

Relationship between HLA-DR Expression by Normal Myeloid Progenitor Cells and Inhibition of Colony Growth by Prostaglandin E

Implications for Prostaglandin E Resistance in Chronic Myeloid Leukemia

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Abstract

The expression of HLA-DR antigens by normal myeloid progenitor cells (CFU-GM) has been linked to inhibition of colony growth by prostaglandin E (PGE), while resistance to the inhibitory effects of PGE in chronic myeloid leukemia (CML) has been attributed to a lower fraction of HLA-DR+ CFU-GM in this disease. However, we have previously shown that virtually all CFU-GM in normal bone marrow (NBM) as well as CML peripheral blood express HLA-DR antigens, which raises the possibility that these surface molecules may not be the sole determinants of a progenitor cell's sensitivity to PGE. In order to evaluate the relationship between HLA-DR expression and prostaglandin inhibition, we partially purified NBM progenitor cells using fluorescence-activated cell sorting to prepare cell fractions with high and low HLA-DR antigen density. Normal progenitor cells with high DR density tended to form monocyte colonies in agar culture, whereas the low DR density fraction was enriched for granulocyte colony-forming cells. Inhibition by PGE was greatest in the high DR+ fraction and was largely restricted to monocyte progenitor cells. Inhibition of CFU-GM by PGE was less in CML than in NBM, but this decreased inhibition correlated with a significantly lower number of monocyte-CFU in CML. These data suggest that high HLA-DR antigen density may select for normal progenitor cells that are committed to monocyte differentiation and are, therefore, more likely to be inhibited by PGE. The relative deficit of monocyte progenitor cells in CML may partially explain the phenomenon of PGE resistance in this disease.

Introduction

HLA-DR antigens are expressed on a variety of hematopoietic cells including B cells, activated T cells, monocytes, and progenitor cells of the erythroid and myeloid lineages (1-6). These Ia-like antigens regulate cellular interactions of the immune response, but their role in the regulation of hematopoiesis is less clear (7-10). Recent reports have suggested that the inhibitory effects of acidic isoferritins or prostaglandin E (PGE)¹ on the

normal granulocyte/monocyte colony-forming cell (CFU-GM) appear to be restricted to those cells that express HLA-DR (11, 12). This is of particular interest because it has been reported that the fraction of DR+ CFU-GM in chronic myeloid leukemia (CML) is lower than in normal bone marrow (NBM), and this has been linked to the resistance of CML progenitor cells to PGE (12, 13).

Different laboratories have varied widely in their determinations of the fraction of normal and CML CFU-GM that express HLA-DR, however, and the association of DR expression with sensitivity to PGE has not been universally accepted (14). It is possible that the extent to which CFU-GM are detected as DR+ may depend on the anti-DR antibody used and its capacity to fix complement, and thus CFU-GM with low DR antigen density would be negative in some studies and weakly positive in others. Unfortunately, complement lysis of CFU-GM is not an ideal technique to investigate DR antigen density, and little is known about biological or functional differences that exist between CFU-GM with low density or high density DR expression.

Previous studies in our laboratory and others with flow cytometry have shown that virtually all CFU-GM of both NBM and CML are DR+, and that the range of Ia antigen density is extremely broad (15, 16). In this report we describe the use of cell-sorting techniques to prepare populations of CFU-GM with different levels of DR antigen expression. These populations have been characterized functionally and used to examine the relationship of DR antigen density to PGE sensitivity. The results indicate that subsets of day 7 CFU-GM express different levels of HLA-DR depending upon their commitment to monocyte or granulocyte differentiation. High density DR+ CFU-GM were enriched for monocyte progenitor cells, and these cells were more sensitive to PGE inhibition compared with granulocyte progenitor cells.

A parallel investigation of progenitor cell subsets and PGE sensitivity in NBM and CML was also done. Sensitivity to PGE inhibition was related to the proportion of monocyte progenitor cells present in either NBM or CML. The observation that overall inhibition by PGE tended to be less in CML may be partly a result of a relative deficiency of monocyte precursor cells in this disease.

Methods

Source of CFU-GMs. Peripheral blood samples (5-10 ml) were obtained from stable-phase CML patients undergoing initial diagnostic testing.

forming cell; CML, chronic myeloid leukemia; FACS, fluorescence-activated cell sorting; FCS, fetal calf serum; G/M, granulocyte/monocyte; GCT, giant cell tumor conditioned medium; IMDMEM, Iscove's modified Dulbecco's minimal essential media; MEM, minimal essential media; NBM, normal bone marrow; PGE₁, prostaglandin E₁.

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1. *Abbreviations used in this paper:* ANAE, alpha naphthyl acetate esterase; CAE, chloroacetate esterase; CFU-GM, granulocyte/monocyte colony-

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After collection into sterile-heparinized syringes, the mononuclear cell fraction was separated by Ficoll-Hypaque diatrizoate gradient sedimentation, washed twice in minimal essential media (MEM; Gibco, Grand Island, NY) containing 2.5% pooled human AB serum (MEM-AB wash), and cryopreserved in the vapor phase of liquid nitrogen in 10% dimethylsulfoxide and 20% heat-inactivated fetal calf serum (FCS) until use. Samples were thawed in the presence of 100 μ g/ml deoxyribonuclease I (Worthington Biochemical Corp., Freehold, NJ) to minimize cell agglutination. Two CML samples were studied before cryopreservation with equivalent results.

NBM was aspirated from healthy volunteers into heparinized syringes after written consent was approved by the Institutional Review Board. The mononuclear cell fraction was separated by Ficoll-Hypaque diatrizoate gradient sedimentation as above.

Monoclonal antibodies. Monoclonal antibodies used in the purification of CML colony-forming cells have been previously characterized and include anti-MY8 (pan-myeloid) (17), anti-Mo1 (pan-myeloid) (18), anti-Mo2 (monocyte) (18), anti-B1 (B cell) (19), anti-T3 and anti-T11 (T cell) (20, 21), HNK1 (natural killer cell) (22), and 31C6 (basophil and natural killer cells) (Hercend, T., and J. Griffin, unpublished observation). Monoclonal antibodies used in the purification of NBM were similar, but also included an anti-glycophorin A monoclonal antibody (clone 39, Sabbath, K., and J. Griffin, unpublished observation).

Partial purification of myeloid progenitor cells. The method of myeloblast purification from the peripheral blood of CML patients has been previously reported and has also been applied to the partial purification of normal bone marrow CFU-GM (23). Briefly, $1-2 \times 10^8$ mononuclear cells from either NBM or CML peripheral blood were incubated with the previously noted monoclonal antibodies for 30 min at 4°C. After two wash steps, affinity-purified rabbit anti-mouse immunoglobulin coupled to sheep erythrocytes by chromic chloride was added (0.7 ml of 10% vol/vol erythrocytes in MEM-AB wash per 10^8 cells). The erythrocyte-monomonuclear cell mixture was pelleted (800 g, 10 min) and incubated at 4°C for 20 min for rosette formation. The rosetted cells were then separated from the nonrosetted cells (containing the progenitor cells) by density gradient sedimentation. Interface cells (unrosetted) were washed twice in MEM-AB. A cytocentrifuge preparation was examined to determine purity.

For CML peripheral blood, this technique typically resulted in a preparation of 85–95% blast forms with a colony-forming efficiency (CFU-GM) of 30–40%. For NBM, partial purification usually resulted in a preparation of ~30–50% blasts, 50% promyelocytes, 0–10% myelocytes, and 0–10% early erythroid precursors, with a CFU-GM colony-forming efficiency of ~10–20%. For some experiments, monocytes were prepared from fresh peripheral blood by adherence as previously described (15).

Immunofluorescence analysis of HLA-DR. In order to determine the relationship of HLA-DR expression to CFU-GM commitment, partially purified progenitor cells from NBM were stained for DR antigen expression by an indirect immunofluorescence assay and subsequently sorted on the basis of HLA-DR antigen density. Briefly, $\sim 1 \times 10^7$ partially purified cells were washed twice in MEM-AB wash, and the pellet was then suspended in 1.0 ml of diluted ascites. Monoclonal antibody 9-49, which identifies a monomorphic DR determinant (24), was used as a 1:250 dilution of ascites for these experiments (kindly provided by Dr. Robert Todd, University of Michigan, Ann Arbor, MI). After incubation for 30 min at 4°C, the cells were washed twice and stained with fluorescein-conjugated goat anti-mouse Ig (Tago Inc., Burlingame, CA) for 30 min at 4°C. After two additional wash steps, the cells were analyzed by flow microfluorimetry (FACS-I, Becton-Dickinson & Co., Oxnard, CA) and separated into a cell fraction with high DR antigen density (40% of DR+ cells with the brightest fluorescence) and a population with low DR density (40% of DR+ cells with the least bright fluorescence). Background fluorescence was measured using an isotype-identical control monoclonal antibody.

CFU-GM assay. Partially purified progenitor cells were plated at $1-2.5 \times 10^3$ cells/culture in Iscove's modified Dulbecco's MEM (IMDMEM), 20% FCS, 0.3% agar over an underlayer of 0.5% agar with

20% GCT (Gibco) (15). Indomethacin (Sigma Chemical Co., St. Louis, MO) at a final concentration of 10^{-6} M, was used in all CFU-GM assays to prevent the endogenous secretion of prostaglandin by small numbers of contaminating accessory cells. Indomethacin by itself did not affect number or type of CFU-GM. Prostaglandin inhibition was evaluated by using PGE₁ (Sigma Chemical Co.) in the range of 10^{-6} – 10^{-10} M in agar. After 7 and 14 d of culture (37°C, 5% CO₂), the overlayers were removed and dried on glass microscope slides. They were subsequently fixed and stained for both naphthol AS-D chloroacetate esterase (CAE, granulocyte lineage) and alpha naphthyl acetate esterase (ANAE, monocyte lineage) as previously described (25). Colonies (>40 cells) and clusters (8–40 cells) were identified as granulocytic if all cells were CAE+, pure monocytic if all cells were ANAE+, and mixed if cells positive for both stains were present within a single colony or cluster. Using these criteria, <5% of colonies and clusters from either CML or NBM could not be classified.

Liquid suspension culture. In order to exclude the possibility that the growth, differentiation, and staining characteristics of NBM and CML CFU-GM were altered by in vitro agar culture, partially purified NBM and CML peripheral blood were also grown in liquid suspension culture over a 7–14-d period. For each treatment group, 1×10^6 partially purified progenitor cells were suspended in 5 ml of IMDMEM, 20% FCS, 10% GCT (Gibco), 10^{-6} M indomethacin, with or without 10^{-6} M PGE₁ in a 60 \times 15-mm petri dish (Falcon Laboratories, Oxnard, CA) on day 0. On days 7 and 14 of culture (37°C, 5% CO₂), the cell population was counted, and cytocentrifuge smears were stained for combined esterase activity and with Wright-Giemsa stain.

Statistical analysis. Significance levels for comparison between treatment groups were determined using two-sided *t* tests. Paired tests were used when appropriate. Significance levels for correlations were derived from the *t* test conversion on the correlation coefficient *r*. The association between monocyte colony number and overall PGE inhibition was estimated by standard multiple regression techniques (26).

Results

Relationship of HLA-DR expression to CFU-GM commitment.

In order to investigate the relationship between PGE inhibition and HLA-DR antigen expression, partially purified progenitor cells from NBM were separated into two fractions on the basis of HLA-DR antigen density by fluorescence-activated cell sorting (FACS) and assayed for CFU-GM in agar, with or without 10^{-6} M PGE₁. In pilot experiments, it was shown that >95% of CFU-GM are recovered in the DR+ fraction. Because of the small numbers of CFU-GM present in the DR– fraction, further analysis of that fraction was not undertaken. Colonies and clusters were counted on day 7 using combined esterase staining to detect monocyte colonies (ANAE+, CAE–) granulocyte colonies (ANAE–, CAE+), and mixed G/M (granulocyte/monocyte) colonies (ANAE+, CAE+). A typical FACS-I histogram of HLA-DR (9-49) expression in this cell population is shown in Fig. 1, and data from five experiments with different bone marrow donors are presented in Table I. In each case, both the absolute number and the relative percentage of pure monocyte colonies was greater in the high HLA-DR antigen group of CFU-GM. The mean percentage of monocyte colonies was significantly greater for the high density HLA-DR group (36 ± 13 vs. $3 \pm 3\%$, $P < 0.005$), as was the mean percentage of mixed G/M plus monocyte colonies (47 ± 19 vs. $11 \pm 10\%$, $P < 0.005$).

Relationship of HLA-DR expression to PGE inhibition. In four of the five sorting experiments, the inhibitory effects of 10^{-6} M PGE₁ on day 7 CFU-GM were assessed (Table II). In each case, the overall inhibition by PGE₁ was greater in the high DR antigen density group, with the mean PGE₁ inhibition being $37 \pm 8\%$ for the high density and $4 \pm 13\%$ for the low density DR+

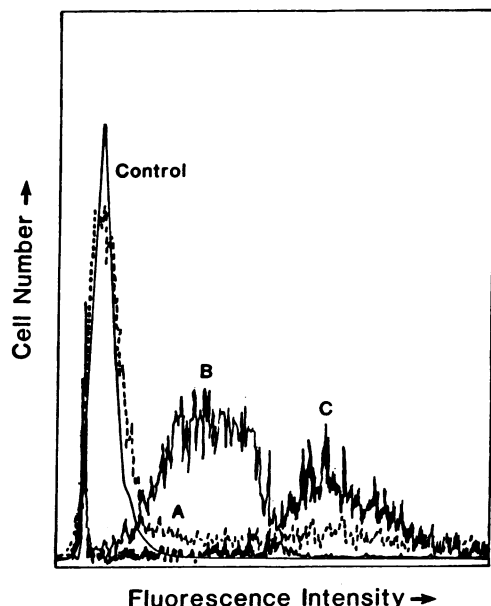


Figure 1. Separation of partially purified NBM progenitor cells into high and low density DR+ fractions by FACS. A, presort fluorescence histogram of HLA-DR as defined by 9-49 monoclonal antibody (34% of cells are positive); B, postsort reanalysis of low density DR+ fraction (99% DR+); C, postsort reanalysis of high density DR+ fraction (99% DR+).

progenitor cells ($p = 0.025$). The inhibitory effects of PGE₁ on individual CFU-GM subsets, however, appeared to be similar for both DR density groups, with the greatest degree of inhibition being observed for monocyte colonies, a moderate degree of inhibition for mixed G/M colonies, and the least amount of inhibition (and sometimes slight enhancement) for granulocyte colonies.

Despite the similar response of CFU-GM subsets in either DR+ fraction to 10^{-6} M PGE₁, we considered the possibility that the dose response of colony inhibition at lower PGE₁ concentrations may differ between the low and high DR+ groups. In order to evaluate this possibility, sorted cells were plated in the presence of PGE₁ in a dose range of 10^{-6} – 10^{-10} M, and the results of one such experiment are shown in Table III. As in previous experiments, high density HLA-DR+ CFU-GM were inhibited to a substantially greater degree than were low density HLA-DR+ CFU-GM. This inhibition was restricted to monocyte and mixed CFU-GM. However, when CFU-GM subsets were analyzed individually, the degree of colony inhibition was similar for the low and high DR+ groups over a broad range of PGE₁ concentrations. It should be noted that accurate quantitation of monocyte progenitor cell inhibition by PGE in the low density DR+ fraction was limited by the extremely low number of monocyte colonies in this group (Table III).

Comparison of CFU-GM subsets in NBM and CML. The experiments described above suggested that inhibition of normal CFU-GM by PGE was partly determined by the proportion of CFU-GM committed to monocyte differentiation, and that the association between PGE sensitivity and DR expression could be due to the fact that monocyte-CFU expressed higher levels of DR antigen than granulocyte-CFU. In order to test this observation further, we evaluated the composition of CFU-GM subsets and their sensitivity to PGE in CML, a disease which is characterized by resistance to PGE inhibition despite the presence of HLA-DR on the majority of CFU-GM (15).

Partially purified progenitor cells from NBM and CML peripheral blood were evaluated for colony and cluster formation on days 7 and 14, with the cumulative results of 18 experiments shown in Table IV. The percentage of ANAE positive colonies from NBM exceeded that of CML by a factor of 3.1 on day 7 (28 ± 7 vs. $9 \pm 6\%$, $P = 0.0006$) and by a factor of 2.3 on day 14 (38 ± 8 vs. $16 \pm 12\%$, $P = 0.2$). Monocytes freshly prepared from

Table I. Relationship of HLA-DR Expression to CFU-GM Commitment in NBM*

Experiment number	HLA-DR antigen density							
	Low				High			
	Gran	Mixed G/M	Mono	Total	Gran	Mixed G/M	Mono	Total
1	182±5‡ (76)§	46±3 (19)	13±2 (5)	241±7	42±3 (31)	18±4 (13)	77±3 (56)	137±5
2	133±10 (91)	6±2 (4)	7±2 (5)	146±11	82±2 (73)	5±2 (4)	26±3 (23)	113±5
3	342±11 (97)	12±2 (3)	0±0 (0)	354±12	318±15 (63)	68±4 (13)	120±10 (24)	506±16
4	122±11 (81)	19±1 (13)	9±1 (6)	150±12	27±3 (45)	10±1 (17)	23±1 (38)	60±4
5	420±17 (98)	2±1 (0.5)	6±1 (1.5)	428±17	159±14 (52)	29±5 (9)	119±4 (39)	307±23
Total (%)	89±10	8±8	3±3		53±16	11±5	36±13	

* Partially purified myeloid progenitor cells from NBM were divided into equal fractions of low and high density HLA-DR (9-49) positive groups by FACS (also see Fig. 1). Cells were subsequently plated in agar containing 10^{-6} M indomethacin and 10% GCT as a source of colony-stimulating activity. Gran, granulocyte; mono, monocyte. ‡ Colony and cluster number and morphology per 10^3 cells was determined on day 7 by combined esterase staining. Results are expressed as mean±SE of duplicate or triplicate cultures. § Numbers in parentheses indicates percentage.

Table II. Relationship of HLA-DR Expression to Inhibition of CFU-GM Subsets by PGE₁ in Normal Bone Marrow*

Experiment number	Percent inhibition of CFU-GM by PGE ₁ (%)‡							
	Low density HLA-DR				High density HLA-DR			
	Gran	Mixed	Mono	Total	Gran	Mixed	Mono	Total
1	4	35	85	14	-48	39	90	41
2	10	17	57	12	13	20	92	32
3	-18	42	NE§	-15	2	34	96	29
4	3	100	100	5	6	83	89	46
Total	0±12	49±36	81±22	4±13	-7±28	44±27	92±3	37±8

* See Table I. Gran, granulocyte; mono, monocyte. ‡ PGE₁ (10⁻⁶ M) inhibition at day 7, expressed as percentage reduction of control colony and cluster number. (Negative sign indicates enhanced growth with PGE₁.) § NE, not evaluable (exp. 3 had no monocyte colonies or clusters in the low HLA-DR density group [Table I]).

CML blood were found to stain adequately for ANAE activity, excluding the possibility that this stain would not detect monocyte colonies in CML.

Effect of PGE₁ on myeloid progenitor cell subsets in NBM and CML. The inhibitory effects of 10⁻⁶ M PGE₁ on granulocyte, mixed G/M, and monocyte colonies and clusters were evaluated at days 7 and 14 for both NBM and CML, with the cumulative results of 16 experiments shown in Table V and Fig. 2. As expected, 10⁻⁶ M PGE₁ markedly inhibited pure monocyte colony and cluster formation in NBM, but it also effectively inhibited pure monocyte colony-forming cells (CFU) in CML. For example, PGE₁ inhibition of NBM monocyte colonies and clusters at day 7 was 95±10% (*n* = 8), compared with 89±21% (*n* = 8) for CML (Table V). The inhibition of mixed colony and cluster formation by PGE₁ was substantial for both NBM and CML, though generally less than the inhibition observed for pure monocyte colonies. Granulocyte colonies and clusters were generally resistant to the inhibitory effects of PGE₁ in both NBM and CML. Overall colony and cluster inhibition by PGE₁, however, was less in CML compared with NBM on both days 7

(13±12 vs. 32±8%, *P* = 0.01) and day 14 (31±10 vs. 43±13%, *P* = 0.2).

Although the degree of inhibition of CFU-GM subsets was similar for NBM and CML at 10⁻⁶ M PGE₁, it was possible that differences in the pattern of colony inhibition may become apparent at lower PGE₁ concentrations. To evaluate this possibility, the inhibiting effects of 10⁻⁶, 10⁻⁸, and 10⁻¹⁰ M PGE₁ were tested, with the data from two representative experiments shown in Table VI. For NBM, the inhibition of ANAE+ colonies (mixed GM plus monocyte) by 10⁻⁶, 10⁻⁸, and 10⁻¹⁰ M PGE₁ was 82±4, 63±6, and 28±11%, respectively (*n* = 2). Corresponding data for CML revealed inhibition of 96±6, 81±17, and 50±17%, respectively (*n* = 3). However, the low number of monocyte colonies in these CML cases does not allow us to conclude with certainty that CML monocyte progenitor cells are as sensitive to PGE as are NBM progenitor cells. The inhibition of granulocyte colonies was negligible for both groups over this PGE₁ dose range.

These data suggest that overall colony inhibition by PGE may be partly determined by the initial percentage of ANAE+

Table III. Dose Response of PGE₁ Inhibition as a Function of HLA-DR Antigen Density

HLA-DR antigen density*	PGE ₁ concentration	Colonies and clusters (day 7) per 10 ³ cells			
		Granulocyte	Mixed G/M	Monocyte	Total
Low	Control	420±17‡	2±1	6±1	428±17
	10 ⁻⁶ M	406±25 (3%)§	0±0 (100)	0±0 (100)	406±25 (5)
	10 ⁻⁷	414±13 (1)	1±1 (50)	0±0 (100)	414±14 (3)
	10 ⁻⁸	425±32 (-1)	1±1 (50)	2±1 (67)	427±32 (0)
	10 ⁻⁹	422±13 (-1)	1±1 (50)	3±1 (50)	426±11 (1)
	10 ⁻¹⁰	443±14 (-5)	2±1 (0)	4±1 (33)	449±14 (-5)
High	Control	159±14	29±5	119±4	307±23
	10 ⁻⁶ M	150±2 (6)	5±1 (83)	13±6 (89)	167±7 (46)
	10 ⁻⁷	152±6 (4)	6±2 (79)	15±2 (87)	174±8 (43)
	10 ⁻⁸	162±4 (-2)	13±1 (55)	34±3 (71)	209±3 (32)
	10 ⁻⁹	162±3 (-2)	21±2 (28)	64±6 (46)	247±7 (20)
	10 ⁻¹⁰	164±8 (-3)	24±2 (17)	88±6 (26)	276±15 (10)

* NBM progenitor cells were separated on the basis of HLA-DR antigen density and plated in varying concentrations of PGE₁ (10⁻⁶ M–10⁻¹⁰ M) as indicated. ‡ See Table I. § Numbers in parentheses indicate percentage PGE₁ colony and cluster inhibition (negative sign indicates enhanced growth with PGE₁).

Table IV. Colony and Cluster Composition of NBM and CML Peripheral Blood*

Day	CFU-GM source	Colony and cluster composition (%)‡			
		Granulocyte	Mixed G/M	Monocyte	Total ANAE positive
7	NBM (n = 9)	73±7 (61–82)§	13±14 (4–22)	14±6 (3–30)	28±7 (18–39)
	CML (n = 9)	92±6 (79–97)	5±7 (1–20)	3±3 (1–9)	9±6 (3–21)
14	NBM (n = 3)	64±8 (55–71)	19±7 (11–24)	17±9 (8–24)	36±8 (29–45)
	CML (n = 3)	84±12 (71–94)	14±11 (5–26)	2±1 (1–3)	16±12 (6–29)

* Myeloid progenitor cells were partially purified from either NBM or CML peripheral blood and plated in agar culture containing 10^{-6} M indomethacin and 10% GCT as a source of colony-stimulating activity. ‡ Colony and cluster morphology was identified by combined esterase staining as described in text. § Results are expressed as mean percentage±SE of duplicate cultures. Numbers in parentheses indicate range.

colonies in either NBM or CML. As shown in Fig. 2, a plot of these two variables (percentage PGE inhibition vs. percentage ANAE+ colonies) suggests a positive correlation, although this is exaggerated by the fact that the percentage of ANAE+ colonies is generally higher for NBM than for CML (Table IV). To correct for this, a regression of PGE inhibition on percentage of ANAE+ colonies and an indicator variable for CML was fit (26). This analysis revealed that PGE inhibition is significantly associated with the initial percentage of ANAE+ colonies ($0.001 < P < 0.005$), regardless of CFU-GM source (NBM or CML).

Differentiation of myeloid progenitor cells in suspension culture. In an attempt to further evaluate the production of monocytes by CML progenitor cells, as well as to determine correlation between Wright Giemsa and combined esterase-staining techniques, partially purified preparations of NBM and CML peripheral blood were grown in liquid suspension culture as described, with or without 10^{-6} M PGE₁. Table VII shows the cell count, cell composition by Wright-Giemsa, and percentage of ANAE+ cells on days 0, 7, and 14 of suspension culture. Although the day 0 population of progenitor cells for NBM and CML differ with respect to enrichment of blast cells (due to more contamination by promyelocytes in the NBM preparation), there were <1% monocytes in either population by Wright-Giemsa staining or ANAE positivity. At day 7, immature monocytes were detected in both NBM and CML preparations (8±4 vs. 12±3%, respectively), although ANAE staining was often weak in these cells. By day 14, however, the majority of cells in the NBM suspension culture were monocytes or macrophages by Wright-Giemsa stain (76±10%), as opposed to a significantly smaller percentage of these cells in the CML suspension culture (19±6%) ($P < 0.025$). Correlation between Wright-Giemsa staining and ANAE positivity in the day 14 control populations was excellent for both NBM and CML (Table VII). As expected,

PGE₁ resulted in a reduction in the percentage of macrophages for both NBM and CML suspension cultures at days 7 and 14.

Discussion

The detection of HLA-DR surface molecules on hematopoietic progenitor cells has created considerable interest, particularly with the finding of an association between DR antigen expression by CFU-GM and the in vitro growth regulation of these cells by inhibitory factors such as acidic isoferitins or PGE (11, 12). Further, the insensitivity of CML CFU-GM to the inhibitory effects of PGE₁ has been related to a lower amount of DR antigen as detected by complement lysis techniques (12, 13). By using more sensitive assay techniques, however, we (15, 17, 23) and others (16) have found that virtually all CFU-GM from both NBM and CML peripheral blood express detectable levels of DR antigen, making it unlikely that DR antigen expression is the sole determinant of CFU-GM sensitivity to PGE.

In this report, we have investigated the possibility that high HLA-DR antigen density on myeloid progenitor cells, rather than the expression of HLA-DR per se, identifies a subset of CFU-GM that is sensitive to the inhibitory effects of PGE₁. Partially purified myeloid progenitor cells from NBM were separated by cell sorting into two fractions on the basis of HLA-DR antigen density, and CFU-GM subsets were determined by ANAE and CAE staining of agar cultures. Partial purification of NBM progenitor cells provided an enriched population of DR+ cells (~30% HLA-DR+), and thus facilitated sorting of CFU-GM on the basis of DR antigen density. Furthermore, partial purification of CFU-GM also minimized the possible effects of accessory cells (monocytes, T lymphocytes) in the inhibition of colony growth by PGE₁.

Analysis of day 7 colonies and clusters revealed a significant

Table V. PGE₁ Inhibition of CFU-GM Subsets from NBM and CML—Cumulative Results

Day	CFU-GM source	Percent inhibition of CFU-GM by 10^{-6} M PGE ₁			
		Granulocyte	Mixed G/M	Monocyte	Total
7	NBM (n = 8)	16±10 (3–27)*	62±18 (40–88)	95±10 (95–100)	32±8 (23–45)
	CML (n = 8)	12±15 (0–35)	54±24 (25–89)	89±21 (54–100)	13±12 (2–37)
14	NBM (n = 3)	24±16 (9–40)	59±51 (0–96)	88±17 (69–100)	43±13 (31–56)
	CML (n = 3)	19±9 (12–29)	74±22 (50–93)	100±0 (—)	31±10 (19–37)

* See Table IV. Results expressed as mean percent inhibition±SE. Numbers in parentheses indicate range.

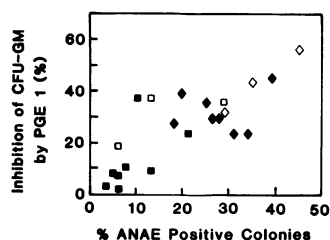


Figure 2. PGE₁-mediated inhibition of CFU-GM as a function of initial percentage of mixed and monocyte colonies as defined by ANAE staining. Closed diamonds: NBM, day 7; open diamonds: NBM, day 14; closed squares: CML, day 7; open squares: CML, day 14.

enrichment of pure monocyte progenitor cells in the high density DR+ fraction, and an enrichment of granulocyte progenitor cells in the low density DR+ fraction. Inhibition of colony growth by PGE tended to be greater in the high DR+ fraction of CFU-GM, and this correlated with the higher percentage of monocyte colonies observed in this progenitor cell population. When each CFU-GM subset was examined individually for PGE₁ sensitivity, inhibition was found to be largely restricted to the monocyte progenitor cells, and this appeared to be true whether these cells were in the high DR+ or low DR+ fractions. These findings suggest that the association between HLA-DR antigen expression by normal CFU-GMs and inhibition by PGE is related to the fact that high levels of HLA-DR select for progenitor cells that are committed to monocyte differentiation. Although inhibition of monocyte progenitor cells by PGE has been observed by previous investigators (27–29), an association between high HLA-DR density and commitment to monocyte differentiation has not been previously described.

It should be noted that these data do not exclude the possibility that HLA-DR antigen density may be functionally important in the mediation of prostaglandin colony inhibition, independent of monocyte progenitor cell enrichment. However, the fact that no major difference was demonstrated in the PGE-mediated inhibition of different CFU-GM subsets in either the high or low HLA-DR groups suggests that DR antigen density may not independently define a progenitor cell's sensitivity to inhibition by PGE. Nevertheless, in view of the difficulty in accurately quantitating the degree of PGE inhibition of monocyte colonies in the low DR density fraction (due to low numbers of monocyte progenitor cells in control cultures), we cannot conclude with certainty that there is equal sensitivity to PGE of monocyte progenitor cells in the low and high DR+ fractions.

The relationship between HLA-DR antigen expression, commitment to monocyte differentiation, and colony inhibition by PGE was evaluated further by studying CFU-GM in CML, a stem cell disorder in which there is relative resistance to the inhibitory effects of PGE (30–33) despite the presence of HLA-DR on virtually all CFU-GM (15). In view of the selectivity of PGE for monocyte precursor cells, we considered the possibility that the lack of PGE inhibition in CML may be related to a relative decrease of monocyte colony-forming units in this disease. Previous investigators using other techniques to identify colony morphology including Luxol Fast Blue staining (32) and Giemsa staining (30, 31) have reported that the ratio of monocyte to granulocyte colonies in CML and NBM is similar. Morphological analysis of colony or cluster subtype, however, may be difficult without the use of lineage-specific stains. Early granulocyte colonies may contain large numbers of myelocytes, metamyelocytes, and band forms that may appear monocytoid. In view of these considerations, we reevaluated prostaglandin-mediated inhibition of CFU-GM in both NBM and CML peripheral blood using combined esterase staining of entire agar overlayers. This technique has the potential advantage of more accurate identification of colony and cluster lineage and avoids the problem of sampling error that may be associated with micropipette manipulation of individual colonies.

The results of 18 experiments demonstrate a statistically significant decrease in the proportion of mixed G/M and monocyte colony-forming cells in CML when compared with NBM. This finding was not related to an inability of CML-derived monocytes to stain for ANAE, since excellent correlation was observed between Wright-Giemsa and ANAE staining of monocytes freshly isolated from CML peripheral blood. Further, cultures of partially purified progenitor cells in suspension culture also revealed a relative decrease of mature monocytes compared with NBM in CML, again with good correlation between Wright-Giemsa morphology and ANAE staining in both CML and NBM cultures.

Despite differences in the composition of CFU-GM subsets in NBM and CML, CFU-GM subsets from either source appeared to be inhibited by PGE to a similar degree, with granulocyte progenitor cells being least sensitive, and monocyte progenitor cells most sensitive, to PGE inhibition. In CML, however, the infrequency of monocyte progenitor cells makes it difficult

Table VI. Dose Response of PGE₁ Inhibition in CFU-GM Subsets of NBM and CML*

CFU-GM source‡	PGE concentration	Colonies and clusters (day 7)§			
		Granulocyte	Mixed G/M	Monocyte	Total
NBM	Control	66±4	4±2	30±2	100±6
	10 ⁻⁶ M	72±2 (–9)	2±1 (50)	5±1 (83)	77±3 (23)
	10 ⁻⁸	71±2 (–8)	3±1 (25)	11±2 (63)	84±2 (16)
	10 ⁻¹⁰	71±2 (–8)	4±2 (0)	17±3 (43)	92±1 (8)
CML	Control	193±10	2±1	7±1	201±10
	10 ⁻⁶	183±8 (5)	0±0 (100)	1±1 (86)	184±7 (8)
	10 ⁻⁸	188±10 (3)	1±1 (50)	2±1 (71)	185±10 (8)
	10 ⁻¹⁰	186±7 (4)	2±1 (0)	4±1 (43)	195±8 (3)

* Two representative experiments of five. ‡ See Table IV. After partial purification of CFU-GM, cells were plated in varying concentrations of PGE₁ in the presence of 10⁻⁶ M indomethacin and 10% GCT as a source of colony-stimulating activity. § Results are expressed as mean number±SE of triplicate cultures per 10³ cells as determined on day 7 by combined esterase staining. || Numbers in parentheses indicate percent PGE₁ colony plus cluster inhibition.

Table VII. Differentiation of Partially Purified Myeloid Progenitor Cells in Suspension Culture*

Cell source	Incubation time	PGE ₁ (10 ⁻⁶ M)	Cell no. (×10 ⁶)	Blasts	Promyelocytes	Myelocytes-granulocytes [‡]	Monocytic cells	ANAE positive cells [¶]
NBM	Day 0		1.0‡	31±8§	50±4	19±6	0±0	1±0
	Day 7	—	10.4	1±1	44±9	47±8	8±4	1±0
		+	7.7	2±1	26±6	69±9	3±2	1±0
	Day 14	—	4.4	1±1	6±3	17±2	76±10	75±12
		+	4.2	3±1	34±8	27±4	36±6	18±13
CML	Day 0		1.0	98±3	2±1	0±0	0±0	1±0
	Day 7	—	15.5	12±5	62±7	14±4	12±3	2±1
		+	11.8	15±3	64±4	20±4	1±1	1±0
	Day 14	—	16.4	0±0	15±3	66±7	19±6	18±4
		+	18.4	5±3	28±5	66±9	1±3	2±1

* Cells for suspension culture were obtained by partial purification of either NBM or CML as previously described. ‡ For each treatment group, 1×10^6 partially purified progenitor cells were suspended in 5 ml of 20% FCS IMDMEM medium containing 10^{-6} M indomethacin and plated in a 60×15 -mm petri dish on day 0. § Differential count expressed as mean percent±SE as determined by Wright-Giemsa staining (300 cells counted). ‖ Myelocyte, metamyelocyte, band, and polymorphonuclear leukocyte. ¶ Mean percent±SE of monocytic cells as determined by ANAE staining (300 cells counted).

to compare their sensitivity to PGE with that of normal monocyte progenitor cells. Nevertheless, these results suggest that the overall resistance to PGE inhibition in CML may be partly explained on the basis of a decreased percentage of monocyte precursors. A significant correlation was demonstrated between the initial percentage of mixed and monocyte colonies and the final degree of prostaglandin inhibition obtained, regardless of the source of progenitor cells (NBM or CML), further supporting this concept (Fig. 2). This correlation is not exact because inhibition of mixed colonies and clusters was rarely complete, and because there was often a small degree of granulocyte inhibition by PGE₁ in both NBM and CML samples. The direct relationship observed between the percentage of monocyte precursor cells and the degree of PGE₁ inhibition of colony and cluster growth suggests that the blunted response to prostaglandin in CML is at least partly due to a decreased proportion of sensitive target cells in this disease, as opposed to deficient progenitor cell HLA-DR antigen expression.

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