Association of Cell Cycle Expression of Ia-Like Antigenic Determinants on Normal Human Multipotential (CFU-GEMM) and Erythroid (BFU-E) Progenitor Cells With Regulation In Vitro by Acidic Isoferritins

By Li Lu, Hal E. Broxmeyer, Paul A. Meyers, Malcolm A.S. Moore, and H. Tzvi Thaler

An association has been established between human Ia-like antigenic determinants, expression during DNA synthesis on multipotential (CFU-GEMM) and erythroid (BFU-E) progenitor cells, and the regulatory action of acidic isoferritins in vitro. Treatment of human bone marrow cells with monoclonal anti-la (NE1-011) plus complement inhibited colony formation of CFU-GEMM and BFU-E by 50%-70%. Reduction of colonies was similar whether bone marrow cells were exposed to anti-la plus complement, high specific activity tritiated thymidine (\(^{3}HTrd\)), or acidic isoferritins. No further decrease was apparent with \(^{3}HTrd\) or acidic isoferritins after la-antigen CFU-GEMM or BFU-E were removed, or with anti-la plus complement or acidic isoferritins after S-phase CFU-GEMM or BFU-E were removed. Anti-la, in the absence of complement, had no effect on colony formation but blocked the inhibition of CFU-GEMM and BFU-E by acidic isoferritins. Demonstration of Ia-antigens on BFU-E and inhibition of BFU-E by acidic isoferritins appeared to require the presence of phytohemagglutinin leukocyte conditioned medium (PHA-LCM) in the culture medium during the 14-day incubation period. These results implicate Ia-antigen cells, acidic isoferritins, and PHA-LCM in the regulation of multipotential and erythroid progenitor cells in vitro.

**HUMAN Ia-LIKE antigens are a group of cell membrane glycoproteins that are products of the major histocompatibility gene complex. The antigens are prominent on B lymphocytes and represent differentiation markers on hematopoietic cells.**

The antigen is expressed on progenitor cells of the granulocyte-macrophage and erythroid lineages, the CFU-GM (colony-forming unit-granulocyte-macrophage)\(^{1}\) and BFU-E (burst-forming unit-erythroid),\(^{5,9,10}\) as well as on subpopulations of myeloblasts, promyelocytes, monocytes, and proerythroblasts.\(^{1,2,8,11}\) They do not appear to be expressed on more mature granulocytes, erythroid cells, or on a population of probable human multipotential stem cells.\(^{15}\) It has recently been shown that certain monoclonal antibodies to human Ia-like antigens\(^{12}\) recognize normal CFU-GM during DNA synthesis (S-phase) and that a leukemia-associated inhibitory activity (LIA),\(^{13,14}\) identified as acidic isoferritins\(^{15}\) and shown to derive from normal monocytes and macrophages,\(^{16}\) acts on CFU-GM expressing Ia-like antigens during S-phase.\(^{12}\)

An in vitro assay for multipotential progenitor cells (CFU-GEMM, colony-forming units-granulocyte, erythroid, monocyte, megakaryocyte) has been developed.\(^{17,18}\) and there is some evidence that CFU-GEMM may represent human multipotential stem cells or a subpopulation of stem cells.\(^{19,20}\) Little is known regarding the regulation of human multipotential stem cells and we decided to investigate whether la-antigens were expressed on CFU-GEMM, if this expression was associated with S-phase of CFU-GEMM using a particular monoclonal antibody plus complement, if colony formation by CFU-GEMM could be suppressed by acidic isoferritins, and if this correlated with an la-antigen subpopulation of CFU-GEMM. These studies were also extended to colony formation of BFU-E, which were scored on the same plates as for CFU-GEMM.

**MATERIALS AND METHODS**

**Cells**

Bone marrow cells were obtained by aspiration from the iliac crest of healthy volunteers who gave informed consent. Unseparated nucleated cells or cells of density less than 1.077 g/ml were obtained after separation with Ficoll-Hypaque, suspended in Iscove’s modified Dulbecco’s medium (IMDM, GIBCO, Grand Island, N.Y.) containing 10% fetal calf serum (FCS), and kept at 4°C until used for treatment or culture.

**Assays**

The colony assay for human CFU-GEMM was carried out according to the procedure of Fauser and Messner.\(^{17,18}\) Bone marrow cells were plated at 2 × 10\(^{5}\), unless otherwise noted, or what was left after antibody and complement treatment, in 35-mm Lux standard tissue culture dishes containing a 1-ml mixture of IMDM, 0.8% methylcellulose, 30% fetal bovine serum, 5% medium conditioned by...
leukocytes from patients with hemachromatosis in the presence of 1% phytohemagglutinin (PHA-LCM)\(^{10}\) and \(5 \times 10^{-3} M\) 2-mercaptoethanol. Cells were plated in dishes with or without acidic isoferritins \((10^{-8} \text{ to } 10^{-10} M)\). Dishes were incubated at 37°C in a humidified atmosphere flushed with 5% CO\(_2\) in air. One unit of a step III preparation of sheep plasma erythropoietin (Connaught Labs, Ltd, Willowdale, Ontario, Canada) was added to each dish on day 4 or 5 unless otherwise noted. Colonies were scored with an inverted microscope after 13–14 days of incubation and were further identified after staining with benzidine in the dishes or after picking out colonies with a fine pipette, putting on a glass slide, and staining with Wright-Giemsa to confirm their mixed nature. Mixed colonies usually contained erythroid, granulocyte, and monocyteic cells, and sometimes contained megakaryocytes.

BFU-E were scored from these same plates. In some cases, and where specifically stated, BFU-E were scored from plates cultured as previously reported\(^{22}\) using the methodology described by Iscove et al.\(^{23}\) Five to ten plates were scored per treatment point.

**Ia-Like Antibody and Complement-Dependent Cytotoxicity Test**

Monoclonal anti-human Ia-like antibodies (M-anti-Ia) (NEI-011) were purchased from New England Nuclear, Boston, Mass. They had been isolated from hybrid 7.2.24 are an IgG\(_\text{a}\) antibody that had been purified by affinity chromatography with protein A, and are greater than 95% homogeneous. They recognize two polypeptides of 28,000 and 33,000 daltons and are found on >99% of B cells, on <1% of T cells, and on monococytes and activated T cells from peripheral blood, and on B-cell lines, B-cell leukemias, and certain myeloid and null cell leukemias (New England Nuclear Communications). This is the same antibody used previously in studies.\(^{12}\) M-anti-Ia-like antibody (Q5/13) was a generous gift from Dr. John Fitchen (V.A. Hospital, Portland, Ore.) and Dr. S. Ferrone, Columbia University, New York, N.Y., and has been described previously.\(^{22}\) The complement-dependent cytotoxicity procedure has been described elsewhere.\(^{13,14,15,16}\) A 1:50 final concentration of antibody was used unless otherwise noted. Rabbit complement was purchased (Low-Tox-H-Rabbit complement, Accurate Chemical Co., Westbury, N.Y.) and used at 1:8 dilution. Cells were washed twice prior to further treatment or to plating.

**Measurement of CFU-GEMM and BFU-E in S-Phase**

Cells were treated with medium, cold thymidine (Tdr, 500 µg), 50 µCi/ml tritiated thymidine \(^{3}H\text{Tdr},\) New England Nuclear, specific activity 20 Ci/m mole), or 50 µCi/µl Tdr plus 500 µg cold thymidine. After a 20-min incubation at 37°C, the cells were washed once in ice-cold thymidine (2000 µg), twice more in IMDM, and then plated in the absence or presence of Tdr with or without further treatment.

**Acidic Isoferritins**

Acidic isoferritins were isolated from the spleen of a patient with chronic myeloid leukemia (CML).\(^{17,18,19}\) This preparation migrated as a single band in the apparent molecular weight region of 550,000 after sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and as two bands with apparent molecular weights of ~19,000 and ~21,000 after treatment with 10% 2-mercaptoethanol at 100°C for 10 min and separation on SDS-PAGE.\(^{15}\) It had an isoelectric point (pI) value of 4.7 after analytical isoelectric focusing and was biochemically similar to acidic isoferritins isolated from normal human blood.\(^{17}\) In several experiments and where noted, crude inhibitory activity (LIA), LIA isolated to the stages of Sephadex G-200 gel filtration, carboxymethylcellulose ion exchange chromatography, or isoclectric focusing were used. Information on these preparations has been reported previously.\(^{14,15}\)

Iron-depleted CML spleen acidic isoferritins were obtained as described previously.

**RESULTS**

Acidic Isoferritins Act on a Subpopulation of BFU-E and CFU-GEMM

Colony formation by BFU-E and CFU-GEMM from low density bone marrow cells were scored on the same plates and the average significant suppression of colony formation by \(10^{-5} M\) CML spleen acidic isoferritins (5 experiments) was 54% ± 5% for BFU-E and 55% ± 6% for CFU-GEMM (Fig. 1, treatment 1; Table 1, treatment 1). Fifty to 60% inhibition of BFU-E \((p < 0.005)\) and 55%–65% inhibition of CFU-GEMM \((p < 0.005)\) colony formation was detected whether the cells were exposed to \(10^{-5} M\)–\(10^{-7} M\) CML spleen acidic isoferritins, crude LIA, LIA purified to the stages of Sephadex G-200, carboxymethylcellulose and isoelectric focusing, or CML spleen acidic isoferritins depleted of iron. (Control numbers for 2 experiments were BFU-E 55 ± 2, 69 ± 4 and CFU-GEMM 8 ± 1, 11 ± 1.)

A Monoclonal Antibody (NEI-011) to Human Ia-Like Antigens Detects Determinants on a Subpopulation of BFU-E and CFU-GEMM Using a Complement-Dependent Cytotoxicity Test

Complement alone was without effect on colony formation (Fig. 1, Table 1 treatments 1, 2, 3: minus ferritin), but treatment of low density bone marrow cells with a 1:50 dilution of m-anti-Ia (NEI-011) in the presence of complement (4–5 experiments) resulted in significant inhibition of colony formation (58% ± 4% for BFU-E and 62% ± 5% for CFU-GEMM).

Similar degrees of inhibition of colony formation were noted with 1:25, 1:50, 1:100, and 1:200 dilution of m-anti-Ia (NEI-011) in the presence of complement. Using the same source and dilution of complement with another m-anti-Ia (Q5/13, 1:50 dilution) resulted, however, in an 85% inhibition of BFU-E \((52 ± 4)\) in 8 ± 1 colonies) and a 93% inhibition of CFU-GEMM \((14 ± 1\) to 1 ± 0.7 colonies) as compared to the 62% inhibition of BFU-E \((52 ± 4)\) to 20 ± 2 colonies) and 71% inhibition of CFU-GEMM \((14 ± 1\).
to 4 ± 1 colonies) with m-anti-Ia (NE1-011). These results suggest that the differences in the extent of lysis of populations of CFU obtained with particular monoclonal antibodies is probably not related to the source or dilution of complement used.

### Table 1. Comparative Effect of m-Anti-Ia (NE1-011) Plus Complement and Treatment With High Specific Activity \(^{3}HTdr\) on Colony Formation by CFU-GEMM and on Inhibitory Activity of Acidic Isoferritins*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Acetic Isoferritins</th>
<th>Colonies</th>
<th>Percent Change From Control (5 Exp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Iscove's medium</td>
<td>-</td>
<td>10 ± 2</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>2. Complement (C')</td>
<td>-</td>
<td>5 ± 0.4</td>
<td>7 ± 0.7</td>
</tr>
<tr>
<td>3. (\alpha\alpha + C')</td>
<td>-</td>
<td>9 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>4. Thymidine (Tdr)</td>
<td>-</td>
<td>4 ± 0.5</td>
<td>7 ± 0.8</td>
</tr>
<tr>
<td>5. (^{3}HTdr)</td>
<td>-</td>
<td>4 ± 0.3</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>6. (^{3}HTdr + Tdr)</td>
<td>-</td>
<td>4 ± 0.4</td>
<td>6 ± 0.8</td>
</tr>
<tr>
<td>7. (^{3}HTdr \rightarrow \alpha\alpha + C')</td>
<td>-</td>
<td>5 ± 0.6</td>
<td>6 ± 0.8</td>
</tr>
<tr>
<td>8. (\alpha\alpha + C' \rightarrow ^{3}HTdr)</td>
<td>-</td>
<td>4 ± 0.5</td>
<td>6 ± 0.8</td>
</tr>
</tbody>
</table>

*Cells were pretreated as shown above and plated, at 2 × 10° cells/ml or what was left after treatment with \(\alpha\alpha + C'\), with or without 10 \(^{3}\)M acidic isoferitins. The results of 5 experiments are given and expressed as the mean ± 1 SEM and the mean percent changes ± 1 SEM of the 5 experiments compared to the Iscove’s medium control values (minus ferritin). The levels of significance compared to the control values within individual experiments are shown to the right of the percent change from control.

NS, not significant.

\(\ast p < 0.01.\)

\(\ast p < 0.005.\)

\(\ast p < 0.0025.\)

\(\ast p < 0.0005.\)
CFU-GEMM were killed by pulse treatment with high specific activity tritiated thymidine (³HTdr) (Fig. 1, Table 1: treatments 1,4,5; minus ferritin) and that the cells were in rapid cycle. The S-phase specificity of the ³HTdr kill in these experiments was substantiated by the fact that cold thymidine (Tdr) had no influence on colony formation (Fig. 1, Table 1: treatment 4) but did countervail the killing effect of ³HTdr when present during the period of pulse treatment (Fig. 1, Table 1: treatment 6). Furthermore, the addition of cold Tdr to the culture plates during the 14 days of incubation did not reverse the killing effect of ³HTdr on BFU-E and CFU-GEMM (Table 2).

Evidence for the detection of Ia antigens (as detected by m-anti-Ia [NEI-011]) on BFU-E and CFU-GEMM during S-phase is given, respectively, in Fig. 1 and Table 1. The kill with m-anti-Ia plus complement (treatment 3) was similar to the kill with ³HTdr (treatment 5). In addition, in one experiment, no further decrease in colony formation was detected with m-anti-Ia plus complement after S-phase cells had been killed by ³HTdr (treatment 7) and no further kill by ³HTdr was noted for those cells surviving treatment with m-anti-Ia plus complement (treatment 8).

Acidic Isoferritins Act on the Cycling Subpopulation of BFU-E and CFU-GEMM Expressing Ia-Antigens

As shown in Fig. 1 and Table 1, acidic isoferritins significantly decreased colony formation of BFU-E and CFU-GEMM pretreated with medium (treatment 1), complement (treatment 2), Tdr (treatment 4), and ³HTdr plus Tdr (treatment 6) but had no effect on BFU-E and CFU-GEMM surviving treatment with m-anti-Ia plus complement (treatment 3), ³HTdr (treatment 5), or sequential treatments with m-anti-Ia plus complement and ³HTdr (treatments 7 and 8).

In order to rule out the possibility that the results that had been described above were due to the number of cells plated, unseparated nucleated bone marrow cells were treated as in Fig. 1 and Table 1 (treatments 1, 2, 3, 5, 6) and plated at 4.0, 2.0, and 1.0 × 10⁴ cells/ml/plate in the absence or presence of 10⁻⁴M acidic isoferritins. As shown in Fig. 2A (BFU-E) and 2B (CFU-GEMM), the plating efficiencies for each group were similar regardless of the number of cells plated within this range. At each cell concentration, the percent decrease of colony formation by acidic isoferritins was similar to that of cells pretreated with m-anti-Ia (NEI-011) plus complement or with high specific activity ³HTdr (p < 0.005 in each case) and the acidic isoferritins had no influence on colony formation already reduced by pretreatment with m-anti-Ia or ³HTdr.

The low numbers of colonies inherent in the assay used to detect CFU-GEMM necessitated that the information in Tables 1 and 2 and in Fig. 2B be analyzed further. When analysis of variance was applied to these data, the treatment points divided into two groups substantiating that the ferritin was acting on CFU-GEMM expressing Ia-antigens during S-phase. There was no evidence of any significant differences among these cells treated with Iscove’s medium (–ferritin), complement (–ferritin), thymidine (–ferritin), or ³HTdr plus thymidine (–ferritin). Moreover, there was no evidence of any significant differences among the second group, which included Iscove’s medium (+ferritin), complement (+ferritin), α1a plus complement (– or + ferritin), ³HTdr (– or + ferritin), ³HTdr plus thymidine (+ferritin), ³HTdr then α1a plus complement or α1a plus complement then ³HTdr (– or + ferritin). There was approximately 9% variability in the numbers of colonies within each of these groups compared with an overall significant reduction of 45%–75% between these groups (p < 0.001).

### Table 2. Cycling Status of Human Bone Marrow BFU-E and CFU-GEMM*

<table>
<thead>
<tr>
<th>Plated in Presence of</th>
<th>Control (Iscove’s Medium)</th>
<th>³HTdr</th>
<th>³HTdr + Excess Tdr</th>
<th>Control (Iscove’s Medium)</th>
<th>³HTdr</th>
<th>³HTdr + Excess Tdr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Tdr</td>
<td>89 ± 4</td>
<td>41 ± 4 (–54)†</td>
<td>76 ± 6 (–15)</td>
<td>5.0 ± 0.4</td>
<td>1.4 ± 0.5 (–72)</td>
<td>4.4 ± 0.8 (–12)</td>
</tr>
<tr>
<td>10 µg Tdr</td>
<td>88 ± 5</td>
<td>33 ± 3 (–63)</td>
<td>83 ± 3 (–6)</td>
<td>4.0 ± 0.7</td>
<td>1.6 ± 0.5 (–60)</td>
<td>4.0 ± 1.0 (0)</td>
</tr>
<tr>
<td>40 µg Tdr</td>
<td>84 ± 7</td>
<td>33 ± 7 (–61)</td>
<td>82 ± 7 (–2)</td>
<td>4.0 ± 0.7</td>
<td>1.8 ± 0.4 (–55)</td>
<td>4.0 ± 0.6 (0)</td>
</tr>
</tbody>
</table>

*Human low density bone marrow cells (≤1.077 g/cm³) were exposed to Iscove’s medium, 50 µCi ³HTdr (20 Ci/mMole) or 50 µCi ³HTdr plus 500 µg Tdr for 20 min. The cells washed once in ice-cold Tdr (2000 µg/ml) and twice more in Iscove’s medium and plated as described for CFU-GEMM in the absence or presence of 10 or 40 µg Tdr.

†Percent change from control.

‡Significance, p value, or not significant (NS).

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In the absence of complement, a 1:50 dilution of m-anti-la (NE1-011) had no influence on colony formation but did block the inhibitory activity of $10^8M$ acidic isoferritins (BFU-E: control medium, $127 \pm 6$; ferritin, $71 \pm 5$; anti-la, $130 \pm 8$; anti-la plus ferritin, $122 \pm 5$; CFU-GEMM: control, $10 \pm 0.7$; ferritin, $5 \pm 0.7$; anti-la, $11 \pm 1$; anti-la plus ferritin, $10 \pm 1$). In this experiment, 6 plates were scored per treatment.

We have previously reported that BFU-E were not affected by crude preparations of LIA, yet in the present studies, crude and purified preparations of acidic isoferritins decreased colony formation of BFU-E scored in the same plates as for colony formation of CFU-GEMM. In order to investigate this apparent discrepancy, bone marrow cells from the same donors were treated with complement or m-anti-la (NE1-011) plus complement and cultured for BFU-E using the same source of stimulatory activity (PHA-LCM) as described for the CFU-GEMM assay or were cultured for BFU-E as described in our prior report, which did not include PHA-LCM in the culture medium. Untreated cells from these same donors were plated in the absence and presence of $10^8M$ CML spleen acidic isoferritins. As shown in Table 3, BFU-E cultured in the absence of PHA-LCM did not express Ia-antigenic determinants and were not suppressed by acidic isoferritins, whereas BFU-E cultured in the presence of PHA-LCM expressed Ia-antigens and were suppressed by acidic isoferritins. In the latter experiments (+ PHA-LCM) the results were similar whether erythropoietin was added at day 0 or after 4–5 days of culture (data not shown).

**DISCUSSION**

A role for Ia-antigens in the regulation of the proliferation of human hematopoietic cells has been postulated, but evidence for an association between Ia-antigens on target cells and the regulatory action of molecules in vitro has been confined to cells of the granulocyte-macrophage lineage. The present study has extended this information to the erythroid system (BFU-E) and to cells with multipotential differentiation capacity (CFU-GEMM) with some evidence for self-renewal in vitro. Heteroantiseria against Ia-antigens in the presence of complement have been shown to remove greater than 90% of human BFU-E, and similar results have been published recently for CFU-GEMM using a particular monoclonal anti-Ia antibody (Q5/13) that we have substantiated in this report. We chose, however, to study the effects of a monoclonal anti-la (NE1-011), which has already been shown to be associated with the action of acidic isoferritins and E-type prostaglandins against CFU-GM. In the present study, BFU-E and CFU-GEMM were shown to express Ia-antigens that were detected during S-phase by complement cytotoxicity. The acidic isoferritins acted only on cells in S-phase and did not act on those noncycling BFU-E and CFU-GEMM on which we could not detect Ia-

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**Table 3. Comparison of Colony Formation From BFU-E Cultured With and Without PHA-LCM: Influence of Anti-la + Complement and Acidic Isoferritins**

<table>
<thead>
<tr>
<th>Percent Change in Colony Formation*</th>
<th>Acidic Isoferritins</th>
</tr>
</thead>
<tbody>
<tr>
<td>-PHA-LCM</td>
<td>$-8 \pm 6$</td>
</tr>
<tr>
<td>+PHA-LCM</td>
<td>$-9 \pm 4$</td>
</tr>
<tr>
<td>Complement (NS)</td>
<td>(NS)</td>
</tr>
<tr>
<td>$-0.025$–$0.0005$</td>
<td>($&lt;0.005$–$0.0005$)</td>
</tr>
</tbody>
</table>

*Bone marrow cells were cultured for BFU-E without PHA-LCM or for BFU-E in the CFU-GEMM assay with PHA-LCM in 3 separate experiments. The control number of colonies were: − PHA-LCM $127 \pm 7$, $61 \pm 3$, $18 \pm 2$ and + PHA-LCM $92 \pm 3$, $31 \pm 4$, $14 \pm 1$.

†Levels of significance, p values or not significant (NS).
antigens using monoclonal anti-Ia- (NE1-011) plus complement. In addition, the action of these molecules was blocked by pretreatment of cells with anti-Ia in the absence of complement, suggesting that acidic isoferritin “receptors” may be in close proximity to Ia-antigenic determinants. Other evidence relating Ia-antigen-positive cells and interactions in vitro has been reported, as Ia-antigen-positive T cells exert a suppressive influence on BFU-E, 28 and acidic isoferritin inhibitory activity derives from an Ia-antigen-positive subpopulation of human monocytes. 29

The recognition by monoclonal antibodies of Ia-antigens detected during a certain phase of the cell cycle is not surprising, as monoclonal antibodies have been reported to recognize subpopulations as well as epitopic regions of human Ia-like molecules, 23, 30, 31 murine I-A and I-E/C region products have been shown to be independently regulated at various stages of the cell cycle of an established B-lymphoma cell line, 23 and some monoclonal antibodies recognize Ia-antigens present on murine T lymphocytes but not B lymphocytes. 32 Moreover, a low density appearance of Ia-antigens recognized by NE1-011 on portions of the cell cycle of BFU-E and CFU-GEMM other than S-phase has not been ruled out definitively. In fact, Ia expression on BFU-E has been shown to be heterogeneous using monoclonal antibodies to nonpolymorphic determinants and fluorescence-activated cell sorting. 10 If the Ia-antigens not detected by this treatment protocol are at a lower density on non-S-phase cells, it would suggest that antigenic density may be of relevance for this interaction.

Previous results by us had suggested that probable human pluripotent stem cell populations with extensive self-renewal capacity that are detected in continuous marrow culture were Ia-antigen-negative, 1 a finding similar to that for murine CFU-S 24 and consistent with the report that human cell line K562, with multipotentialities, was Ia-antigen-negative. 35 Detection of a subpopulation of Ia-antigen-positive CFU-GEMM does not necessarily conflict with these results, since the pluripotential stem cell compartment in mice is known to be composed of a heterogeneous group of cells differing in size, density, charge, volume, cell cycle status, and self-renewal capacity, 36 and the two assays may be detecting different cells within a hierarchy of stem cell differentiation, proliferation, and self-renewal capacity.

It had been reported by others that regenerating marrow contained rapidly proliferating CFU-GEMM, but that steady-state marrow contained CFU-GEMM that were in a slow or noncycling state. 37 The reasons for the differences between those studies 37 and ours are not known at present, but may relate to differences in culture conditions. BFU-E have also been reported to be in a slowly cycling state but differences in cycle status of BFU-E, which correlated to culture conditions, have been reported. 38

We reported previously that crude LIA had no influence on human BFU-E, 22 but the crude as well as the most purified samples of acidic isoferritins suppressed colony formation of the Ia-antigen-positive subpopulation of BFU-E. In this article, BFU-E cultured under the original conditions 22, 23 were Ia-antigen-negative and nonresponsive to acidic isoferritins, whereas the same bone marrow specimens cultured in the CFU-GEMM assay allowed detection of the Ia-antigen-positive subpopulation of BFU-E that was responsive to inhibition by acidic isoferritins. Detection of Ia-antigen-positive BFU-E may be due to the presence of PHA-LCM in the culture, since it has been reported that lymphokines can stabilize and/or allow reexpression of Ia-antigens on mouse macrophages. 39, 40

The effect of acidic isoferritins on stem cells and progenitor cells in vivo remains to be established, but the finding that acidic isoferritin inhibitory activity derives only from normal bone marrow and blood cells of the mononuclear phagocytic lineage 16 implicates mononuclear phagocytes as potential suppressor cells of stem cell proliferation and erythropoiesis, at least in vitro. Others have also implicated monocytes as suppressors of the colony formation in vitro of BFU-E. 42, 43

ACKNOWLEDGMENT

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Association of cell cycle expression of Ia-like antigenic determinations on normal human multipotential (CFU-GEMM) and erythroid (BFU-E) progenitor cells with regulation in vitro by acidic isoferritins

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