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Regulation of cell surface receptors for different hematopoietic growth factors on myeloid leukemic cells

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There are clones of myeloid leukemic cells which are different from normal myeloid cells in that they have become independent of hematopoietic growth factor for cell viability and growth. The ability of these clones to bind three types of hematopoietic growth factors (MGI-1GM=GM-CSF, IL-3=multi-CSF and MGI-1M=M-CSF=CSF-1) was measured using the method of quantitative absorption at 1°C and low pH elution of cell-bound biological activity. Results of binding to normal myeloid and lymphoid cells were similar to those obtained by radioreceptor assays. The results indicate that (i) the number of receptors on different clones of these leukemic cells varied from 0 to 1300 per cell. The receptors have a high binding affinity. (ii) Receptors for different growth factors can be independently expressed in different clones. (iii) There was no relationship between expression of receptors for these growth factors and the phenotype of the leukemic cells regarding their ability to be induced to differentiate. (iv) The number of receptors on the leukemic cells was lower than on normal mature macrophages. (v) Myeloid leukemic cells induced to differentiate by normal myeloid cell differentiation factor MGI-2 (=DF), or by low doses of actinomycin D or cytosine arabinoside, showed an up-regulation of the number of MGI-1GM and IL-3 receptors. (vi) Induction of differentiation of leukemic cells by MGI-2 also induced production and secretion of the growth factor MGI-1GM, and this induced MGI-1GM saturated the up-regulated MGI-1GM receptors. It is suggested that up-regulation of these receptors during differentiation is required for the functioning of differentiated cells.

Key words: cell differentiation/hematopoietic growth factors/myeloid leukemic cells/surface receptors

Introduction

The development of blood cells requires a program for the multiplication of stem cells and their differentiation into various types of mature cells with different functions. Normal myeloid progenitor cells are dependent for their viability and multiplication on the presence of myeloid cell growth factors called macrophage and granulocyte-inducing proteins type 1 (MGI-1) or colony-stimulating factors (CSF) (reviewed in Sachs, 1974, 1978, 1982, 1985, 1986; Metcalf, 1985). Four different myeloid growth factors have been identified which induce the development of normal cell clones containing either only macrophages (MGI-1M=M-CSF=CSF-1), only granulocytes (MGI-1G=G-CSF), both granulocytes and macrophages (MGI--1GM=GM-CSF), or granulocytes, macrophages, eosinophils, mast cells, megakaryocytes or erythroid cells (multi-CSF=interleukin 3) (IL-3). The genes for these four myeloid growth factors (murine and/or human) have been cloned (Fung *et al.*, 1984; Gough *et al.*, 1984; Yokota *et al.*, 1984; Kawasaki *et al.*, 1985; Lee *et al.*, 1985; Miyatake *et al.*, 1985; Wong *et al.*, 1985; Nagata *et al.*, 1986) and do not show any apparent similarities.

In addition to these growth factors there is another type of myeloid regulatory protein which does not have growth factor activity, that can induce differentiation in normal myeloid precursor cells and in certain clones of myeloid leukemic cells (Fibach and Sachs, 1976; Lotem *et al.*, 1980; Sachs, 1980, 1982; Liebermann *et al.*, 1982; Weisinger and Sachs, 1983; Weisinger *et al.*, 1986). This differentiation factor has been called MGI-2 (Lotem *et al.*, 1980; Sachs, 1980, 1982, 1985; Sachs and Lotem, 1984), differentiation factor (DF) (Tomida *et al.*, 1984) or differentiation-inducing factor (DIF) (Olsson *et al.*, 1984), and our experiments indicate that there is more than one type of MGI-2 (Lotem and Sachs, 1978,1984,1985; Symonds and Sachs, 1982; Sachs, 1985). The development of normal cell clones containing mature macrophages and/or granulocytes from undifferentiated myeloid precursor cells indicates that normal myeloid cell multiplication induced by the growth factors is coupled to their differentiation. We have shown that normal myeloid precursor cells produce differentiation factor MGI-2 when incubated with different myeloid growth factors (Sachs, 1980,1982; Lotem and Sachs, 1982,1983), so that this serves as an efficient mechanism to couple growth and differentiation in normal cells. There are myeloid leukemic cells, which unlike normal myeloid precursors, can remain viable and multiply in the absence of externally added myeloid growth factors, and do not differentiate into mature cells by the addition of these growth factors (see Sachs, 1985,1986). In these cells, the growth factors do not induce production of differentiation factor MGI-2 (Sachs, 1980,1982; Lotem and Sachs, 1982,1983).

To induce growth or differentiation by these regulatory proteins, the cells presumably need specific cell surface receptors to recognize, bind and transduce the appropriate signals (Cuatrecasas, 1974; Kahn, 1976). Different studies with radiolabeled MGI-1M, MGI-1GM, MGI-1G and IL-3 have shown the existence of such receptors on normal myeloid cells and some leukemic clones (Stanley and Guilbert, 1981; Palaszynski and Ihle, 1984; Nicola and Metcalf, 1985; Walker and Burgess, 1985; Walker *et al.*, 1985).

The present experiments were carried out to study the receptors for three different myeloid growth factors (MGI-1GM, MGI-1M and IL-3) on normal cells and growth factor independent clones of myeloid leukemic cells, and the regulation of these receptors during differentiation. We have shown that the method of quantitative cell-associated absorption of growth factor activity and its elution at low pH (Bowen-Pope and Ross, 1985) can demonstrate the existence of receptors for different myeloid growth factors on normal and leukemic myeloid cells and can be used to study their binding characteristics and regulation. The results indicate that receptors for different growth factors can be independently expressed in different clones of growth factor independent myeloid leukemic cells; that the number of recep-

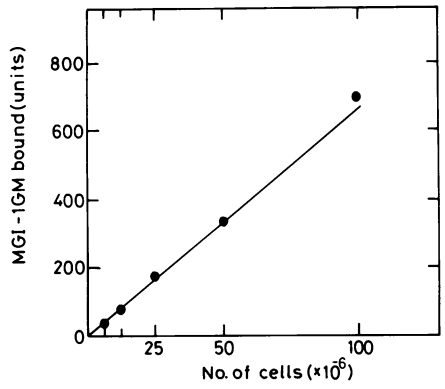

Fig. 1. Binding of MGI-1GM to different numbers of myeloid leukemic cells. Different numbers of clone 7-M12 leukemic cells were incubated with 2×10^5 units MGI-1GM for 30 min at 1°C and binding determined as described in Materials and methods.

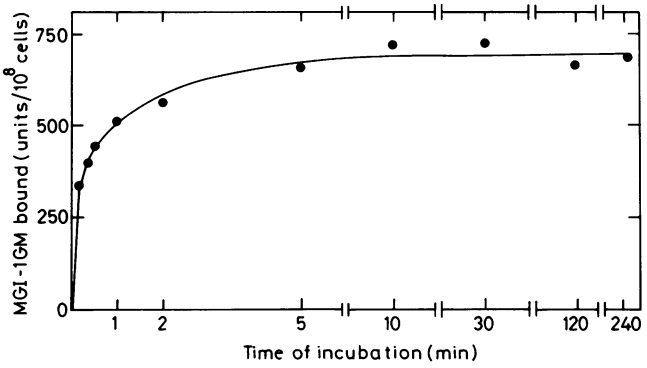

Fig. 2. Time dependence of binding of MGI-1GM to myeloid leukemic cells. Fifty $\times 10^6$ clone 7-M12 leukemic cells were incubated at 1°C for different periods of time with 2×10^5 units MGI-1GM and binding determined as described in Materials and methods.

tors for MGI-1GM and IL-3 is up-regulated during induction of differentiation by the differentiation factor MGI-2, or low doses of actinomycin D or cytosine arabinoside and that MGI-2 can induce differentiation-competent myeloid leukemic cells to produce and secrete MGI-1GM which then saturates the receptors which are up-regulated.

Results

Quantitative analysis of binding of biologically active MGI-1GM (GM-CSF), IL-3 and MGI-1M (M-CSF, CSF-1) to a clone of myeloid leukemic cells

The kinetics of absorption and elution of biologically active myeloid cell growth factors was first determined with a growth factor independent and differentiation-competent clone of myeloid leukemic cells (clone 7-M12). Incubation at 1°C of increasing numbers of clone 7-M12 cells with a fixed amount of MGI-1GM activity derived from Krebs ascites tumor cells (Lipton and Sachs, 1981) followed by three washes and low pH elution (52 mM acetic acid) for 5 min (Bowen-Pope and Ross, 1985) (see Materials and methods), showed a linear relationship between the amount of cell bound and low pH elutable MGI-1GM activity and the number of cells in the incubation mixture (Figure 1). Low pH elution for up to 30 min did not increase the amount of MGI-1GM that was eluted after 5 min. Unlike receptors for platelet-derived growth factor (Huang *et al.*, 1982), replacing the acetic acid with protamin sulfate did not result in any elutable

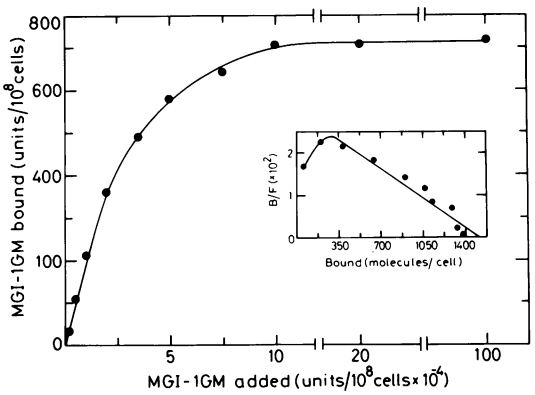

Fig. 3. Saturability of MGI-1GM receptors on myeloid leukemic cells. Fifty $\times 10^6$ clone 7-M12 leukemic cells were incubated at 1°C for 30 min with different amounts of MGI-1GM and binding determined as described in Materials and methods. The inset shows the Scatchard analysis of binding where B/F = bound/free MGI-1GM.

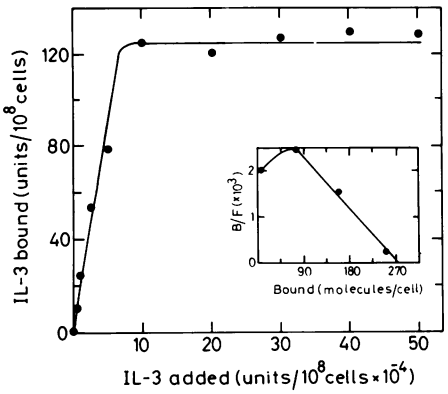

Fig. 4. Saturability of IL-3 receptors on myeloid leukemic cells. Fifty $\times 10^6$ clone 7-M12 leukemic cells were incubated at 1°C for 30 min with different amounts of IL-3 and binding determined as described in Materials and methods. Inset shows the Scatchard analysis.

MGI-1GM activity from the cells. These results were obtained under conditions which minimize possible non-specific binding, namely in the presence of 10% horse serum (Figure 1) or 1 mg/ml bovine serum albumin. No growth factor activity was eluted from clone 7-M12 cells without prior incubation with growth factor in the assay. The time dependence of binding of MGI-1GM at 1°C has shown that maximum binding to clone 7-M12 was reached within 5 – 10 min (Figure 2). Adding increasing amounts of MGI-1GM gave a typical saturation curve, and at saturation ~0.7% of the MGI-1GM added was absorbed onto the cells (Figure 3). Binding for 30 min at 23 or 37°C showed only a 10 – 15% increase in the amount of cell bound and eluted MGI-1GM activity. Experiments at 1°C with IL-3 activity derived from WEHI-3B myelomonocytic leukemic cells (Ihle *et al.*, 1982) have shown a similar saturation of binding (Figure 4) and ~0.1% of the input IL-3 activity was bound at saturation (Figure 4).

Purified recombinant mouse GM-CSF (DeLamarter *et al.*, 1985) and purified IL-3 (Goldwasser *et al.*, 1983) have specific activities of 8.4×10^8 and 3.3×10^8 myeloid colony-forming units/mg protein, respectively, which according to our assay conditions (see Materials and methods) correspond to specific activities of 2.4×10^8 units/mg for MGI-1GM and 2.2×10^8 units/mg for IL-3. Based on the true molecular weights of MGI-1GM (12 000, Miyatake *et al.*, 1985; 14 285, DeLamarter

Table I. Binding of myeloid growth factors to myeloid leukemic cells

Clone no.	Mouse strain	D^+ or D^-	Growth factor activity bound[a]					
			MGI-1GM		IL-3		MGI-1M[b]	
			Units/10^8 cells	Receptors/cell	Units/10^8 cells	Receptors/cell	Units/10^8 cells	Receptors/cell
2	SL	D^-	650 ± 226	1190 ± 413	308 ± 49	600 ± 83	48 ± 10	251 ± 52
5		D^-	10 ± 3	18 ± 5	0	0	0	0
11		D^+	76 ± 21	140 ± 39	66 ± 10	129 ± 20	37 ± 5	193 ± 26
1	SJL/J	D^-	95 ± 32	173 ± 58	0	0	0	0
6		D^-	493 ± 163	901 ± 297	3 ± 1	6 ± 2	0	0
7-M12		D^+	690 ± 271	1270 ± 500	129 ± 23	252 ± 45	0	0
10		D^-	0	0	218 ± 60	425 ± 117	0	0
WEHI-3B[c]	BALB/c	D^-	1190 ± 127	2190 ± 233	328 ± 115[c]	640 ± 224	0	0
HL-60	Human	D^+	0	0	0	0	0	0

[a]Cells were washed in cold medium with 10% horse serum and 50 × 10^6 cells were pelleted and resuspended in 2 ml cold medium containing 2 × 10^5 units of MGI-1GM, IL-3 or MGI-1M. The cells were then incubated at 1°C for 30 min (MGI-1GM and IL-3) or 4 h (MGI-1M), washed three times, eluted at pH 3.1, and the eluted activity analyzed for growth factor activity as described in Materials and methods.
[b]The number of receptors for MGI-1M was calculated assuming a sp. act. of 1.6 × 10^8 units/mg (Stanley and Guilbert, 1981) (5 × 10^7 units/mg under our assay conditions) and a mol. wt of 28 000 (Stanley and Guilbert, 1981).
[c]WEHI-3B cells have surface-bound IL-3 activity. The binding of MGI-1GM, and MGI-1M to these cells was measured after removal of the IL-3 from the cell surface by low pH elution and two washes with medium and 10% horse serum.

et al., 1985) and IL-3 (~15 000, Fung *et al.*, 1984; Yokota *et al.*, 1984) deduced from the nucleotide sequence of the cloned genes, a Scatchard analysis of our binding data indicate that clone 7-M12 cells have a single class of ~1500 MGI-1GM receptors per cell with an apparent K_d of 2 × 10^{-9} M (Figure 3) and 270 IL-3 receptors with an apparent K_d of 2.6 × 10^{-9} M (Figure 4). In contrast, incubation of clone 7-M12 cells with a high amount of MGI-1M activity derived from L cells (2 × 10^5 units per 10^8 cells) for 30 min to 6 h at 1°C did not show any detectable binding of MGI-1M (Table I).

We also determined whether growth factor bound to cells is spontaneously released from the cells. Results with MGI-1GM bound to clone 7-M12 cells indicate that incubation in medium at 23 or 37°C for 4–6 h resulted in the release of almost the same amount of MGI-1GM activity that was eluted by a 5-min incubation at low pH at 1°C (Figure 5). The spontaneous release of MGI-1GM was slower at 1°C than at 23°C, and after 4 h was only ~30% of the amount released at 23°C (Figure 5). The rapid binding (Figure 2) and slow spontaneous dissociation (Figure 5) of bound MGI-1GM are typical characteristics of high affinity cell surface receptors for a variety of hormones (Kahn 1976).

Binding of MGI-1GM, IL-3 and MGI-1M to different clones of myeloid leukemic cells: independent expression of receptors for myeloid growth factors

Differentiation-competent (D^+) and differentiation-defective (D^-) clones of myeloid leukemic cells originating in different strains of mice, and one line of human myeloid leukemia (HL–60), were used to determine the ability of different clones of myeloid leukemic cells to bind the three myeloid growth factors. All these clones are independent of myeloid growth factor for cell viability and cell multiplication. The viability, growth rate in liquid cultures and cloning efficiency in agar of all the leukemic clones used were not affected by adding MGI-1GM, IL-3 or MGI-1M at a concentration that induced the formation of 100 macrophage and/or granulocyte colonies by 5 × 10^4 normal nucleated bone marrow cells. The results (Table I), indicate that these growth factor independent myeloid leukemic cells can express receptors for all three types of myeloid growth factors (clones 2 and 11), no or almost no receptors for any of these growth factors (clone 5 and human HL-60), only for MGI-1GM

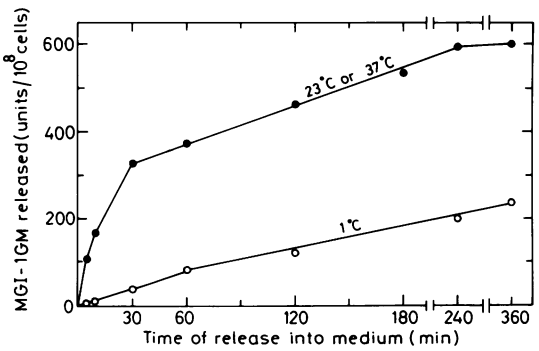

Fig. 5. Reversibility of binding of MGI-1GM to myeloid leukemic cells. Fifty × 10^6 clone 7-M12 leukemic cells were incubated at 1°C for 30 min with 2 × 10^5 units MGI-1GM. The cells were then washed, incubated for different periods of time in 4 ml EM with 10% horse serum, and the amount of MGI-1GM released into medium was determined. ○ – ○, release into the medium at 1°C; ● – ●, release into the medium at 23 or 37°C. Incubation at 37°C showed a similar curve for release to that obtained at 23°C.

(clones 1,6), only for IL-3 (clone 10) or for both IL-3 and MGI-1GM but not for MGI-1M (clone 7-M12). WEHI-3B leukemic cells (Table I), which constitutively produce IL-3, were similar to clone 7-M12 which does not produce any of these growth factors, in having receptors for IL-3 and MGI-1GM. The number of receptors on the growth factor independent leukemic clones with the highest numbers of these receptors (Table I) was ~5-fold lower for MGI-1GM and ~3-fold lower for IL-3 than those found on normal resident macrophgaes (Table II). The number of MGI-1M receptors on these leukemic cells was ~20-fold lower than on normal resident macrophages (Table II). There was no correlation between the type of receptors expressed and the phenotype of the cells as regards their susceptibility for induction of differentiation by the normal myeloid differentiation factor MGI-2 (Table I). These results indicate that growth factor independent clones of myeloid leukemic cells can or cannot have receptors for the growth factors MGI-1GM, IL-3 or MGI-1M and that receptors for myeloid growth factors can be independently expressed in different clones.

Specificity of binding of MGI-1GM, MGI-1M and IL-3 to myeloid cells

The four radiolabeled myeloid growth factors bind to normal

Table II. Binding of myeloid growth factors to normal myeloid cells and to normal and malignant lymphoid cells

Cells	Growth factor activity bound[a]					
	MGI-1GM		IL-3		MGI-1M	
	Units/10^8 cells	Receptors/cell	Units/10^8 cells	Receptors	Units/10^8 cells	Receptors/cell
Normal granulocytes	1100 ± 315	2011 ± 576	336 ± 52	655 ± 102	55 ± 20[b]	286 ± 104[b]
Normal resident macrophages	3115 ± 710	5696 ± 1298	1108 ± 86	2159 ± 167	830 ± 163	4340 ± 852
Normal exudate macrophages[c]	6610 ± 600	12 087 ± 1097	1007 ± 163	1963 ± 320	6200 ± 436	32 426 ± 2280
Normal bone marrow	1400 ± 263	2560 ± 481	246 ± 65	480 ± 104	0	0
Normal lymph nodes	0	0	12 ± 5	23 ± 10	0	0
Normal thymus	0	0	5 ± 1	10 ± 2	0	0
Malignant lymphoid cells						
EL-4	0	0	0	0	0	0
EL-4 + Con A[d]	0	0	0	0	0	0
YAC	0	0	0	0	0	0
L1210	38 ± 8	70 ± 15	170 ± 15	343 ± 29	0	0

[a]No growth factor activity could be eluted from any of these cells before incubation with growth factor in the assay for receptor binding as in Table I.
[b]The amount of MGI-1M bound to granulocytes could all be accounted for by the 5–7% macrophages in the mixture. Therefore, granulocytes probably do not bind MGI-1M.
[c]The total amount of MGI-1GM and IL-3 bound to macrophages was corrected by subtracting the amount bound to the granulocytes in the cell exudate (35% granulocytes) from the amount bound to the mixed cell population.
[d]EL-4 cells were treated with 20 μg/ml Con A for 24 h. The conditioned medium of these cells contain 84 000 units MGI-1 for macrophage and granulocyte colonies per 10^8 EL-4 cells.

Table III. Up-regulation of MGI-1GM receptors during induction of differentiation by normal myeloid differentiation factor MGI-2

Input MGI (units/10^8 cells)		MGI–1GM bound[a]			
MGI-1GM	MGI-2	Direct elution		Binding and elution	
		Units/10^8 cells	Receptors/cell	Units/10^8 cells	Receptors/cell
0	0	0	0	76 ± 21	140 ± 39
0	300	2540 ± 478	4667 ± 895	2630 ± 312	4833 ± 573
4×10^5	0	0	0	63 ± 12	115 ± 22
8×10^4	300	3000 ± 416	5512 ± 764	2983 ± 466	5481 ± 856

[a]D^+ clone 11 myeloid leukemic cells were incubated for 3 days with MGI-2, MGI-1GM or both MGI-2 and MGI-1GM. The MGI-1GM activity was eluted from the cells at low pH either directly (without adding MGI-1GM as in the usual assay) to measure the number of occupied receptors, or after the usual binding assay to measure the total binding capacity.

mouse bone marrow cells at 0°C (Walker *et al.*, 1985) and cells of the lymphoid series do not bind radiolabeled MGI-1GM (Walker and Burgess, 1985) or MGI-1M (Byrne *et al.*, 1981). To determine the cell specificity of binding in our assay that includes elution at low pH, we tested binding to normal and malignant lymphoid cells and to normal myeloid cells. The results (Table II) indicate that there was no binding of either MGI-1GM or MGI-1M activity to the normal and malignant lymphoid cells with T-cell markers and that only minute amounts of IL-3 bound to normal lymph node and thymus cells. Interestingly, however, L1210 cells which are lymphoid leukemic cells with no T or B cell markers (Shevach *et al.*, 1972) showed binding of MGI-1GM and IL-3 corresponding to 70 and 340 receptors per cell, respectively, but no binding of MGI-1M (Table II).

Normal bone marrow cells, mature granulocytes and macrophages bound MGI-1GM (2000–12 000 receptors per cell) and IL-3 (500–2000 receptors per cell) but only macrophages also bound MGI-1M (4000–30 000 receptors per cell) (Table II). The number of MGI-1M and IL-3 receptors on these normal cells calculated from our data are in the same range as those found by using radiolabeled MGI-1M binding to normal bone marrow cells and macrophgaes (Guilbert and Stanley, 1980) and IL-3 binding to IL-3 dependent cell lines (Palaszynski and Ihle, 1984). The results, therefore, indicate that the method of absorption and low pH elution of biological activity from different cell types can be effectively used to analyse the number, affinity and specificity of receptors for different forms of biologically active hematopoietic growth factors and to detect even very low numbers of receptors.

Up-regulation of MGI-1GM and IL-3 receptors during differentiation of myeloid leukemic cells

During differentiation of myeloid leukemic cells there is an increase in the expression of various cell surface receptors which mediate cellular functions such as binding and phagocytosis of immune complexes and chemotaxis (Lotem and Sachs, 1974, 1977; Symonds and Sachs, 1979). Cells of non-myeloid origin display an induction of specific hormone receptors during differentiation (Lin and Beckner, 1983). We have also shown that growth factor independent myeloid leukemic cells regain the normal cell requirement for myeloid growth factors for viability and growth during differentiation (Lotem and Sachs, 1982,1983). We have, therefore, now determined whether differentiating myeloid leukemic cells express an increased number of cell surface receptors for myeloid growth factors compared to undifferentiated and growth factor independent myeloid cells.

Induction of differentiation of D^+ clone 11 myeloid leukemic cells by differentiation factor MGI-2 derived from Krebs ascites cells has shown that at 3 days after induction of differentiation, when most of the cells are still not mature, the number of recep-

Table IV. Up-regulation of MGI-1GM and IL-3 receptors after induction of differentiation by low doses of actinomycin D or cytosine arabinoside

Incubation of cells with:[a]	Growth factor bound					
	MGI-1GM		IL-3		MGI-1M	
	Units/10^8 cells	Receptors/cell	Units/10^8 cells	Receptors/cell	Units/10^8 cells	Receptors/cell
None	76 ± 21	140 ± 39	66 ± 10	129 ± 20	37 ± 5	193 ± 26
MGI-2	2540 ± 478	4667 ± 895	1260 ± 316	2457 ± 616	35 ± 5	187 ± 27
Act. D (low)	320 ± 96	590 ± 176	254 ± 10	495 ± 20	30 ± 6	156 ± 31
Ara C (low)	226 ± 58	415 ± 106	208 ± 16	405 ± 31	22 ± 4	114 ± 21
Dex.	46 ± 15	84 ± 28	48 ± 10	94 ± 20	20 ± 4	104 ± 21
Act. D (high)[b]	0	0	0	0	0	0
Cyclohexamide[b]	0	0	0	0	0	0

[a]D^+ clone 11 myeloid leukemic cells were incubated for 3 days at 3×10^5 cells per ml with 300 units MGI-2, or dexamethasone (400 ng per ml = 1 μM), or at 8×10^5 cells/ml with low dose (7 ng per ml) actinomycin D (Act. D) or low dose (90 ng per ml), cytosine arabinoside (ara C). Binding to cells induced to differentiate by MGI-2 was determined after removal of surface-bound MGI-1GM at low pH as in Table III. Cells were washed three times and were assayed for binding as in Table I. No growth factor activity was eluted directly from cells treated with actinomycin D, cytosine arabinoside or dexamethasone and the medium conditioned by cells treated with these compounds did not contain any detectable growth factor activity.
[b]One microgram per ml actinomycin D or 5 μg per ml cyclohexamide was added for 3 h, the cells washed twice and binding of growth factor determined.

tors for MGI-1GM increased ~40-fold (Tables III, IV), the number of IL-3 receptors increased ~20-fold (Table IV) and there was at this time no up-regulation of receptors for MGI-1M (Table IV). Moreover a direct low pH elution of MGI-2 induced cells (without prior incubation with MGI-1GM as in the absorption assay) gave a similar amount of cell-bound MGI-1GM (Table III). This indicates that during induction of differentiation by MGI-2 there is a 40-fold increase in the number of MGI-1GM receptors, all of which are occupied by MGI-1GM. Incubation of the leukemic cells for 3 days with a high concentration of MGI-1GM, which does not induce differentiation of these leukemic cells, did not induce an increase in the number of MGI-1GM receptors (Table III).

These results suggested that induction of differentiation by MGI-2 not only up-regulates receptors for MGI-1GM but also induces the synthesis of MGI-1GM which then occupies the induced receptors. This supposition was confirmed by experiments which showed that conditioned medium from the leukemic cells incubated for 3 days with MGI-2 (300 units/10^8 cells) (Table III) contained a high amount of MGI-1GM activity (4×10^4 units/10^8 cells). This was not due to contamination of the MGI-2 with lipopolysaccharide (LPS), which can also induce production of MGI-1 in these leukemic cells (Weiss and Sachs, 1978), since the MGI-2 induced production of MGI-1GM activity was not inhibited by polymyxin B which completely inhibits the activity of LPS. To determine whether every type of MGI-1 producing cell expresses receptors for MGI-1, we induced production of MGI-1 activity in the T cell lymphoma EL-4 with Concanavalin A (Con A). The induced synthesis of 8.4×10^4 units MGI-1 per 10^8 Con A treated EL-4 cells did not result in expression of detectable receptors for any of the three growth factors (Table II).

Clone 11 myeloid leukemic cells can be induced to differentiate not only by the normal differentiation factor MGI-2, but also by low concentrations of compounds such as actinomycin D, cytosine arabinoside or dexamethasone (Lotem and Sachs, 1974; Sachs, 1978,1982). Incubation with low concentrations of actinomycin D or cytosine arabinoside for 3 days, which induced a lower degree of differentiation than MGI-2, induced a 3- to 4-fold increase in the number of MGI-1GM and IL-3 receptors, and gave no change in the number of MGI-1M receptors (Table IV). Unlike induction of differentiation with MGI-2 these induced receptors were not occupied. There was no increase in the number of receptors for any of these growth factors by incubation of cells with dexamethasone (Table IV). A high concentration of actinomycin D (1 μg/ml) or cycloheximide (5 μg/ml) completely abolished the capacity of these cells to bind MGI-1GM, IL-3 or MGI-1M (Table IV). This shows that the receptors are labile structures which have to be actively synthesized. The present results indicate that differentiation of myeloid leukemic cells induced by MGI-2 and some other compounds is accompanied by an up-regulation of the number of MGI-1GM and IL-3 receptors, and that MGI-2 induced cells also produce MGI-1GM activity which occupies the induced receptors.

Discussion

We have analyzed the binding of three hematopoietic growth factors to leukemic and normal myeloid cells using the method of quantitative absorption at 1 °C and low pH elution of biological activity (Bowen-Pope and Ross, 1985). The results show that this binding assay can detect cell surface receptors even when the number of receptors per cell is small. Binding of MGI-1GM (GM-CSF) and IL-3 to myeloid leukemic cells was rapid and saturable (saturation within 10 min), with 0.7 and 0.1% of the added MGI-1GM and IL-3, respectively, bound to the cells at saturation. This is in the same range as results obtained by binding radiolabeled MGI-1GM, IL-3 and MGI-1M to cells (Das *et al.*, 1980; Palaszynski and Ihle, 1984; Walker and Burgess, 1985). Our results on the binding of MGI-1M (M-CSF, CSF-1) to normal bone marrow cells and mature macrophages are in agreement with those obtained using radiolabeled MGI-1M (Guilbert and Stanley, 1980; Byrne *et al.*, 1981; Stanley and Guilbert, 1981) and our calculated number of IL-3 receptors on macrophages is the same range as that found on IL-3 dependent cell lines (Palaszynski and Ihle, 1984). In addition, our results on MGI-1GM receptors on macrophages are similar to those obtained by a radioreceptor assay with a macrophage cell line (Park *et al.*, 1986b). The comparison of our calculated number of receptors using quantitative absorption and low pH elution of bioactivity and those obtained by radioreceptor assays are thus generally in good agreement. Furthermore, this binding and elution assay with leukemic cells with only one type of specific receptor can detect a specific type of growth factor in a mixture containing several types and can be used to separate one type of growth factor from a mixture.

MGI-1GM or MGI-1M did not bind to normal lymphoid cells

and to 2 T lymphoma cell lines and only small amounts of IL-3 were bound to normal lymphoid cells. Interestingly, however, L1210 lymphoid leukemia cells that have no T or B cell markers (Shevach *et al.*, 1972) bind both MGI-1GM and IL-3 (70 and 370 receptors per cell, respectively). Since IL-3 also binds to a pre B cell line (Palacios and Garland, 1984) and activates the synthesis of 20-α-hydroxysteroid dehydrogenase in spleen lymphocytes from nude mice (Ihle *et al.*, 1981), the binding of IL-3 to L1210 cells may reflect the activity of IL-3 on a variety of cell types.

The binding of MGI-1GM to the myeloid leukemic cells was rapid and reversible after removing the unbound activity. The rapid binding and the slow release of bound growth factor at neutral pH fit the behavior of high affinity surface receptors. The analysis of binding according to the slope of the Scatchard plot or the concentration at which binding was half maximal (Bowen-Pope and Ross, 1985) has shown that clone 7-M12 myeloid leukemic cells have a single class of MGI-1GM receptors with an apparent K_d of 2.0×10^{-9} M and IL-3 receptors with an apparent K_d of 2.6×10^{-9} M. This K_d is considerably higher than the K_d of receptors for MGI-1M receptors on macrophages (2.8×10^{-11} M) which show almost no reversibility of binding (Stanley and Guilbert, 1981) or IL-3 receptors on IL-3 dependent cell lines ($1.7-5.4 \times 10^{-11}$ M) (Palaszynski and Ihle, 1984), but is in the same range as the K_d of mouse MGI-1GM receptors on bone marrow cells (Walker and Burgess, 1985) or on a macrophage cell line (Park *et al.*, 1986b), the human CSF-2α receptors (IL-3 like) (Park *et al.*, 1986a) and receptors for other hormones such as interleukin 1 (Dower *et al.*, 1985), platelet-derived growth factor (Huang *et al.*, 1982) and glucagon (Rojas and Birnbaumer, 1985).

Analysis of binding of MGI-1GM, IL-3 and MGI-1M to different clones of myeloid leukemic cells that are all independent of myeloid cell growth factors for cell viability and growth has shown that there can be an independent expression of receptors for different hematopoietic growth factors. There are clones that express all three types of receptors, none or almost none of these receptors, only MGI-1GM receptors, only IL-3 receptors or both of these but not MGI-1M receptors. The number of receptors expressed on the leukemic cells was lower than on normal mature macrophages and there was no correlation between the number and type of receptors and the phenotype of the leukemic cells as regards their inducibility for differentiation. The human HL-60 cells showed no binding of any of the three mouse growth factors as was also shown by the radioreceptor assay with mouse MGI-1GM (Walker and Burgess, 1985). These human leukemic cells, however, bind the human MGI-1GM (Gasson *et al.*, 1986) but not the human CSF-2α (IL-3 equivalent) (Park *et al.*, 1986a).

Differentiation-competent myeloid leukemic cells induced to differentiate by the myeloid differentiation-inducing protein MGI-2 regain the requirement for myeloid growth factor for viability and growth which can be fulfilled by MGI-1GM, IL-3 or MGI-1M (Lotem and Sachs, 1982). We have shown that induction of differentiation with MGI-2 to a stage when most of the cells are still not mature results in a 40-fold and 20-fold increase in the number of receptors for MGI-1GM and IL-3, respectively, to a level similar to that found on normal resident macrophages. Furthermore, all the MGI-2 induced MGI-1GM receptors were already occupied by MGI-1GM molecules even when the cells were induced by MGI-2 devoid of any MGI-1GM activity, and the cells were shown to be induced by MGI-2 to produce MGI-1GM activity. This differentiation-associated autocrine-like synthesis of MGI-1GM and saturation of the receptors can explain how cells induced by MGI-2 do not die in the absence of externally added MGI-1 at a time when the cells become MGI-1 dependent for viability (Fibach and Sachs, 1976; Lotem and Sachs, 1982).

In addition to up-regulation of MGI-1GM and IL-3 receptors during differentiation of the leukemic cells induced by MGI-2, induction of differentiation by low doses of actinomycin D or cytosine arabinoside (Lotem and Sachs, 1974), which induced a lower degree of differentiation than MGI-2, also increases 3- to 4-fold the number of receptors for MGI-1GM and IL-3. MGI-1GM, however, does not up-regulate its own receptors, nor IL-3 and MGI-1M receptors in these leukemic cells, unlike other growth factors such as IL-2 (Reem *et al.*, 1985) or epidermal growth factor (EGF) (Clark *et al.*, 1985) which up-regulate their own receptors in some other cell types. Incubation with a high concentration of actinomycin D or cycloheximide completely abolishes the expression of MGI-1GM, IL-3 and MGI-1M receptors. This indicates that the receptors for these growth factors are labile and continue to be synthesized and degraded. Hematopoietic growth factors can enhance the functional activity of mature cells (Ziboh *et al.*, 1982; Tushinsky and Stanley, 1983; Vadas *et al.*, 1983; Chen and Lin, 1984; Gasson *et al.*, 1984). The up-regulation of these receptors during differentiation may thus be required for functioning of the differentiated cells.

Materials and methods

Cells and cell culture

Normal mouse lymph node, thymus, bone marrow, mature macrophages and granulocytes were obtained from 3-month-old SJL/J mice. Thymus and lymph node cells were teased out of the organs into cold phospahte-buffered saline (PBS) pH 7.4, and bone marrow cells were obtained from the femurs of normal mice. Resident macrophages were obtained from the peritoneal cavity of normal mice and exudate macrophages from mice injected 3 days earlier with a 2 ml solution of 10% sodium caseinate (DIFCO Lab., Detroit, MI) (Lotem and Sachs, 1985). Granulocytes were obtained 16 h after the injection of caseinate. Granulocytes and macrophages were collected in PBS with 6 U/ml heparin (Novo, Cophenhagen). The cells were washed twice in cold PBS and once in Dulbecco's modified Eagle's medium (EM) (H-21 Grand Island Biological Co., Gibco, NY) with 10% horse serum (Gibco, NY) before testing for binding of growth factors to receptors.

The tumor cell lines were all cultured in EM with 10% horse serum. The lines used were EL-4 and YAC T cell lymphomas and L1210 non-T, non-B lymphoid leukemia (Shevach *et al.*, 1972); myeloid leukemic clones 2, 5 and 11 (Fibach *et al.*, 1973) derived from a spontaneous myeloid leukemia in an SL mouse (Ichikawa, 1969); myeloid leukemic clones 1, 6, 10 and 7-M12 derived from four independently arising X-ray induced leukemias in SJL/J mice (Lotem and Sachs, 1977); differentiation-defective WEHI-3B myelomonocytic leukemia line originating in a BALB/c mouse (Walker and Burgess, 1985); and human HL-60 promyelocytic leukemic cells (Collins *et al.*, 1977). Clones of myeloid leukemia cells that can be induced to differentiate to mature macrophages and/or granulocytes by the normal myeloid cell differentiation factor MGI-2 (DF) are defined as D^+ clones. Differentiation-defective clones, which include clones that are induced by MGI-2 to an intermediate stage of differentiation but not mature cells and clones that are not induced by MGI-2 even to this intermediate stage, are called D^- clones (Lotem and Sachs, 1974; Sachs, 1985).

Myeloid cell growth and differentiation factors

The growth and differentiation factors were obtained from conditioned medium from the following sources. Krebs II ascites tumor cells that produce MGI-1GM (GM-CSF) (Lipton and Sachs, 1981). This MGI−1GM activity is not inhibited by antiserum against MGI-1M (Lotem *et al.*, 1980). These cells also produce differentiation factor MGI-2 (DF) (Lipton and Sachs, 1981). WEHI-3B myelomonocytic leukemia cells that produce IL-3 (multi-CSF) (Ihle *et al.*, 1982). L929 fibroblasts that produce MGI-1M (M-CSF, CSF-1) (Stanley and Heard, 1977). These conditioned media were concentrated 100-fold using high molecular weight polyethylene glycol as described (Lotem and Sachs, 1981). To obtain MGI-2 without MGI-1GM from Krebs ascites cells, 2 ml of 100-fold concentrated conditioned medium were dialysed against Tris-HCl (10 mM pH 8.5) and loaded on a 1.6 × 10 cm DEAE-Sephacel (Pharmacia, Uppsala, Sweden) column equilibrated in the same buffer. The column was washed with 50 ml starting buffer and then eluted with 300 ml linear gradient of 0−0.2 M NaCl in the same buf-

fer. Fractions (3.5 ml) were collected and assayed for MGI-1 and MGI-2 activity and the fractions that contained only MGI-2 activity (at 0.05 M NaCl) (Lipton and Sachs, 1981) were pooled. To obtain MGI-1GM without MGI-2 from this source, 1 ml of concentrated conditioned medium was dialysed against sodium phosphate buffer (0.1 M pH 6.3) containing 1 M ammonium sulfate and loaded on a 1.6 × 10 cm phenyl-Sepharose column (Pharmacia). The column was washed with 100 ml of the same buffer, then with a 100 ml linear gradient of 1 M ammonium sulfate to water, followed by a 100 ml linear gradient of 0–70% ethylene glycol. Most of the MGI-1GM activity did not bind to the column at 1 M ammonium sulfate and was separated from the MGI-2 activity which eluted near the water.

Growth factor activity was assayed in 35-mm Petri dishes by seeding 5×10^4 normal nucleated bone marrow cells in 0.8 ml of 0.33% agar on top of a 2.5 ml harder agar base (0.5%) that contained different amounts of the material to be assayed. Both layers contained EM and 20% horse serum (Pluznik and Sachs, 1965) and the medium was also supplemented with 1% normal rabbit serum to increase the cloning efficiency. Colonies of macrophages and/or granulocytes containing 50 or more cells were counted after 7 days incubation at 37°C and the number of units of growth factor activity (1 unit = 1 colony) was determined from the linear part of the concentration curve. Other assays of growth factor activity use only a single 1 ml layer of agar and 7.5×10^4 cells (Walker *et al.*, 1985) and would, therefore,yield a 5-fold higher number of colony forming units compared to our assay with the same amount of tested material. This was taken into account in comparing our data to that published with this other assay. Differentiation factor (MGI-2) activity was assayed using differentiation competent (D^+) growth factor independent clone 11 myeloid leukemic cells and measuring the amount of lysozyme produced by these cells after 4 days incubation as described (Krystosek and Sachs, 1976). One unit of MGI-2 activity is defined as the amount that induces the production of 1 μg equivalent of lysozyme per 5×10^6 cells after 4 days.

Assay for quantitative absorption and elution of growth factor activity

The cells were washed twice in cold EM with 10% horse serum. Generally, 50×10^6 cells were pelleted in 50 ml tubes and resuspended in a volume of 2 ml containing different amounts of MGI-1GM, IL-3 or MGI-1M activity in cold EM and 10% horse serum. The tubes were kept on ice (1°C) for 30 min (with MGI-1GM or IL-3) or 4 h (MGI-1M) unless otherwise stated, mixed gently every 15 min, transferred to new 10-ml tubes and centrifuged in the cold at 800 *g* for 4 min. The cell pellet was washed three times with 10 ml cold medium with serum and the pellet was then resuspended in 0.5 ml saline (0.15 M NaCl) containing 52 mM acetic acid (pH 3.1) (Bowen-Pope and Ross, 1985). After 5 min on ice with one mixing (vortex) after 2 min, the cells were again centrifuged and the supernatant collected, diluted to 2–4 ml with medium and serum (the pH was increased to 6.5) and assayed for growth factor activity at a final concentration of 5–40%. The amount of absorbed and eluted growth factor activity was determined from the linear part of the concentration curve and expressed as units eluted per 10^8 cells. Incubation of the growth factors for 5 min at pH 3.1 did not affect their activity. Each experiment was repeated at least three times.

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