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Vitamin D: Myeloid Differentiation and Proliferation

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A. Background

The active metabolite of vitamin D ($1,25(OH)_2D_3$) is a major physiologic regulator of mineral metabolism in humans. The $1,25(OH)_2D_3$ facilitates calcium absorption from the intestine, acts to mobilize calcium from bone, and possibly stimulates renal reabsorption of calcium. In contrast to the current progress achieved in elucidating the role of $1,25(OH)_2D_3$ in mineral metabolism, the mechanism of action and biologic significance of vitamin D metabolites in the hematopoietic system are just beginning to be studied.

Early reports suggested that vitamin D-deficient rickets patients frequently had anemia, extramedullary hematopoiesis, and mononuclear phagocytes that poorly performed chemotaxis and phagocytosis. A murine myeloblastic leukemia cell line (M-1) was discovered to undergo macrophage-like differentiation in the presence of $1,25(OH)_2D_3 > 24,25(OH)_2D_3 > 25(OH)_2D_3$ [1]. Development of leukemia was inhibited or the survival time of mice prolonged after M-1 cells were injected into syngeneic mice and the mice were treated with $1,25(OH)_2D_3$.

B. Vitamin D and Differentiation of HL-60 Cells

Preliminary evidence by Miyaura et al. [9] suggested that the cells from the human promyelocytic leukemia line known as HL-60 differentiated to granulocytes when cultured with $1,25(OH)_2D_3$. Further studies by others as well as ourselves showed that the HL-60 became predominantly macrophage-like after culture with $1,25(OH)_2D_3$ [2, 7, 8]. Likewise, we have studied a variant of HL-60 (HL-60 blast) that is unable to undergo differentiation [7]. As shown in Table 1, after 7 days of treatment with as little as 10^{-10} *M* $1,25(OH)_2D_3$, significant differentiation occurred as measured by four test parameters of macrophage differentiation. In contrast, the HL-60 blast cells were unresponsive at each concentration of $1,25(OH)_2D_3$.

Morphological and functional changes began to be minimally expressed after 7 days with a $1,25(OH)_2D_3$ incubation as short as 18 h [7]. The differentiation response became more pronounced the longer the hormone remained in the culture medium, suggesting that the presence of $1,25(OH)_2D_3$ is required and must be maintained over a long period of time relative to transient events occurring at the molecular level.

The mechanism by which $1,25(OH)_2D_3$ induces differentiation is unknown. Preliminary evidence by Tanaka et al. [12] implicated the presence of cytosolic $1,25(OH)_2D_3$ receptors in HL-60 and suggested that differentiation may be occurring by a mechanism similar to that of

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Table 1. Functional and morphologic changes in HL-60 and HL-60 blast cells by various concentrations of $1.25(OH)_2D_3$[a]

Added concentration of $1,25(OH)_2D_3$[b] (M)	Cell line	NBT reduction (%)	Phagocytic cells (%)	Morphology		
				Myeloblasts and promyelo-blasts (%)	Intermediate to mature[c] (%)	Nonspecific acid esterase-positive[d] (%)
0	HL-60	2± 3	2±2	99±2	1±2	2
	HL-60 blast	0	1	100	0	0
10^{-11}	HL-60	10± 2	2±3	95±3	5±4	3
	HL-60 blast	0	0	100	0	0
10^{-10}	HL-60	18±11	13±7	82±5	18±7	10
	HL-60 blast	0	0	100	0	0
10^{-9}	HL-60	37±19	20±7	66±9	34±6	25
	HL-60 blast	0	0	100	0	0
10^{-8}	HL-60	64±13	26±4	45±12	55±9	54
	HL-60 blast	0	0	100	0	0
10^{-7}	HL-60	82± 8	44±9	32±5	67±6	82
	HL-60 blast	3	5	96	4	0
10^{-6}	HL-60	86±12	60±3	27±6	78±14	98
	HL-60 blast	1	0	100	0	0

[a] HL-60 and HL-60 blast cells were cultured in the presence or absence of various concentrations of $1.25(OH)_2D_3$. After 7 days cells were assessed for the various differentiation parameters. Cell viability was >99%. All data are expressed as the percentage of total cells assayed. At least 200 cells were assessed for each parameter. The HL-60 cell data represent the mean±standard deviation of triplicate assays

[b] Basal $1,25(OH)_2D_3$ in 10% fetal bovine serum is 1.6×10^{-11} M

[c] Intermediate to mature cells include monocytes and macrophages

[d] Represents the average of two experiments

classical steroid hormone action. We reported the unqualified identification of the $1,25(OH)_2D_3$ receptor in HL-60 cells and the existence of a positive correlation between $1,25(OH)_2D_3$-induced differentiation and the occurrence of occupied $1,25(OH)_2D_3$ receptors [7].

Aliquots of high salt nuclear extracts, prepared by first labeling intact HL-60 and HL-60 blast cells with ^3H $1,25(OH)_2D_3$, were analyzed by high salt sucrose density gradient centrifugation. As illustrated in Fig. 1a, virtually all of the bound ^3H $1,25(OH)_2D_3$ migrated in a single 3.3 S peak, characteristic of $1,25(OH)_2D_3$ receptors. This peak was completely obliterated by the mercurial reagent, PCMBS (Fig. 1a), indicating dissociation of the $1,25(OH)_2D_3$ hormone–receptor complex. Finally, to confirm the 3.3 S macromolecule as the $1,25(OH)_2D_3$ receptor, monoclonal antibodies to the $1,25(OH)_2D_3$ receptor were incubated with the nuclear extract before sedimentation. The monoclonal antibody 4A5λ shifted the migration of the 3.3 S macromolecule to the 7–8 S position (Fig. 1a), a finding consistent with the sedimentation properties of the hormone–receptor–antibody complex. In contrast, the 8D3 monoclonal antibody is specific for chick intestinal receptor and therefore had no effect on the sedimentation of this 3.3 S macromolecule (Fig. 1a). Figure 1b shows a similar sedimentation pattern for the nuclear extract derived from HL-60 blast. A PCMBS-dissociable 3.3 S macromolecule that was recognized by 4A5λ (but not 8D3) antibody demonstrated the presence of a small, yet detectable, amount of $1,25(OH)_2D_3$ receptor in HL-60 blast cells.

We measured the specific uptake of ^{3}H 1,25$(OH)_2D_3$ into intact HL-60 under conditions that are normally used to grow these cells in culture. Figure 2a demonstrates that ^{3}H 1,25$(OH)_2D_3$ uptake by intact HL-60 cells was a specific and saturable process. Accordingly, Scatchard transformation of these data yielded an equilibrium dissociation constant for 1,25$(OH)_2D_3$ internalization (K_{int}) of 5.4 nM (Fig. 2b) and extrapolation to the abscissa predicts 4000 1,25$(OH)_2D_3$ receptor molecules per HL-60 cell. The K_{int} (5.4 nM) is nearly identical to the concentration that induces 50% of HL-60 cells to differentiate. This correspondence, plus the resistance of the relatively receptor-poor HL-60 blast, strongly suggests that 1,25$(OH)_2D_3$-induced differentiation of HL-60 cells to monocytes/macrophages is occurring via receptor-mediated events.

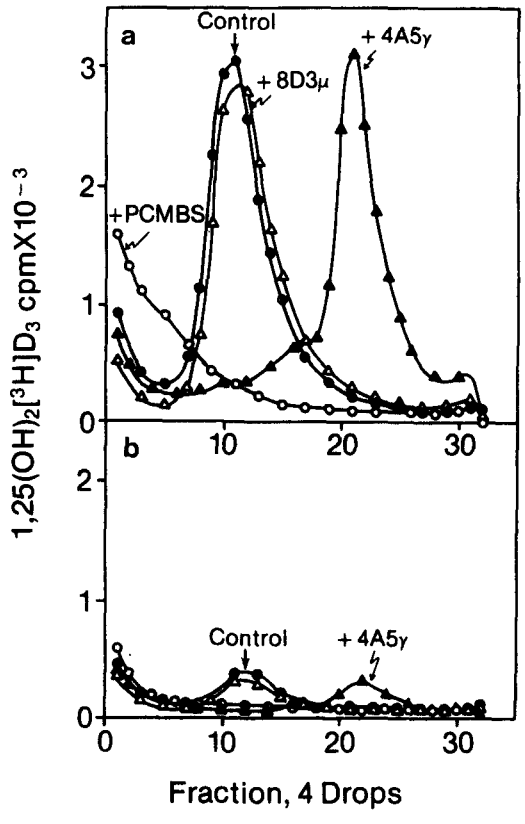

Fig. 1a, b. Sucrose density gradient analysis of ^{3}H 1,25$(OH)_2D_3$ binding in nuclear extract of HL-60 **a** and HL-60 blast **b** cells. Prior to ultracentrifugation, samples were incubated in the presence (*open circles*) or absence (*full circles*) of PCMBS, the presence of 4A5λ monoclonal antibody (*full triangles*), or the presence of 8D3μ monoclonal antibody (*open triangles*)

C. Effect of 1,25$(OH)_2$ D_3 and its Fluorinated Analogs on Myeloid Differentiation

I. Normal Myeloid Stem Cells

We examined the effect of 1,25$(OH)_2D_3$ and two fluorinated analogs of 1,25$(OH)_2D_3$ on differentiation of the normal human myeloid stem cell (GM-CFC) [6]. We studied the two fluorinated analogs of 1,25$(OH)_2D_3$, known as 24,24-F_2-1,25$(OH)_2D_3$ and 26,26,26,27,27,27,-1,25$(OH)_2D_3$ (26,27-F_6-1,25$(OH)_2D_3$), because both compounds are as active or more active than 1,25$(OH)_2D_3$ in calcium reabsorption and probably have a longer in vivo half-life than 1,25$(OH)_2D_3$.

We found that the 1,25$(OH)_2D_3$ and its fluorinated analogs markedly induced human myeloid GM-CFC to differentiate to colonies containing only macrophages. In the absence of 1,25$(OH)_2D_3$, normal human bone marrow GM-CFC differentiated to approximately 55% neutrophil, 10% mix,

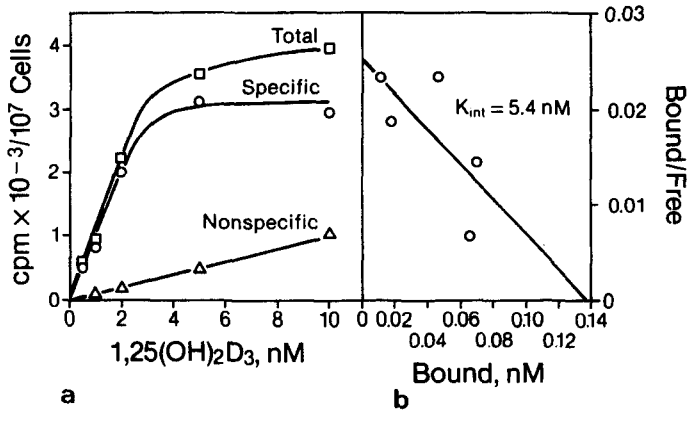

Fig. 2a, b. Determination of the euqilibrium dissociation constant of 1,25$(OH)_2D_3$ internalization of intact HL-60 cells. Saturation analysis **a** was determined by incubating intact cells under normal growth conditions for 4 h with 10% serum along with various concentrations of tritiated 1,25$(OH)_2D_3$ in the presence of or absence of 100-fold excess nonradioactive 1,25$(OH)_2D_3$. Specific binding was transformed by Scatchard analysis and the data line-fitted by linear regression, **b** to yield K_{int} = 5.4 nM (abscissa intercept = 4000 molecules per cell, $r = -0.71$)

and 25% macrophage colonies. Nearly 95% of the colonies were composed of only macrophages in culture plates containing 10^{-7}–10^{-8} *M* 1,25$(OH)_2D_3$. Some 55% of the colonies were composed of only monocytes/macrophages in plates containing 10^{-9} *M* 1,25$(OH)_2D_3$. Similar results were observed with the fluorinated analogs.

The 1,25$(OH)_2D_3$ and fluorinated analogs (10^{-7}–10^{-9} *M*) increased the absolute number of macrophage colonies, rather than merely increasing the relative proportion of macrophage colonies, by selectively inhibiting granulocytic differentiation of GM-CFC. Plates containing either 10^{-7} or 10^{-8} *M* 1,25$(OH)_2D_3$ developed approximately 75 and 90 macrophage colonies, respectively per 10^5 cultured marrow cells. Likewise, plates with 10^{-9} *M* 1,25$(OH)_2D_3$ developed about 65 macrophage colonies per 10^5 cultured marrow cells. In contrast, 35 macrophage colonies per 10^5 cultured marrow cells developed in control plates containing no 1,25$(OH)_2D_3$. Similar results were observed with the fluorinated analogs.

The hypothesis that 1,25$(OH)_2D_3$ may be a possible inducer of differentiation of GM-CFC to macrophages is appealing because of the known ability of 1,25$(OH)_2D_3$ to modulate bone resorption. Osteoclasts resorb bone. Evidence suggests that osteoclasts may develop from monocyte/macrophage cells [4] and one study suggested that 1,25$(OH)_2D_3$ may modulate the number of osteoclasts [5]. Therefore, 1,25$(OH)_2D_3$ might modulate bone resorption by inducing GM-CFC to differentiate to monocytes and macrophages and eventually to osteoclasts. Likewise, in vitro, the monocytes can directly resorb bone [10].

Caution must be exercised in the overinterpretation of the data. The plasma concentration of 1,25$(OH)_2D_3$ in humans is approximately 7.7×10^{-11} *M* [3]. Our studies showed that only concentrations $\geqq 10^{-9}$ *M* 1,25$(OH)_2D_3$ induced significant macrophage differentiation of myeloid progenitor cells in vitro. Therefore, 1,25$(OH)_2D_3$ may not have a physiologic role in the induction of differentiation of human myeloid stem cells to macrophages. Likewise, patients who received superphysiologic doses of 1,25$(OH)_2D_3$ have not been reported to have an increased concentration of blood monocytes [11]. The true hematopoietic role of vitamin D metabolites in vivo is unknown and will require careful experimentation.

II. Leukemic Myeloid Stem Cells

Interest has developed in attempting to induce differentiation of myeloid leukemic blast cells to functional cells that are no longer capable of proliferation (Kœffler HP, 1983). It can be shown that 1,25$(OH)_2D_3$, 24,24-F_2-1,25$(OH)_2D_3$, and 26,27-F_6-1,25$(OH)_2D_3$ induce macrophage differentiation of leukemic myeloid colony-forming cells (Table 2). The potency of induction of differentiation of leukemic cells by 1,25$(OH)_2D_3$ and the fluorinated analogs was almost equivalent – concentrations of 10^{-8} *M* induced about 90% of CML and 50% of AML myeloid leukemic colony-forming cells to differentiate to colonies containing macrophage-like cells. These concentrations of the agents can be achieved in patients. The CML and AML control dishes containing no 1,25$(OH)_2D_3$, developed only 30% and 15% macrophage colonies, respectively. The study suggests that the myeloid stem cell can be induced to undergo terminal macrophage differentiation and be removed from the proliferative pool.

D. Effect of 1,25$(OH)_2$ D_3 on Maturation of Human Leukemic Cells in Liquid Culture

We also examined the effect that 1,25$(OH)_2D_3$ would have on proliferation and differentiation in liquid culture of freshly isolated myeloid leukemic blast cells from nine patients (Fig. 3). Blast cells of two patients (1, 2) were at the early myeloblast M1 (French–American–British classification) stage of development; blast cells of three patients (3, 4, 5) were at the myeloblast (M2) stage of differentiation; blasts from two patients (6, 7) were at the myelomonoblast stage (M4); cells from patient 8 were CML myeloid blasts and cells from patient 9 were CML lymphoid blasts.

Table 2. Effects of 1,25$(OH)_2D_3$ and fluorinated analogs of 1,25$(OH)_2D_3$ on differentiation and proliferation of leukemic human myeloid colony-forming cells

Cell source	Vitamin D analog	Concentration (H)	No. of colonies (% of control)[a]	Colony morphology[b] (%) N	NM	M	B
Chronic myelogenous leukemia (4 patients)	1,25$(OH)_2D_3$	0	100	62±6	7±1	31±2	
		10^{-10}	98±5	66±5	4±1	30±4	0
		10^{-9}	110±6	42±2	6±1	52±5	0
		10^{-8}	101±6	5±1	3±1	92±1	0
		10^{-7}	76±7	0	0	100±1	0
Acute nonlymphocytic leukemia (4 patients)	1,25$(OH)_2D_3$	0	100	12±2	0	18±3	70±5
		10^{-10}	103±8	7±3	4±1	17±4	72±6
		10^{-9}	112±8	7±1	3±1	28±3	53±5
		10^{-8}	89±6	6±1	3±1	53±4	38±6
		10^{-7}	80±6	1±1	4±3	60±5	36±6
Chronic myelogenous leukemia	24,25-F_2-1,25-$(OH)_2D_3$	0	100	62±6	7±1	31±2	0
		10^{-10}	106±9	55±6	2±1	43±4	0
		10^{-9}	109±7	38±3	5±2	51±4	0
		10^{-8}	83±5	0	4±1	96±2	0
Acute nonlymphocytic leukemia	24,24-F_2-1,25$(OH)_2D_3$	0	100	12±2	0	18±3	70±5
		10^{-10}	104±8	5±2	2±1	8±1	84±4
		10^{-9}	116±6	3±1	4±1	26±3	67±3
		10^{-8}	85±7	1±1	7±1	56±4	36±3

[a] Marrow cells were obtained from 4 acute nonlymphocytic leukemia (AML) patients, and peripheral blood cells were obtained from 4 chronic myelogenous leukemia (CML) patients. The low density, nonadherent, mononuclear cells were cultured in the presence of 2.5% T lymphocyte CM (source of CSF) and various concentrations of 1,25$(OH)_2D_3$ and fluorinated analogs of 1,25$(OH)_2D_3$. The CMLV and AML cultures contained a mean of 121±7, and 31±8 (±standard error) myeloid colonies, respectively

[b] N neutrophilic colonies; NM neutrophil/macrophage mixed colonies; M monocyte/macrophage colonies; B blast cell colonies

Net cellular proliferation was examined after the blast cells (5×10^5/ml) from each of the leukemic patients were exposed for 6 days to 1,25$(OH)_2D_3$ or culture media alone (control). The median (range) of day 6 cell counts ($\times 10^5$/ml) in flasks containing 10^{-7} M 1,25$(OH)_2D_3$, 10^{-6} M 1,25$(OH)_2D_3$, and culture medium alone (control) was 5 (2–9), 4 (2–8), and 7 (5–9), respectively. The 1,25$(OH)_2D_3$ had an inhibitory effect on cellular proliferation with the higher concentration of 1,25$(OH)_2D_3$ (10^{-6} M) producing a significant inhibition of growth as compared with control flasks ($P < 0.05$) (Fig. 3).

The 1,25$(OH)_2D_3$ induced maturation of leukemia cell maturation to monocytes, macrophages, metamyelocytes, and granulocytes. At day 6, the median percentage of mature myeloid cells in flasks containing 10^{-7} M and 10^{-6} M 1,25$(OH)_2D_3$ or culture medium alone was 36%, 40%, and 5%, respectively. The percentage of mature myeloid cells at day 6 of culture was significantly ($P < 0.05$) higher for the flasks containing 10^{-6} M 1,25$(OH)_2D_3$ as compared with day 6 control flasks. Macrophages and granulocytes are able to reduce NBT, but their immature progenitors can not. We examined the ability of the leukemic cells from the nine patients to reduce NBT after culture with 1,25$(OH)_2D_3$ for 6 days (Fig. 3). The median percentage of NBT-reducing cells in control, 10^{-7} M, and 10^{-6} M 1,25$(OH)_2D_3$-containing flasks was 10%, 33%, and 49%, respectively. The number of NBT-reducing cells was significantly ($P < 0.05$) greater in the flasks containing

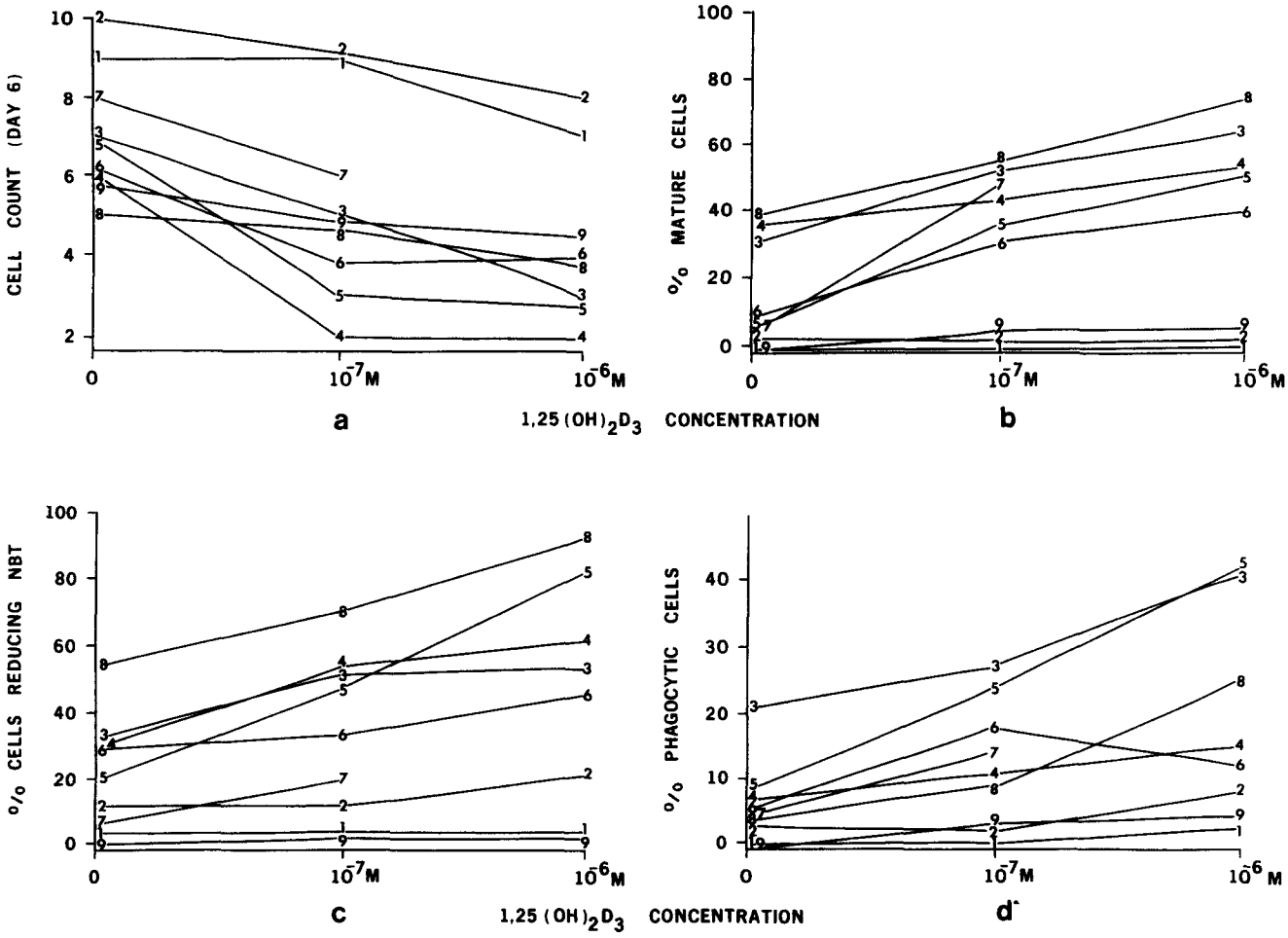

Fig. 3a–d. In vitro study of $1,25(OH)_2D_3$ on myeloid blast cells from leukemic patients **a** effect of $1,25(OH)_2D_3$ on proliferation of leukemic blast cells, **b** effect of $1,25(OH)_2D_3$ on morphological maturation of leukemic blast cells; **c** effect of $1,25(OH)_2D_3$ on NBT reduction by leukemic blast cells; **d** effect of $1,25(OH)_2D_3$ on *Candida* phagocytosis by leukemic blast cells. Cells were cultured in media containing 0, 10^{-7}, or 10^{-6} *M* $1,25(OH)_2D_3$, harvested on day 6, and viable cell counts, morphology, and function of the cells were determined

10^{-6} *M* $1,25(OH)_2D_3$ as compared with control flasks.

We examined the ability of the blast cells from the leukemic patients to phagocytose *Candida* after culture with $1,25(OH)_2D_3$ for 6 days (Fig. 3). Median percentage of blasts cells which phagocytosed *Candida* in control, 10^{-7} *M*, and 10^{-6} *M* $1,25(OH)_2D_3$-containing flasks were 5%, 11%, and 14%, respectively. The percentage of phagocytic cells was significantly ($P < 0.05$) increased in the flasks containing 10^{-6} *M* $1,25(OH)_2D_3$ as compared with control flasks. This study suggests that high concentrations of $1,25(OH)_2D_3$ can induce terminal differentiation of leukemic cells in liquid culture.

E. $1,25(OH)_2$ D_3: In Vivo Myelodysplastic Study

A group of 18 myelodysplastic patients were treated with $1,25(OH)_2D_3$ (Table 3). All patients received weekly escalating (0.5 μg) doses of $1,25(OH)_2D_3$ until a daily dose of 2 μg was reached. The median duration of therapy with $1,25(OH)_2D_3$ was 12 weeks (range 4 to > 20 weeks). Tables 3, 4 and Fig. 4 show the results of treatment. During the study, the peak granulocyte, macrophage, and platelet blood concentrations increased in most patients as compared with their starting values. As a group, the peak granulocyte, macrophage, and platelet values during the study increased significantly as compared with initial and final peripheral blood cell levels (Table 3). In contrast, by the end of the trial, the pe-

Table 3. Preleukemic patients: clinical characteristics and 1,25$(OH)_2$ vitamin D_3 therapeutic response

Patient	Age/sex	Time from diagnosis to treatment (months)	Duration of treatment (weeks)	Response (weeks)	Diagnosis before treatment[a]	End of treatment
1	70/F	4	13	Prog	RAEB-TR	AML (M2)
2	34/F	3	14	PR; Prog	RAEB	AML (M4)
3	51/M	30	12	Prog	RAEB	AML (M2)
4	60/M	11	12	MR; Prog	RAEB-TR	AML (M2)
5	59/M	20	12	Prog	RAEB-TR	AML (M2)
6	65/F	27	12	MR	PASA	PASA
7	36/F	10	12	MR	RAEB	RAEB
8	59/M	23	8	MR; Prog	PASA	PASA
9	66/F	6	15	MR	RAEB	RAEB
10	52/M	7	12	S	PASA	PASA
11	71/M	35	8	Prog	PASA	RAEB
12	78/F	3	12	S	RAEB	RAEB
13	74/M	18	12	S	CMML	CMML
14	79/M	5	6	MR	RAEB	RAEB
15	71/M	7	10	Prog	RAEB	AML (M1)
16	65/M	5	4	Prog	RAEB	AML (M1)
17	80/M	6	7	Prog	RAEB	Death
18	61/M	12	>20	MR	PASA	PASA

[a] RAEB refractory anemia with excess blasts; RAEB-TR RAEB in transformation; PASA primary acquired sideroblastic anemia; CMML chronic myelomonocytic leukemia; AML acute myelogenous leukemia with M subclassification according to FAB classification. Prog progression; PR partial response; MR minor response; S stable

Table 4. Treatment of myelodysplastic patients with 1,25$(OH)_2D_3$[a]

	Median	Range	P[c]
Granulocytes (per microliter blood)			
Start[b]	2 300	180–11 520	
Peak	3 750	486–29 820	0.0007
End	3 110	96–24 480	0.0347
Macrophages (per microliter blood)			
Start	50	3– 8 435	
Peak	300	43–16 898	0.0022
End	80	3–10 200	0.1578
Platelets ($\times 10^3$ per microliter blood)			
Start	58	12–300	
Peak	105	23–610	0.0003
End	65	4–358	0.9622
Marrow blasts (%)			
Start	10	3–20	
End	15	4–70	0.0068
Serum calcium (mg/dl)			
Start	8.9	8.2–10.4	
Peak	10.8	9.7–17.0	0.0002
End	10.3	9.6–12.4	0.0002

[a] Results represent data from 18 preleukemic patients

[b] Start, initial value before therapy; peak, highest value during therapy; end, value on last day of therapy

[c] Wilcoxon signed rank test. Values are compared with baseline (starting) values and $p \leqq 0.05$ is significant

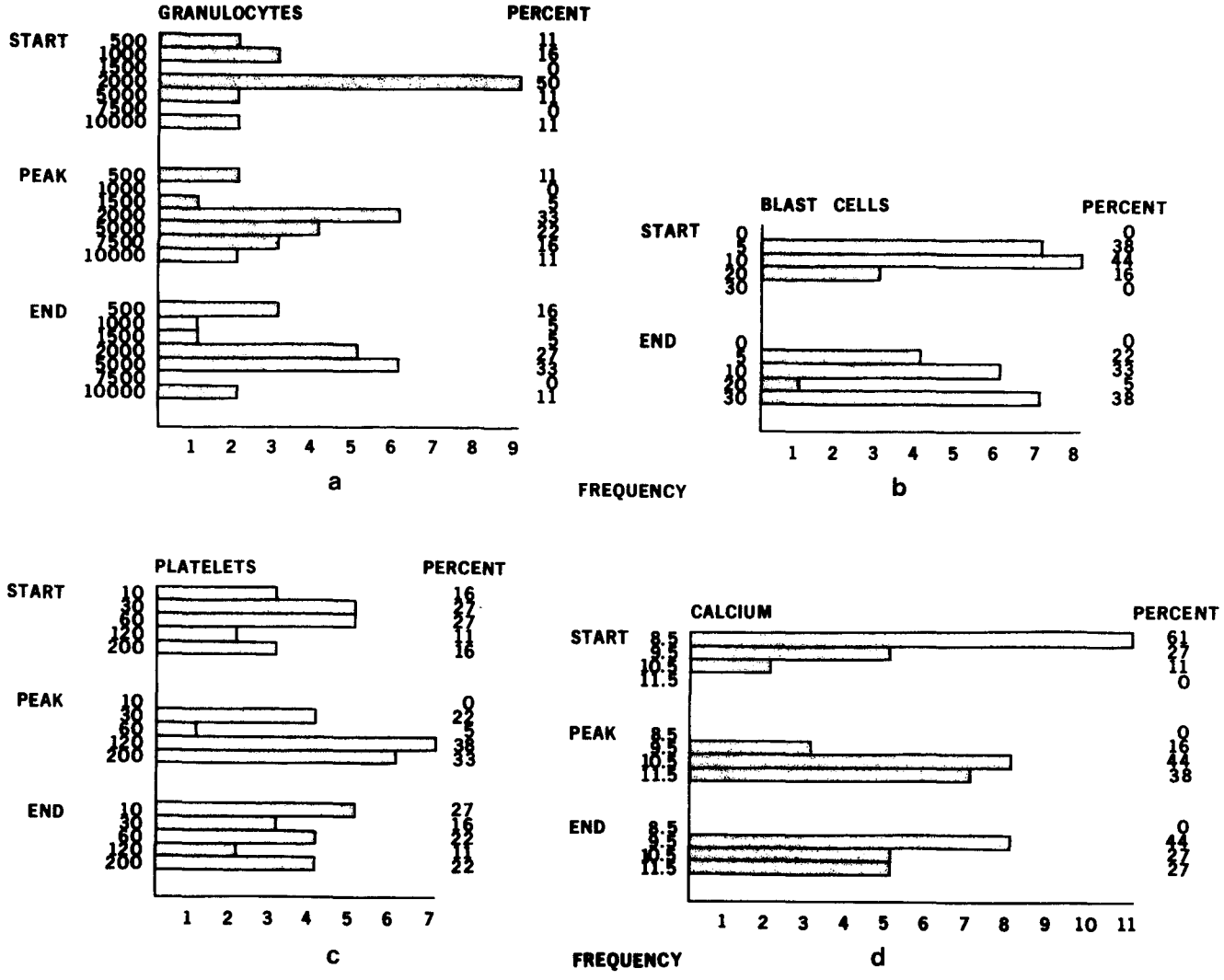

Fig. 4a–d. In vivo study of 1,25$(OH)_2D_3$ in preleukemic patients **a** effect of 1,25$(OH)_2D_3$ on peripheral blood granulocyte counts; **b** effect of 1,25$(OH)_2D_3$ on peripheral blood platelet counts; **c** effect of 1,25$(OH)_2D_3$ on percentage of bone marrow myeloblasts; **d** effect of 1,25$(OH)_2D_3$ on serum calcium concentration (mg/dl). Start, baseline value; peak, highest value during study; end, value on last day of study

ripheral blood cell values were not significantly different than at the initiation of the trial. Likewise, the percentage of marrow blasts rose from a median value of 10% (range 3%–20%) at the initiation of the study to a median value of 15% (4%–70%) by the completion of the study.

Patient 2 had a 50% improvement in absolute blood granulocyte, monocyte, and platelet counts for greater than 5 weeks of treatment (partial response, Table 3). This patient, however, developed AML on week 11 of receiving 1,25$(OH)_2D_3$. Seven patients (4, 6, 7–9, 14, 18) had an improvement of either their absolute granulocyte, monocyte, or platelet peripheral blood counts for greater than 4 weeks of treatment (minor response). However, two of these patients (4, 8), developed AML while receiving 1,25$(OH)_2D_3$. Three patients (10, 12, 13) had no change in granulocyte, monocyte, or platelet peripheral blood counts. By the conclusion of the therapeutic trial, seven patients progressed to AML with greater than 10% blast cells in the peripheral blood and greater than 30% blast cells in the bone marrow (Table 3). The serum calcium increased in each of the patients while receiving 1,25$(OH)_2D_3$. The serum calcium was a median 8.9 mg/dl (range 8.2–10.4) prior to therapy; a peak median 10.8 mg/dl (range 9.7–17) during therapy; and a median 10.3 mg/dl (range 9.6–12.4) at the end of study (Table 4, Fig. 4). Nine patients had serum calcium levels above 11 mg/dl, and six of these patients had symptoms of hepercalcemia.

We attempted to improve the peripheral blood cytopenia and bone marrow ineffective hematopoiesis in patients with myelodysplastic syndromes by having the patients ingest pharmacologic doses of $1,25(OH)_2D_3$. The in vivo administration of $1,25(OH)_2D_3$ produced only a very temporary improvement in hematopoietic parameters in a minority of the patients. Patients received 2 μg/day $1,25(OH)_2D_3$ which would be expected to produce serum levels of about 2×10^{-10} *M* $1,25(OH)_2D_3$. This dosage of drug induced hypercalcemia in 6 of 18 patients. However, this concentration of $1,25(OH)_2D_3$ induced only about 20% of HL-60 cells to mature in vitro and 10^{-9} *M* $1,25(OH)_2D_3$ had only a small effect on maturation in vitro of fresh leukemic blast cells. Severalfold higher concentrations of $1,25(OH)_2D_3$ can not be given to patients because the drug will precipitate life-threatening hypercalcemia. The future development of vitamin D analogs that induce heamtopoietic cell differentiation without hypercalcemia might be medically useful for selected preleukemic and leukemic patients. The induction of differentiation of myeloid leukemic cells to functional end cells offers an appealing therapeutic prospect. However, the future role of biologic modifiers in the treatment of hematopoietic malignancies remains undefined at this time.

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