lymphocytes, amniotic cell cultures, skin fibroblasts, bone marrow and single cell clones, when the yields from harvests are low. Hum Genet 55:363–366
25. Robertson M, Ritz I (1990) Biology and clinical relevance of human natural killer cells. Blood 76:2421–2432
26. Rogers SY, Bradbury D, Kozlowski R (1994) Evidence for internal autocrine regulation of growth in acute myeloblastic leukemia cells. Exp Hematol 22:593–598
27. Rothe G, Schmitz G (1996) Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies. Leukemia 10:877–895
28. Rovigatti U, Mirro J, Kitchingman G, Dahl G, Ochs J, Murphy S, Stass S (1984) Heavy chain immunoglobulin gene rearrangement in acute nonlymphocytic leukemia. Blood 63:1023
29. Sandberg AA (1990) The chromosomes in human cancer and leukemia, 2nd edn. Elsevier Science, New York
30. Seabright M (1971) A rapid banding technique for human chromosomes. Lancet 2:971–972
31. Schmetzer, H, Gerhartz HH (1987) Immunological phenotyping in situ of myeloid colonies in agar cultures. Exp Hematol 15:877–882
32. Schmetzer H, Gerhartz H (1993) Acute myeloid leukemia can be oligoclonal. Leukemia 7:1965–1970
33. Schmetzer H, Wilmanns W, Gerhartz H (1996) Detection of acute myeloid leukemic cells in complete remission and in extramedullary sites by clonal analysis. Acta Haematol 96:83–92
34. Schumacher HR, Shirt MA, Kowal-Vern A (1991) Acute leukemia and related entities. Arch Pathol Lab Med 115:331–337
35. Smith M, Singer C, Pallister C (1995) The effect of hemopoietic growth factors on the cell cycle of AML progenitors and their sensitivity to cytosine arabinoside in vitro. Br J Haematol 90:767–772
36. Sundman-Engberg B, Tidefelt U, Paul C (1996) Effect of cytokines on the toxicity of cytostatic drugs on leukemic cells in vitro and in vivo. Eur J Haematol 56:1–6
37. Takahashi N, Nakai S, Honjo T (1980) Cloning of human immunoglobulin μ gene and comparison with mouse μ gene. Nucleic Acids Res 8:5983–5991
38. Trent JM, Davis JR, Durie BG (1983) Cytogenetic analysis of leukemic colonies from acute and chronic myelogenous leukemia. Br J Cancer 47:103–109
39. Trycoff CM, Abboud MR, Laver J (1994) Rapid exit from G0/Gl phases of cells: an enhanced ex vivo expansion potential. Exp Hematol 22:1264–1272
40. Vujanovic NL, Yasumura S, Hirabayashi H, Lin WC, Watkins S, Herberinan RB, Whiteside TL (1995) Antitumor activities of subsets of human IL-2-activated natural killer cells in solid tissues. J Immunol 154:281–289
41. Wong GC, Witek J, Temple PA, Wilkens KM, Leary AC, Luxenberg DP, Jones SS, Brown EC, Kay RM, Orr EC, Shoemaker C, Golde DW, Kaufinan RJ, Henrick RM, Wang EA, Clark SC (1985) Molecular cloning of the complementary DNA and purification of the natural and recombinant proteins. Science 228:810–812
42. Yanagi Y, Yasunobu Y, Leggett K, Clark SP, Aleksander J, Mak TW (1984) A human T-cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. Nature 308:145–147