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Association between colony forming units-granulocyte macrophage expression of Ia-like (HLA-DR) antigen and control of granulocyte and macrophage production. A new role for prostaglandin E.

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Research Article

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A NEW ROLE FOR PROSTAGLANDIN E

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ABSTRACT The expression of Ia-like antigens on human colony forming units-granulocyte macrophage (CFU-GM) is related to S-phase of the cell cycle, and associated with the control of normal granulocyte and macrophage production by prostaglandin E and acidic isoferritins in vitro. Ia-antigen expression by CFU-GM is lost within 3–6 h of culture at 37°C and occurs simultaneously with loss of responsiveness to inhibition by these factors. Culture of bone marrow CFU-GM in a limited exposure suspension culture with 1 μM–1pM prostaglandin E (PGE_1 or PGE_2), but not prostaglandin $F_2\alpha$ or dibutyryl-cyclic-3′-5′-AMP results in the detection of CFU-GM Ia-antigen after 24 h. Antigen expression is associated with an absolute increase in total and S-phase CFU-GM, and restoration of responsiveness to inhibition by prostaglandin E and acidic isoferritins. The detection of Ia-antigen on CFU-GM after suspension culture with prostaglandin E results both from Ia-antigen reexpression as well as stimulation of noncycling cells to enter S-phase, express Ia-antigen and give rise to CFU-GM sensitive to inhibition by prostaglandin E and acidic isoferritins. The sensitivity of CFU-GM to inhibition by these factors after suspension culture with prostaglandin E is identical to that of the same cells tested prior to the suspension culture. These studies provide evidence for a direct regulatory association between Ia-antigen expression and control of myeloid progenitor cell differentiation, and suggest a role for prostaglandin E in the control of CFU-GM cell cycle, Ia-antigen expression, and growth regulation.

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INTRODUCTION

Analysis of the antigenic profiles of hematopoietic stem cells and their progeny indicate that human granulocyte-macrophage progenitor cells (CFU-GM),[1] express Ia-like HLA-DR antigen on their surface (1–6). The pattern of Ia-antigen expression on myeloid cells; their absence on multipotential stem cells (4); their heterogenous expression on myeloid precursors (3, 4, 7) and their disappearance with progressive differentiation(1, 3, 7) suggests that these antigens may be involved in cellular interactions that regulate hematopoietic cell proliferation (1, 4).

At present, considerable evidence indicates that human CFU-GM proliferation can be regulated by diffusible factors, and two naturally occurring humoral factors, prostaglandin E (8–10) and acidic isoferritins (11–13) have been postulated to act as negative regulators. Recent analyses of these mechanisms indicate that Ia-antigen expression characterizes a state of CFU-GM responsiveness to humoral control. The inhibitory effects of both prostaglandin E and acidic isoferritin on human CFU-GM proliferation are selective for a subpopulation of colony-forming cells that express this antigen (8–11). Selective removal of Ia-antigen positive CFU-GM by cytolytic treatment with several monoclonal anti-human-HLA-DR antibodies results in virtually complete loss of CFU-GM responsiveness to inhibition.

In vitro, the expression of human CFU-GM Ia-antigen is transient and lost with time in culture, anal-

[1] *Abbreviations used in this paper*: CFU-GM, colony-forming units-granulocyte macrophage; [^{3}H]Tdr, tritiated thymidine.

ogous to that shown for murine macrophages (14–16), and occurs coincidentally with the loss of CFU-GM responsiveness to inhibition by prostaglandin E and acidic isoferritins (8, 11). In patients with chronic myeloid leukemia, CFU-GM Ia-antigen expression is absent or greatly diminished, and correlates with hyporesponsiveness of leukemic colony-forming cells to inhibition (8, 11).

The progression of cells through the cell cycle is often associated with changes in surface molecular expression. Analysis of the representation of CFU-GM Ia-antigen in relation to the cell cycle indicates that Ia-antigen or an epitopic determinant is detected only during S-phase (11). Consistent with these observations, acidic isoferritins have been shown to be S-phase specific inhibitors of normal CFU-GM proliferation (12, 13).

Prostaglandins of the E series have been shown to augment the proportion of human CFU-GM (17) and murine CFU-S (18) in S-phase of the cell cycle after limited exposure in vitro. The relationship between cycle-related Ia-antigen expression and response to growth regulation in vitro suggested that prostaglandin E might be used to investigate events associated with the control of myeloid progenitor cell proliferation. This report now demonstrates an association between cell cycle-related expression of human CFU-GM Ia-antigen and sensitivity of CFU-GM to growth inhibition by both prostaglandin E and acidic isoferritins. In addition, a new role for prostaglandin E in the regulation of CFU-GM differentiation is described.

METHODS

Bone marrow cells were obtained from normal adult volunteers by iliac crest aspiration. Heparinized marrow aspirates were collected after informed consent and according to established protocols.

Biophysical separation of colony-forming cells. Granulocyte-macrophage progenitor cells were enriched by neutral density centrifugation in isotonic Percoll (1.074 g/ml, 270 mosmol) (Pharmacia Fine Chemicals, Piscataway, NJ) as previously described (8). Mononuclear cells were recovered in the buoyant cell fraction, washed twice, and resuspended in supplemented McCoy's 5A modified medium containing 10% heat-inactivated fetal calf serum (Biofluids Inc., Rockville, MD).

Assay for granulocyte-macrophage colony formation. The capacity of normal human bone marrow cells to form colonies and clusters of granulocytes and macrophages was assayed in soft agar culture as described (8, 9). Indomethacin, at a final concentration of 1 μM, was routinely incorporated into all CFU-GM assays to prevent endogenous prostaglandin production (9). All cultures were incubated in a fully humidified 5% CO_2 atmosphere, and colonies (>50 cells) and clusters (4–50 cells) enumerated in quadruplicate plates after 7 d.

HLA-DR (Ia)-specific monoclonal antibodies. Monoclonal anti-human HLA-DR antibody (NEI-011) (7s, IgG_2) was purchased from New England Nuclear, Boston, MA. Anti-human HLA-DR monoclonal antibodies L243 and $OKIa_1$ were purchased from Becton, Dickinson, Sunnyvale, CA and Ortho Pharmaceutical Corp., Raritan, NJ, respectively. The characteristics and specificities of these monoclonal antibodies have been described (8, 11).

Complement-dependent cytotoxicity assay. All antibody plus complement (C')-mediated cytotoxicity reactions were carried out at a standard concentration of 1 million low density marrow cells, either freshly isolated or after suspension culture, per 0.1 ml of antibody dilution or C' as previously described (8). Rabbit complement was used at a final dilution, previously determined for each batch, which provided maximal cytotoxic activity when used with these or other C' fixing antibodies, while having no nonspecific cytotoxic effect when used alone.

Measurement of CFU-GM in S-phase. The fraction of CFU-GM in DNA synthesis, S-phase of the cell cycle, was assessed by exposure of bone marrow cells to high specific activity tritiated thymidine ([^{3}H]Tdr-sp. act.: 20 Ci/mmol, New England Nuclear) prior to agar culture (11). Specificity controls included media-treated cells, cells receiving unlabeled Tdr only, and cells receiving 100-fold excess Tdr (300 μg) 5 min before exposure to [^{3}H]Tdr. All groups were washed extensively, and resuspended to the identical cell concentration such that 100,000 cells were added to each agar culture.

Suspension culture. Low density bone marrow cells were maintained in liquid culture at 37°C, 5% CO_2 in air, prior to soft agar culture. All cultures were established in replicate at a concentration of $1.5–2.0 \times 10^6$ cells in 1.0 ml of McCoy's medium with 10% HI-FCS both in the absence and presence of various molar concentrations of prostaglandin E or other test compound. Indomethacin, 1 μM, was included in the suspension phase. Cultures were maintained for 3, 6, and 24 h. The number of S-phase and Ia^+ CFU-GM and their response to inhibition by prostaglandin E and acid isoferritins were assayed in soft agar before suspension culture. Control cells treated to remove S-phase and Ia^+ CFU-GM were placed in suspension culture and then assayed in soft agar for the fraction of cells in S-phase, Ia^+, and responsive to growth inhibition after the suspension culture. Bone marrow cells were not subjected to every treatment in every assay. Absolute CFU-GM were expressed per 1×10^5 cells before primary treatment and/or placed into suspension culture.

Reagents. Prostaglandins, dibutyryl-3'-5'-cyclic adenosine monophosphate and indomethacin were purchased from Sigma Chemical Co., St. Louis, MO. A standard source of acidic isoferritins (pH < 5.0 after isoelectric focusing) was used throughout these studies (13, 19). This fraction of ferritin was kindly provided by Dr. H. Broxmeyer and Dr. J. Bognacki, and contains >94% acidic isoferritins by radioimmunoassay.

RESULTS

Association of Ia-antigens with CFU-GM cell cycle and regulatory responsiveness to prostaglandin E and acidic isoferritins. Reported studies (9, 11, 12) suggested that the inhibitory effect of prostaglandin E on human CFU-GM might be cell-cycle related. Experiments were therefore conducted to evaluate the CFU-GM cell-cycle specificity of prostaglandin E, and these results compared to the S-phase associated expression of CFU-GM Ia-antigen (11) and the CFU-GM inhi-

bitory specificity of acidic isoferritins (11, 12). A representative experiment (1 of 12) is described (Table I). The responsiveness of colony and cluster forming CFU-GM to inhibition by both prostaglandin E and acidic isoferritins was lost after treatment with either [^{3}H]Tdr or anti-Ia-antibody plus C′. Loss of CFU-GM sensitivity to inhibition by prostaglandin E after [^{3}H]Tdr treatment was observed over a prostaglandin E concentration range of 1 μM–1 pM (not shown). The ability of excess unlabeled thymidine to block the effects of [^{3}H]Tdr confirmed the specificity of the thymidine suicide technique. The fraction of CFU-GM that could be inhibited by prostaglandin E and acidic isoferritins was equivalent to the fraction of CFU-GM sensitive to cytolysis by antibody plus C′ or exposure to [^{3}H]Tdr. In 12 experiments, this effect was observed equally for colonies as well as clusters (% S-phase CFU-GM: colonies 47±3, clusters 43±2; % Ia$^+$ CFU-GM: colonies 44±4; clusters 47±1), although in some experiments, a greater number of colony forming CFU-GM were in S-phase and Ia$^+$ when compared with cluster-forming CFU-GM.

Modulation of CFU-GM Ia-antigen expression. As previously reported (8, 11), expression of Ia-antigen on human CFU-GM maintained in suspension culture at 37°C was transient and could not be detected beyond 3–6 h (Table II). Once lost, constitutive Ia-antigen reexpression was not observed throughout the 24-h culture period (seven experiments). However, the inclusion of prostaglandin E_1 in the suspension culture resulted in detection of CFU-GM Ia-antigen expression after 24 h. A low level of Ia-antigen was noted in some experiments, after 6 h of suspension culture with prostaglandin E, but probably represents incomplete Ia-antigen loss, or low level of antigen reexpression. In most experiments, no Ia-antigen was detected at 6 h. GM-colony stimulating factors (GCT conditioned medium) did not induce Ia-antigen expression to a significant degree. The loss of Ia-antigen detection with time in culture at 37°C, and the apparent reexpression of Ia-antigens in the presence of prostaglandin E were independent of the inclusion of indomethacin in the culture system (not shown).

TABLE I

Selective Effects of Prostaglandin E and Acid Isoferritins on CFU-GM in S-Phase or Expressing Ia-antigenic Determinants

Treatment prior to assay in soft agar	Addition*	CFU-GM Colonies	CFU-GM Clusters
None	Media	143±6‡	350±11
	PGE_1 (0.1 μM)§	84±2 (42)‖	185±5 (47)
	A.F. (1 nM)§	82±2 (43)	189±4 (46)
[^{3}H]Tdr	Media	77±4 [46]¶	185±5 [47]
	PGE	80±4	183±5
	A.F.	85±4	180±5
Tdr + [^{3}H]Tdr	Media	141±4	355±11
	PGE	84±2	187±3
	A.F.	82±2	186±3
C′	Media	140±3	355±9
	PGE	83±1 (41)	184±3 (48)
	A.F.	82±2 (41)	188±3 (47)
αIa + C′**	Media	85±2 [40]	181±4 [49]
	PGE	87±4	180±5
	A.F.	84±4	178±3

* Added at initiation of agar cultures.

‡ Mean±SEM of quadruplicate plates scored for total CFU-GM (colonies > 50 cells, clusters 4–50 cells) on day 7 of culture.

§ Prostaglandin E (PGE); acidic isoferritins (AF).

‖ Percentage inhibition of CFU-GM proliferation in parentheses.

¶ Percentage CFU-GM is S-phase or expressing Ia-antigen in brackets.

** Anti-Ia-antibody (NEI-011) was used at a final dilution of 1:100.

TABLE II

Time Course for Prostaglandin E-mediated Reexpression of Ia-antigens Lost during Incubation at 37°C

Time in suspension culture	Present throughout suspension culture	Percentage Ia$^+$ CFU-GM* Colonies	Percentage Ia$^+$ CFU-GM* Clusters	Number of experiments
h				
0	—	51±3‡	47±2	(7)
3	Media	52±5	47±5	(4)
	1 μM PGE_1	54±5	50±2	(2)
	GM-CSF§	56±3	50±1	(2)
	GM-CSF + 1 μM PGE	63±4	56±4	(2)
6	Media	3±5	2±2	(4)
	1 μM PGE_1	12±4	11±8	(2)
	GM-CSF	5±5	6±4	(2)
	GM-CSF + 1 μM PGE	7±8	4±6	(2)
24	Media	1±2	−1±2	(7)
	1 μM PGE_1	54±3	46±3	(7)
	GM-CSF	−6±1	7±3	(3)
	GM-CSF + 1 μM PGE	52±1	47±8	(3)

* The percentage of day 7 CFU-GM expressing Ia-antigen was determined as a percentage of control cells treated with C′ alone. Anti-HLA-DR antibody (NEI-011) was used at a final dilution of 1:100.

‡ Mean±SEM of the average percent Ia$^+$ CFU-GM as determined from quadruplicate plates for each experiment. A negative value indicates a greater number of CFU-GM detected in αIa + C′ treated cultures than in C′ treated cultures.

§ GCT-conditioned medium (10% vol/vol) was used as a source of GM-CSF.

Analysis of the association between Ia-antigen, cell cycle status and inhibition by prostaglandin E and acidic isoferritins following suspension culture. To determine if loss of Ia^+ CFU-GM with time in culture was related to the exit of CFU-GM from S-phase, and if the detection of CFU-GM Ia-antigen after culture with prostaglandin E resulted solely from Ia-antigen reexpression or occurred coincident with induction of noncycling cells into S-phase; bone marrow cells were treated with [^{3}H]Tdr or anti-Ia-antibody plus C′ both before and after suspension culture.

Untreated or complement-treated control cells maintained in media alone for 24 h at 37°C clearly demonstrated the loss of detectable Ia^+ CFU-GM during the culture period. However, no decrease in the proportion of CFU-GM in S-phase was observed (Ta-

TABLE III
Cell Cycle Status and Ia-Antigen Expression of Granulocyte-Macrophage Colony and Cluster Forming Cells after Suspension Culture

Treatment prior to suspension culture	Present throughout suspension	Treatment following suspension culture	CFU-GM	
			Colonies	Clusters
			Mean±SEM	
Media	Media	Media	39±5	156±3
		[^{3}H]Tdr	21±1 (47)*	100±3 (36)
		C′	40±2	149±5
		αIa + C′‡	36±2 [10]*	145±5 [3]
	PGE_1 1 μM	Media	76±2	222±4
		[^{3}H]Tdr	24±1 (68)	105±3 (53)
		C′	73±3	216±3
		αIa + C′	23±1 [68]	106±5 [53]
^{3}H-Tdr	Media	Media	26±2	121±2
		[^{3}H]Tdr	23±1 (12)	119±1 (2)
		C′	20±1	123±2
		αIa + C′	21±1 [−5]	122±2 [1]
	PGE_1 1 μM	Media	44±2	162±3
		[^{3}H]Tdr	23±3 (48)	110±7 (33)
		C′	50±2	175±7
		αIa + C′	25±1 [50]	113±3 [36]
C′	Media	Media	43±3	208±5
		[^{3}H]Tdr	18±1 (58)	121±6 (42)
		C′	47±3	220±2
		αIa + C′	39±5 [16]	210±12 [5]
	PGE_1 1 μM	Media	82±3	289±2
		[^{3}H]Tdr	27±4 (67)	133±9 (54)
		C′	78±6	264±8
		αIa + C′	29±3 [73]	130±7 [51]
αIa + C′	Media	Media	30±1	128±1
		[^{3}H]Tdr	30±2 (0)	124±3 (3)
		C′	30±2	130±5
		αIa + C′	30±2 [2]	125±4 [4]
	PGE_1 1 μM	Media	65±5	183±8
		[^{3}H]Tdr	29±4 (55)	123±9 (36)
		C′	63±4	187±6
		αIa + C′	32±2 [49]	131±2 [31]

* Percent S-phase ( ) or Ia-antigen [ ] positive CFU-GM.
‡ Anti-Ia-antibody (NEI-011) was used at a final dilution of 1:100.

bles III, IV). The inability to detect Ia^+ CFU-GM was associated with the lack of CFU-GM responsiveness to inhibition by prostaglandin E and acidic isoferritins (Table IV). Likewise, treatment of bone marrow cells with [^{3}H]Tdr or anti-Ia-antibody plus C′ before suspension culture resulted in the absence of Ia^+, S-phase (Tables III, IV), and regulatory sensitive (Table IV) CFU-GM, when these cells were maintained in medium alone for 24 h. Incubation of control (untreated or C′-treated) cells with 1 μM prostaglandin E for 24 h resulted in the restoration of CFU-GM responsiveness to inhibition by prostaglandin E and acidic isoferritins (Table IV), and an increase in the proportion of total CFU-GM (colonies and clusters) in S-phase, with the coincident detection of Ia^+ CFU-GM equivalent to the fraction of S-phase clone-forming cells (Table III). In a similar fashion culture of cells, pretreated to remove all S-phase and Ia^+ CFU-GM before being placed in suspension culture with 1 μM prostaglandin E for 24 h, was associated with the detection of S-phase and Ia^+ CFU-GM (Table III, Table IV) and the demonstration of CFU-GM sensitivity to growth inhibition by prostaglandin E and acidic isoferritins (Table IV). In every experiment in which prostaglandin and acid ferritin responsive CFU-GM were detected/induced after suspension culture with prostaglandin E, this population of CFU-GM could be eliminated by treatment to remove S-phase or Ia^+ cells. In all cases, an absolute rise in total CFU-GM was observed in cultures incubated with prostaglandin E when compared with cells maintained in media alone. In most experiments, following culture with prostaglandin E a preferential increase in colony-forming cells over cluster forming cells was observed (percent rise CFU-GM: colony 87±16; cluster 44±5, $n = 11$. $P < 0.005$).

The detection of Ia-antigens on CFU-GM, their association with S-phase of the cell cycle, loss of Ia-antigen detection in culture and expression following culture with prostaglandin E could also be demonstrated using two additional monoclonal anti-human HLA-DR antibodies L-243 and $OKIa_1$ (not shown). In all cases, responsiveness of CFU-GM to inhibition by prostaglandin E and acid isoferritins was associated with the expression of Ia-antigen.

Dose titration of the effects of prostaglandin E on CFU-GM cell cycle, Ia-antigen expression and regulatory response. The association of CFU-GM inhibition by prostaglandin E and acidic isoferritins coincident with prostaglandin-induced CFU-GM S-phase and Ia-antigen expression was analyzed over serial dilutions of 1 μM–0.1 fM prostaglandin E_1 (Fig. 1).

TABLE IV

Cell Cycle Status, Ia-Antigen Expression, and Response to Inhibition by Prostaglandin E and Acid Isoferritins of CFU-GM after Suspension Culture

Treatment prior to suspension culture	Present throughout suspension culture	% CFU-GM*		% Inhibition CFU-GM	
		S-phase	Ia^+	PGE_1 (0.1 μM)	Acid isoferritins (1 nM)
Media	Media	38±1‡	4±3	0±0	3±1
	1 μM PGE_1	57±3	55±2	40±1	40±1
[^{3}H]Tdr	Media	4±1	0±0	0±0	0±0
	1 μM PGE_1	33±1	32±1	34±1	34±1
C′	Media	43±2	10±1	10±2	7±1
	1 μM PGE_1	40±2	33±4	39±1	37±1
αIa + C′	Media	2±1	3±1	4±1	4±1
	1 μM PGE_1	39±2	35±1	36±1	36±2

* The percentage of CFU-GM in S-phase or expressing Ia-antigen was determined as a percentage of control cells treated with unlabeled Tdr or C′, respectively. Anti-HLA-DR antibody (NEI-011) was used at a final dilution of 1:100.

‡ Mean±1 SEM of quadruplicate plates scored for total CFU-GM (colonies > 50 cells; clusters 4–50 cells) on day 7.

Total CFU-GM in S-phase or Ia^+ prior to suspension culture: S-phase—colonies 41±3%, —clusters 40±2%; Ia^+—colonies 51±3%, —clusters 42±3%. Average inhibition of CFU-GM prior to suspension culture: PGE (0.1 μM) —colonies 42±1%, —clusters 47±3%; acid isoferritin (1 nM) —colonies 40±2%, —clusters 44±2%.

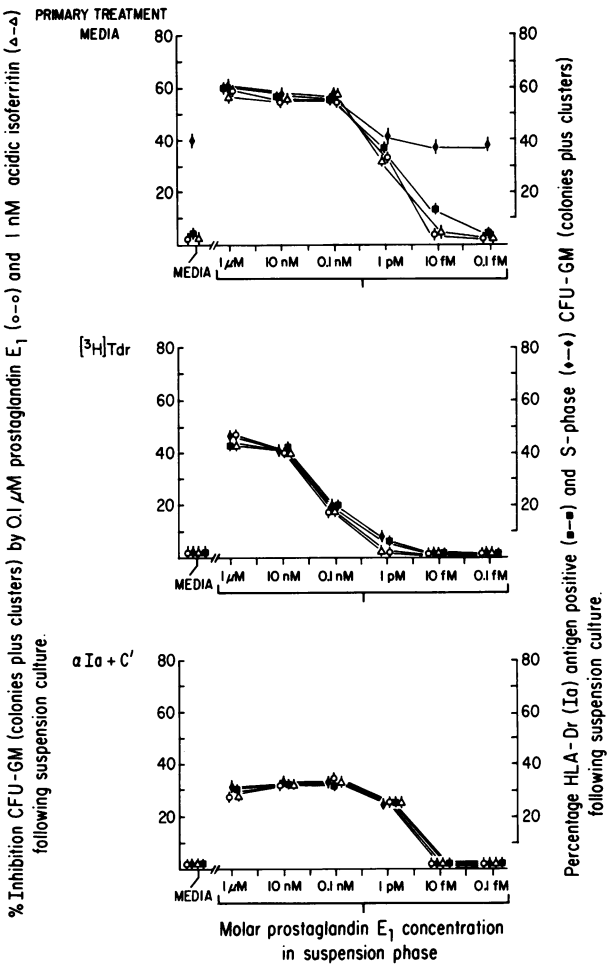

FIGURE 1 Titration analysis of the effects of prostaglandin E on induction of CFU-GM cell cycle, Ia-antigen expression, and responsiveness to growth inhibition by both prostaglandin E and acidic isoferritins. Bone marrow cells were treated with media, [^{3}H]Tdr and α Ia + C' before suspension culture with 1 μM through 0.1 fM PGE, and again following culture. The response of CFU-GM in each group to prostaglandin E and acidic isoferritin was determined. Monoclonal anti-human HLA-DR antibody NEI-011 was used at a final dilution of 1:100 throughout.

Suspension culture of untreated cells with molar prostaglandin E concentrations of 1 μM through 1 pM increased the fraction of CFU-GM in S-phase, and induced CFU-GM Ia-antigen expression. The induction of CFU-GM Ia-antigen expression by prostaglandin E_1 at concentrations of 1 μM–0.1 nM was associated with complete restoration of CFU-GM responsiveness to inhibition by prostaglandin E and acidic isoferritin equivalent to that observed on fresh marrow cells assayed before suspension culture. Prostaglandin-induced CFU-GM Ia-antigen expression and responsiveness to inhibition was essentially lost by 10 fM. The ability of prostaglandin E to augment the fraction of CFU-GM in S-phase was not observed beyond I pM. Throughout the dose range of 1 μM–0.1 nM prostaglandin E, a direct correlation was observed between CFU-GM response to inhibition by prostaglandin E and acidic isoferritin and sensitivity to treatment with anti-antibody plus C' or [^{3}H]Tdr. However, at prostaglandin E concentrations <0.1 nM, CFU-GM prostaglandin and ferritin responsiveness correlated only with the degree of Ia-antigen expression. Treatment of bone marrow cells with [^{3}H]Tdr and anti-Ia-antibody plus C', and suspension culture in media alone, resulted in the detection of an Ia-antigen negative, noncycling, prostaglandin E and acidic isoferritin-unresponsive CFU-GM population. However, when incubated with prostaglandin E_1, Ia^+, and S-phase CFU-GM were detected in identical proportions. Furthermore, CFU-GM responsiveness to inhibition by both prostaglandin E and acidic isoferritins could be demonstrated, and was directly proportional to the number of Ia^+ and S-phase CFU-GM detected.

Titration of CFU-GM growth regulation following suspension culture. The response of CFU-GM to inhibition by both prostaglandin E_1 and acidic isoferritins before and after suspension culture with media or 1 μM prostaglandin E was analyzed over a dose-response titration for both inhibitors (Fig. 2). After suspension culture with prostaglandin E, the sensitivity of CFU-GM to inhibition by both factors was identical to that of the same marrow cells tested prior to the suspension culture.

Specificity of prostaglandin E in the suspension culture. The specificity of prostaglandin E for the induction of CFU-GM Ia-antigen expression and restoration of CFU-GM responsiveness to inhibition by prostaglandin E and acidic isoferritin after suspension preculture was investigated (Fig. 3). Before suspension culture, CFU-GM proliferation could be inhibited by both prostaglandin E and acidic isoferritins, and removal of S-phase and Ia^+ CFU-GM resulted in the loss of sensitivity to inhibition. Following suspension culture in media alone, loss of CFU-GM responsiveness to inhibition was observed coincident with lack of significant CFU-GM Ia-antigen expression. In the presence of 1 μM prostaglandin E_2, regulatory responsiveness and Ia-antigen expression was restored. Prostaglandin $F_2\alpha$ and dibutyryl-cyclic AMP were unable to mimic the effects of prostaglandin E.

DISCUSSION

Analysis of Ia-antigen expression on human CFU-GM in vitro indicates that an Ia-antigen, or an epitopic region, is preferentially expressed during S-phase of

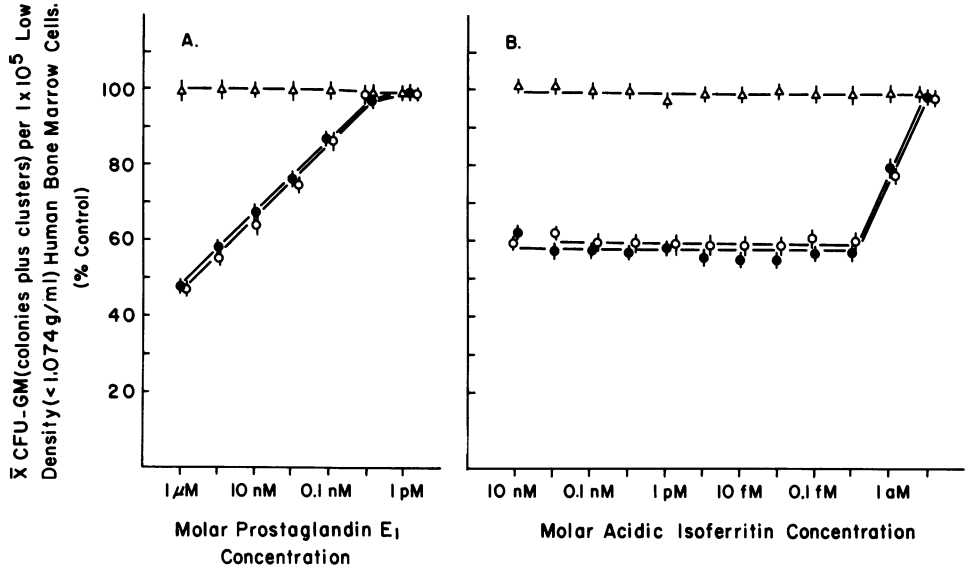

FIGURE 2 Analysis of CFU-GM response to prostaglandin E_1 (A) and acidic isoferritin (B) before and after suspension culture with media or 1 μM prostaglandin E_1. Data is expressed as mean±SEM of quadruplicate cultures, as a percentage of control. ●, 0 h primary agar culture; △, post 24 h suspension media; ○, post 24 h suspension 1 μM PGE^1.

the CFU-GM cell cycle (11) and is associated with the humoral control of CFU-GM proliferation by prostaglandin E (8) and acidic isoferritin (11) in vitro. The present communication extends this concept of a regulatory role for Ia-like antigen in hematopoietic differentiation. These studies describe a role for prostaglandin E in the regulation of CFU-GM Ia-antigen expression and the control of CFU-GM responsiveness to humoral regulation. In addition, the specificity of prostaglandin E as an S-phase selective inhibitor of CFU-GM proliferation is established.

Monoclonal antibody analysis of human CFU-GM Ia-antigen expression by other laboratories (5, 6) have demonstrated the presence of these antigens on virtually all CFU-GM, and are in contrast with the cycle-related detection by the antibodies used in this and other studies (8, 11). Heterogeneity of Ia-antigen expression (20) and changes in HLA-DR antigen density (21) relative to the cell cycle have been reported for both murine and human B-cell lines. The masking and unmasking of epitopic regions or changes in antigen density can result in monoclonal antibodies with different specificities. In this regard, the recognition of HLA-DR epitopes, and subpopulations of human Ia-like molecules by various monoclonal antibodies has been documented (22–25). Therefore, the detection of Ia-antigen only on a subpopulation of CFU-GM may reflect the highly discriminatory capacity of the monoclonal antibodies. However, a low density appearance of Ia-antigen on CFU-GM during phases of the cell cycle other than S-phase, resulting in nondetection with the three monoclonal anti-HLA-DR antibodies tested (8, 11) has not been ruled out. Moreover, it is possible that different monoclonal anti-HLA-DR antibodies may recognize different Ia-antigens that may or may not be cycle-related and may not be involved in the regulatory interactions described in this communication. The relevance of the detection of the cycle-related Ia-antigen expression on human CFU-GM described herein, is their presence only on that population of human CFU-GM responsive to inhibition by prostaglandin E and acidic isoferritin in vitro. The detection of CFU-GM in S-phase after 24 h in suspension culture demonstrates that lack of Ia-antigen detection is not due *a priori* to a lack of S-phase CFU-GM. These data indicate that CFU-GM Ia-antigen expression and not cycle state is the determinant factor tightly associated with control of CFU-GM proliferation in vitro, and may therefore define a normal CFU-GM regulatory phenotype.

The demonstration of a relationship between loss of CFU-GM Ia-antigen expression and response to growth inhibition with time in culture supports, but in itself does not provide sufficient evidence for a direct relationship between Ia-antigen expression and growth control. A regulatory network would require that reinduction of CFU-GM Ia-antigen expression and/or stabilization be possible, and directly associated with return to or maintenance of a state of CFU-GM responsiveness. The in vitro conversion of Ia^- mouse

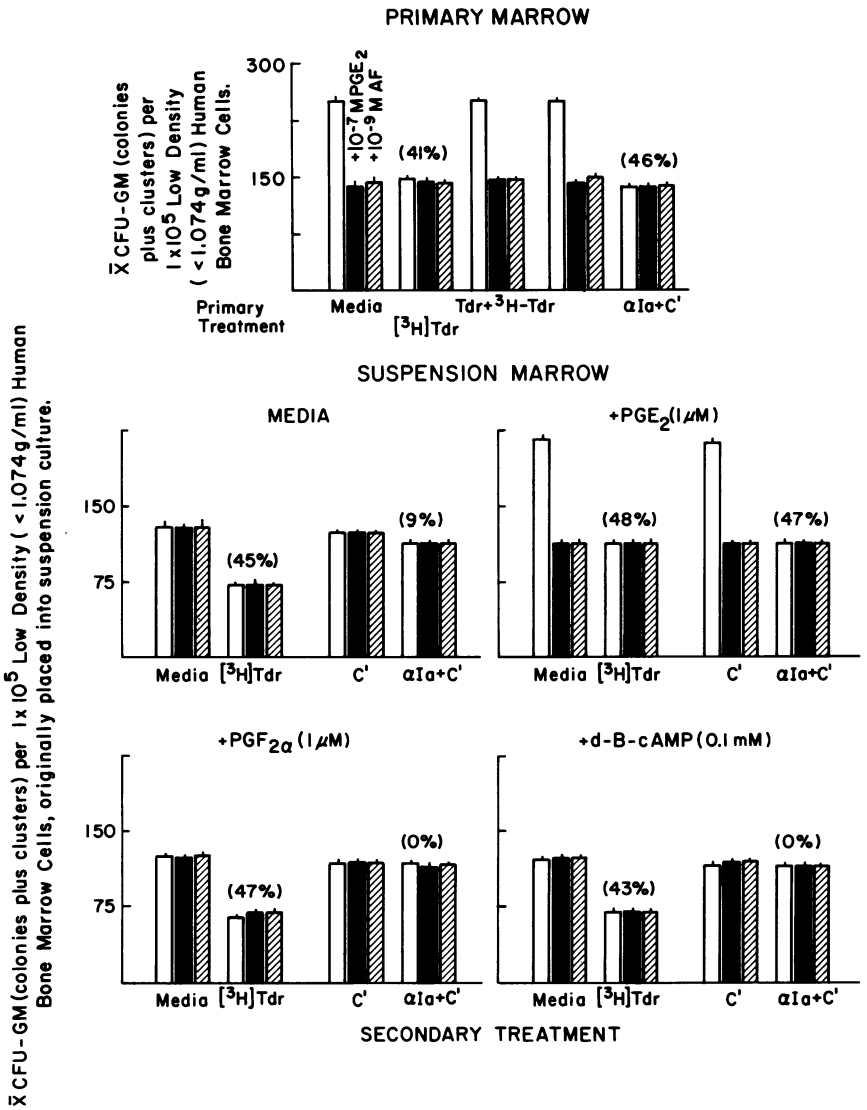

FIGURE 3 Comparison of the ability of prostaglandin E_2, prostaglandin $F_2\alpha$, and dibutyryl-3′5′-cyclic AMP to induce CFU-GM, Ia-antigen expression, cell-cycle, and responsiveness to growth regulation by prostaglandin E_1 and acidic isoferritin in suspension culture. Monoclonal anti-human HLA-DR antibody NEI-011 was used at a final dilution of 1:100 throughout. Data is expressed as mean±SEM of quadruplicate cultures scored on day 7. The percentage of CFU-GM in S-phase ([^{3}H]-Tdr) and expressing Ia-antigen (αIa + C′) are given in parentheses and correspond to the unshaded histograms.

macrophages to an Ia^+ state, and stabilization of macrophage Ia-antigen expression by lymphokine containing supernates has been reported (14–16). A role for prostaglandin E in the regulation of CFU-GM Ia-antigen expression is now described.

Exposure of bone marrow cells to prostaglandin E in vitro for 24 h results in the detection of Ia-antigen on CFU-GM, otherwise not detected on cells cultured in media alone; and the coincident reappearance of CFU-GM, which possess normal responsiveness to both prostaglandin E and acidic isoferritin. Time sequence analysis indicates that culture with prostaglandin E does not result in CFU-GM Ia-antigen stabilization, but rather, results in new antigen expression.

In those groups of cells treated to remove all S-phase and Ia^+ CFU-GM prior to suspension culture with prostaglandin E, both S-phase and Ia^+ CFU-GM were detected following the suspension culture, and in a

one-to-one relationship. No S-phase or Ia^+ CFU-GM were detected in identical cultures maintained without prostaglandin. This suggests that the mechanism of action of prostaglandin E in the induction of CFU-GM Ia-antigen expression is via the stimulation of noncycling cells, possibly earlier myeloid stem cells capable of giving rise to CFU-GM, into cycle. The normal regulatory responsiveness detected appears to be a direct consequence of Ia-antigen expression by these CFU-GM.

Interpretation of CFU-GM antigen induction/reexpression and detection of normal regulatory responsiveness of CFU-GM that were not treated before culture with prostaglandin E is not as clear. In all experiments, culture of untreated bone marrow cells with prostaglandin E for 24 h results in an absolute increase in the total number of colony- and cluster-forming CFU-GM, an augmentation of the fraction of total CFU-GM in S-phase, the detection of Ia^+ CFU-GM equivalent to the total number of S-phase CFU-GM, and the restoration of normal regulatory control. The majority of S-phase and Ia^+ CFU-GM detected probably derive from noncycling cells whose entry into S-phase and coincident expression of Ia-antigen is induced by prostaglandin E. This is consistent with the absolute increase in the number of CFU-GM detected. However, the data presented are not inconsistent with Ia-antigen reexpression on those S-phase CFU-GM which can be detected in culture with media alone. Comparison of the number of S-phase and Ia^+ CFU-GM detected following culture with prostaglandin E with the numerical increase in total CFU-GM detected in these cultures indicates that more S-phase and Ia^+ CFU-GM are present than can be accounted for solely by assuming that all new CFU-GM detected were in S-phase and expressing Ia-antigen at the end of the suspension culture. Under these circumstances the difference apparently represents Ia-antigen reexpression on those S-phase, Ia-antigen negative CFU-GM detected in the control cultures.

Timed addition experiments indicate that in order to observe CFU-GM Ia-antigen expression at 24 h in the system described, prostaglandin E must be added to the cultures within the first 3 h in suspension (unpublished observation). Kinetic analysis indicates that constitutive prostaglandin E production by mouse macrophages (26) and human monocytes is not detected during the first 3 h in culture. These results negate the contribution of endogenously produced prostaglandin E in this system. This fact is supported by the lack of constitutive CFU-GM Ia-antigen expression regardless of the absence or presence of indomethacin in the suspension culture. The inability of dibutyryl cyclic AMP to mimic the effect of prostaglandin E in modulation of CFU-GM cell cycle, HLA-DR expression, and regulatory responsiveness suggests that prostaglandin E may not mediate this effect via the adenyl cyclase system. In this regard, the E series prostaglandins have been shown to also act as calcium ionophores (27), and may mediate this effect by altering transmembrane calcium potentials.

The enhancement of CFU-GM Ia-antigen expression, entrance into cell-cycle and sensitivity to growth inhibition by prostaglandin E observed in suspension preculture contrasts with its inhibitory effect on CFU-GM proliferation observed using agar culture alone. It is possible that in agar one observes a net effect of both positive and negative influences on CFU-GM differentiation. However, investigation of these effects directly in the agar cultures is severely impaired by the semisolid agar matrix. The addition of prostaglandin E and acidic isoferritins to agar cultures at time points 12–24 h after initiation has no effect on CFU-GM proliferation (unpublished observation). This result may occur as a result of loss of CFU-GM Ia-antigen expression (8, 11). The addition of small quantities of prostaglandin E (10 nM) to agar cultures at initiation, did not restore CFU-GM sensitivity to a larger concentration of prostaglandin E (0.1 μM) or acidic isoferritin (1 nM) added 24 h later (unpublished observation). In contrast, these same concentrations of prostaglandin E restored Ia-antigen expression and sensitivity to growth inhibition of CFU-GM maintained in suspension culture before culture in soft agar. It appears, that the effects of prostaglandin E observed in suspension culture may not occur in agar. In this regard, preliminary evidence indicates that the reexpression of CFU-GM, Ia-antigen and sensitivity to growth inhibition observed using prostaglandin E in suspension culture require the interaction of hematopoietic cells and T lymphocytes, possibly mediated by cell-to-cell contact.

The ability of prostaglandins of the E series to promote CFU-GM differentiation reported in this study do not contradict the demonstrated inhibitory capacity of these compounds reported by this and other laboratories (8, 9, 22, 24, 28–30). It is now apparent that the effects of prostaglandin E are biphasic. At low, as well as high, concentrations, prostaglandin E appears to play a role in CFU-GM differentiation by promoting or modulating CFU-GM cell cycle and coincidently, modulating CFU-GM growth regulation as a consequence of Ia-antigen expression. The demonstration of circulating prostaglandin E levels in the range of 0.1 nM to 10 pM in human plasma (31) and the insensitivity of human CFU-GM to inhibition at these levels (9) places the CFU-GM differentiating role of prostaglandin E well within physiological range. The

ultimate degree of CFU-GM expansion to mature granulocytes and macrophages is dependent upon GM-CSF levels (32, 33). At high prostaglandin concentrations, particularly as a consequence of GM-CSF induced monocyte-macrophage prostaglandin production (20, 26, 28, 34), clonal CFU-GM expansion is limited (8–10, 28) without compromising the differentiation of earlier stem cells into the CFU-GM compartment.

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