Expression of Ia-Antigens on Normal and Chronic Myeloid Leukemic Human Granulocyte-Macrophage Colony-Forming Cells (CFU-GM) is Associated with the Regulation of Cell Proliferation by Prostaglandin E

By Louis M. Pelus, Stephen Saletan, Richard T. Silver, and Malcolm A. S. Moore

The presence of Ia-antigens and their relationship to the inhibitory effect of prostaglandin E on the proliferation of human CFU-GM was studied in normals and patients with chronic myeloid leukemia. Consistent reduction of normal colony formation to approximately 50% of baseline levels was observed using a monoclonal anti-human Ia antibody in a complement-dependent cytoxicity assay titrated over serial dilutions. Elimination of the Ia-antigen-bearing CFU-GM population was associated with virtually a complete loss of responsiveness to the inhibitory effects of prostaglandin E. Maintenance of bone marrow cells in short-term suspension culture at 37°C prior to agar culture resulted in the loss of detectable Ia-antigen on the CFU-GM and, similarly, loss of response to prostaglandin. In contrast, most patients with chronic myeloid leukemia showed greatly reduced levels of Ia-antigens on their CFU-GM in fresh marrow together with lack of prostaglandin sensitivity, suggesting a correlation with the abnormal growth regulation observed in these patients. In two chronic myeloid leukemia patients, levels of Ia-antigen higher than that observed in the majority of patients could be detected and correlated with a residual response to prostaglandin E. These results suggest a relationship in normals between the expression of Ia-antigens on CFU-GM and the physiologic response to regulation by prostaglandin E, and a possible mechanism for the aberrant regulatory response in patients with chronic myeloid leukemia.

REGULATION of granulocyte-macrophage progenitor cell (CFU-GM) expansion involves a balance between responsiveness to specific humoral growth stimulatory glycoproteins, termed colony-stimulating factors (GM-CSF), and specific growth inhibitory molecules. Prostaglandins of the E series function as selective inhibitory regulators of normal CFU-GM proliferation, with a high degree of specificity for colony-forming cells committed to monocytoid differentiation. Acidic isofernitins also serve as regulators of normal CFU-GM proliferation, and like prostaglandin E, manifest an inhibitory effect directly on the proliferation of normal CFU-GM. Maintenance of bone marrow cells in short-term suspension culture at 37°C prior to agar culture resulted in the loss of detectable Ia-antigen on the CFU-GM and, similarly, loss of response to prostaglandin. In normals between the expression of Ia-antigens on CFU-GM and their subsequent disappearance during differentiation suggests a role for Ia-antigenic determinants in the regulation of stem cell proliferation by prostaglandin E. These results suggest a relationship in normals between the expression of Ia-antigens on CFU-GM and the physiologic response to regulation by prostaglandin E, and a possible mechanism for the aberrant regulatory response in patients with chronic myeloid leukemia.
MATERIALS AND METHODS

Patient Population

Bone marrow aspirates were obtained from 13 patients with chronic myeloid leukemia undergoing routine posterior iliac crest bone marrow aspiration for the assessment of clinical status and response to therapy at New York Hospital (12 patients) or Memorial Sloan-Kettering Cancer Center (1 patient). Table 1 lists the pertinent clinical and hematologic characteristics. All patients were in the chronic phase of CML (absence of fever and malaise; percentage of blasts and promyelocytes <10% in all marrow aspirates and <3% in all peripheral blood smears). Overall length of time in chronic phase (3.3 yr) exceeded slightly the reported mean percentage of blasts and promyelocytes <10% in all marrow aspirates.

Table 1. Clinical and Hematologic Characteristics of Patients With Chronic Myelogenous Leukemia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>49</td>
<td>17–71</td>
</tr>
<tr>
<td>Time since diagnosis (yr)</td>
<td>3.3</td>
<td>0.5–10</td>
</tr>
<tr>
<td>Percent Philadelphia-positive metaphases*</td>
<td>100%</td>
<td>(100%)</td>
</tr>
<tr>
<td>Leukocyte count (x 10^9/liter)</td>
<td>33.6</td>
<td>3.7–45.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42</td>
<td>33–47</td>
</tr>
<tr>
<td>Platelet count (x 10^9/liter)</td>
<td>314</td>
<td>106–624</td>
</tr>
</tbody>
</table>

Sex

- Male 10
- Female 3

Splenomegaly

- Present 4
- Absent 9

Therapy‡

- None 8
- Hydroxyurea 3
- Busulfan 1
- L-PAM 1

*More than 5 metaphases studied for 12/13 patients. All patients were documented Philadelphia-positive.

‡ Data for 12/13 patients. One patient had platelet counts < 1.5 x 10^9/ml.

Buoyant cell fraction, washed twice and resuspended in supplemented McCoy's 5A modified medium containing 10% heat inactivated fetal calf serum (HI-FCS) (Biofluids Inc., Rockville, Md.). Neutral density separation in Percoll at 1.074 g/ml routinely resulted in the recovery of buoyant mononuclear cells devoid of red cells and mature granulocytes with less than 10% contamination with morphologically recognizable earlier granulocytic cells (metamyelocytes and band forms). The dense cell fraction consisted of erythrocytes and mature granulocytoid cells, with less than 1% mononuclear cell contamination.

Assay for Granulocyte-Macrophage Colony Formation

The ability of normal and patient bone marrow cells to form colonies of granulocytes and macrophages was assayed in soft agar culture. One-hundred thousand low density mononuclear cells (<1.074 g/ml) were plated in a 1.0 ml layer of 0.3% agar (Difco Laboratories Inc., Detroit, Mich.) in supplemented McCoy's medium. Colony formation was stimulated by the inclusion of 10% conditioned medium (v/v) from the Sp-GCT human monocytic cell line (GIBCO, Grand Island, N.Y.). Prostaglandins were added directly to the culture dish prior to the addition of the bone marrow suspension. Indomethacin, at a final concentration of 10^-6 M, was routinely incorporated into all CFU-GM assays to prevent endogenous prostaglandin production. At this concentration, indomethacin inhibits greater than 90% of the prostaglandin production by normal mouse macrophages and murine and human leukemic cell lines and greater than 95% of the prostaglandin production by normal and myeloid leukemia and bone marrow cells (L.M. Pelus, unpublished). Cultures were incubated in a humidified 5% CO2 atmosphere, and after 7 days, colonies and clusters enumerated in quadruplicate plates.

Ia-Specific Antibodies

Monoclonal anti-human HLA-DR antibody (NEI-O11) (7s, IgG2) was purchased from New England Nuclear Corp., Boston, Mass. This monoclonal antibody has been purified by affinity chromatography with protein A and is >95% homogeneous. This antibody precipitates the Ia-antigen complex (consisting of two polypeptide chains of 28,000 and 33,000 daltons), fixes complement, and reacts with human B cells, B-cell lines, B-cell leukemias, certain normal myeloid cells, and some myeloid and null-cell leukemias, but not with T cells. Monoclonal anti-human HLA-DR (clone L243) (IgG2) was purchased from Becton-Dickinson, Sunnyvale, Calif. This antibody has been purified by ion-exchange chromatography, fixes complement, precipitates a nonpolymorphic HLA-DR antigen (28,000 and 34,000 daltons), and reacts with B cells, monocytes/macrophages, thymic epithelium, B-cell-dependent areas of spleen and lymph node, B-cell lymphomas, and activated T cells. Anti-human HLA-DR, OK1a, (IgG1) was purchased from Ortho Pharmaceuticals Corp., Raritan, N.J., as a lyophilized ascitic fluid. This antibody fixes complement and reacts with B cells, some monocytes/macrophages, 20% of null cells, and activated T cells. Anti-human HLA-DR monoclonal antibody (clone 7.2) was kindly provided by Drs. J. Hansen and B. Torok-Storb, Seattle, Wash. Anti-Ia clone 7.2 is identical to NEI-O11.

Complement-Dependent Cytotoxicity Assay

Dilutions of antibody were prepared in McCoy's medium supplemented with 10% HI-FCS. All cytotoxicity reactions were carried out at a standard concentration of 10^6 low density bone marrow cells/0.10 ml of antibody dilution or complement. Routinely, 5 x 10^6 cells were incubated with antibody for 30 min at 4°C, washed,
resuspended in an identical volume of a 1:8 dilution of rabbit complement (Low Tox-H rabbit complement, Accurate Chemical Co., Westbury, N.Y.), incubated for 45 min at 37°C, and subsequently washed 3 times. This dilution of complement was previously determined to provide maximal cytotoxic activity when used with this or other complement-fixing antibodies, while having no effect when used alone.

**Suspension Culture**

Low density (<1.074 g/ml) bone marrow cells were resuspended at a concentration of 3 x 10^6 cells in 1 ml of McCoy’s medium plus 10% FCS and replicate samples incubated at 4°C, 25°C (room temperature), and 37°C for 3, 6, or 24 hr. After incubation, the appropriate replicates were washed, treated with either complement or anti-Ia antibody (NEI-011) plus complement, and placed into agar culture with GM-CSF and in the absence or presence of prostaglandin E.

**Individual Colony Morphology**

Morphological examination of proliferating clones was performed in situ following fixation with 5% glutaraldehyde, methanol dehydration, and staining with Luxol’s Fast Blue and Harris’ hematoxylin as described.6,13

**Reagents**

Prostaglandin and indomethacin were purchased from Sigma Chemical Co., St. Louis, Mo. Stock solutions were prepared in absolute ethanol and stored at -70°C.

**Statistical Analysis**

Statistical evaluation was performed using the Student’s t distribution.

**RESULTS**

**Expression of Ia-Antigens on a Subpopulation of CFU-GM**

The responsiveness of granulocyte-macrophage colony-forming cells to inhibition by exogenously added prostaglandin E was analyzed before and after treatment with a monoclonal anti-Ia antibody (NEI-011) plus complement. In four experiments, addition of prostaglandin E to cultures of low density bone marrow cells resulted in the dose-dependent inhibition of colony formation over a concentration range of 10^-2M to 10^-10M (Fig. 1). In contrast, treatment of bone marrow cells with anti-Ia antibody plus complement prior to culture resulted in the cytolytic reduction of total CFU-GM, with the resulting colony-forming population being unresponsive to all but the highest prostaglandin E concentration tested. Complement alone was without effect on the observed prostaglandin dose-response curve in comparison to untreated cells. In addition, treatment with anti-Ia antibody alone did not block or alter the inhibitory effect of prostaglandin E.

Lack of responsiveness of CFU-GM after anti-Ia antibody (NEI-011) plus complement cytotoxicity was observed using antibody concentrations of 1/50 through 1/3000 (Table 2). The inability of prostaglandin E to inhibit the proliferation of CFU-GM surviving antibody plus complement treatment occurred equally using CFU-GM from individual bone marrow samples treated with a single concentration of anti-Ia antibody plus complement or CFU-GM from a single marrow sample tested in an antibody titration study (Table 2).

Throughout this dilution range, inhibition of CFU-GM proliferation of between 40% and 50% was routinely observed (mean 48% ± 4%, n = 38). Beyond a dilution of 1/3000, the detection of Ia-antigens by cytotoxic reduction decreased rapidly and was not observed beyond a 1/5000 anti-Ia antibody dilution. The observed inhibition of colony formation was independent of the concentration of GM-CSF (GCT conditioned medium) used to stimulate clonal growth over an eightfold dilution range (not shown). Likewise, the lack of responsiveness to inhibition by prostaglandin E of CFU-GM surviving the anti-Ia antibody plus complement cytotoxic assay was independent of optimal or suboptimal GM-CSF concentrations.

A reduction in all morphologically distinct colony and cluster types was observed following cytotoxic antibody treatment. In one experiment, the relative inhibition of morphologically identified monocyte-
Table 2. The Effects of Antibody Dilution on the Inhibition of Granulocyte-Macrophage Colony Formation and Responsiveness to Inhibition by Prostaglandin E

<table>
<thead>
<tr>
<th>Molar Prostaglandin E Concentration*</th>
<th>CFU-GM (Colonies and Clusters) per 10^5 Low Density Bone Marrow Cells</th>
<th>Anti-la (1:500) + C'</th>
<th>Anti-la (1:500) + C'</th>
<th>Anti-la (1:500) + C'</th>
</tr>
</thead>
<tbody>
<tr>
<td>A‡</td>
<td>0</td>
<td>281 ± 6§</td>
<td>146 ± 14 (48%)</td>
<td>399 ± 6</td>
</tr>
<tr>
<td></td>
<td>10^8 M</td>
<td>164 ± 10</td>
<td>147 ± 4</td>
<td>203 ± 15</td>
</tr>
<tr>
<td></td>
<td>10^8 M</td>
<td>196 ± 4</td>
<td>148 ± 5</td>
<td>240 ± 3</td>
</tr>
<tr>
<td>B†</td>
<td>0</td>
<td>319 ± 4</td>
<td>184 ± 5 (43%)</td>
<td>193 ± 5 (42%)</td>
</tr>
<tr>
<td></td>
<td>10^8 M</td>
<td>182 ± 8</td>
<td>176 ± 6</td>
<td>174 ± 8</td>
</tr>
</tbody>
</table>

*Prostaglandin was added at the initiation of agar culture.
‡Dilution of anti-la antibody (NEI-011) used in a complement-dependent cytotoxic assay.
§Data from three separate experiments using three different normal marrows each treated at a single antibody concentration.
¶Mean ± standard error of quadruplicate cultures.
*Percentage la-antigen positive CFU-GM.
†Data from one experiment.

macrophage, mixed monocytoid-neutrophilic, neutrophil and eosinophil colonies and/or clusters following treatment with la-antibody (NEI-011) plus complement was 83%, 59%, 21%, and 56%, respectively, of that observed with complement alone.

Analysis of CFU-GM la-Antigen Expression Using Several Anti-human HLA-DR Monoclonal Antibodies

The ability to detect a subpopulation of CFU-GM responsive to inhibition by prostaglandin E could be demonstrated using three different mouse anti-human HLA-DR monoclonal antibodies (Table 3). In each case, the proportion of CFU-GM expressing la-antigenic determinants, as recognized by these antibodies, was equivalent, and cytolysis of this colony-forming population with any of three anti-la antibodies plus complement resulted in the loss of CFU-GM inhibition due to exogenously added prostaglandin E. Treatment with either C' or anti-la antibody alone had no effect on the ability of prostaglandin E to inhibit CFU-GM proliferation.

Table 3. Effects of Various Monoclonal Anti-Human HLA-DR(la) Antibodies on CFU-GM Cloning Efficiency and Responsiveness to Prostaglandin E

| Bone Marrow Cell Treatment* | Monoclonal-Ab(dilution)‡ | C§ | Anti-la (1:500) + Media|| Anti-la (1:500) + PGE, (10^-8 M)||
|----------------------------|--------------------------|---------|-----------------|-----------------|-----------------|
| None                       |                          | 439 ± 13† | 267 ± 14       | 280 ± 10       | 252 ± 15       |
| None                       | +                        | 452 ± 10 | 280 ± 10       | 252 ± 15       | 101 ± 3        |
| Anti-human HLA-DR(la)      |                          | 440 ± 19 | 264 ± 12       | 252 ± 15       | 101 ± 3        |
| Anti-human HLA-DR(la)      |                          | 290 ± 5 (36%)** | 266 ± 16 | 252 ± 15       | 101 ± 3        |
| NEI-O11(clone 7.2) (1:200) |                          | 290 ± 5 (36%)** | 266 ± 16 | 252 ± 15       | 101 ± 3        |
| Anti-human HLA-DR(la)      |                          | 447 ± 25 | 308 ± 9        | 252 ± 15       | 101 ± 3        |
| OK-la, (1:200)             | +                        | 247 ± 18 (39%) | 252 ± 15 | 252 ± 15       | 101 ± 3        |
| None                       |                          | 204 ± 3  | 101 ± 3        | 252 ± 15       | 101 ± 3        |
| None                       | +                        | 193 ± 5  | 108 ± 1        | 252 ± 15       | 101 ± 3        |
| Anti-human HLA-DR(la)      |                          | 206 ± 2 (46%) | 105 ± 1 | 252 ± 15       | 101 ± 3        |
| Anti-human HLA-DR(la)      |                          | 212 ± 4  | 108 ± 2        | 252 ± 15       | 101 ± 3        |
| BD-clone L-243 (1:200)     | +                        | 107 ± 1 (46%) | 106 ± 4 | 252 ± 15       | 101 ± 3        |
| Anti-human HLA-DR(la)      |                          | 183 ± 5  | 102 ± 2        | 252 ± 15       | 101 ± 3        |
| Clone 7.2 (1:200)          | +                        | 103 ± 4 (47%) | 102 ± 4 | 252 ± 15       | 101 ± 3        |

*Bone marrow cells were treated prior to culture in semisolid agar.
†CFU-GM per 10^5 low density (< 1.074 g/ml) human bone marrow cells.
‡Final antibody dilution used. Maximal activity was determined from prior dose–response analysis.
§Rabbit low toxicity complement was used at a 1:8 dilution of lyophilized serum reconstituted in distilled water.
||Added to agar cultures prior to addition of bone marrow cells. All cultures were stimulated by 10% (v/v) GCT-conditioned medium as a source of granulocyte-macrophage-CSF.
†Mean ± standard error of quadruplicate cultures.
**Percentage la-antigen-positive colony-forming cells.
Loss or Absence of Ia-Antigens on CFU-GM is Associated With Lack of Sensitivity to Inhibition

Based on reported evidence that the expression of Ia-antigens on mature cells is transient and lost in culture over time,26–28 we investigated the expression of Ia-antigens on CFU-GM as a function of time in culture at different temperatures and the subsequent responsiveness of CFU-GM to inhibition by prostaglandin E. Maintenance of granulocyte-macrophage colony-forming cells at 4°C, regardless of time in suspension, had no effect on the subsequent detection of Ia-antigens or responsiveness to prostaglandin E. Identical results were observed with bone marrow cells maintained in suspension culture at room temperature (not shown).

In contrast, when incubated at 37°C, a small decline in the number of Ia-antigen-positive CFU-GM was observed in as little as 3 hr and was associated with a similar reduction in prostaglandin responsiveness.

After 6 hr in culture, no significant presence of Ia-antigen could be detected by cytotoxicity. The loss or metabolism of Ia-antigens at 37°C directly correlated with loss of responsiveness to prostaglandin-E-mediated inhibition. This loss of detectable Ia-antigen as well as the decreased prostaglandin sensitivity of CFU-GM maintained at 37°C did not result from a nonselective loss of cells during the culture period, since the cloning efficiency based on the original number of cells placed into suspension culture was consistent at all time points tested. Identical results were observed regardless of the inclusion of exogenous GM-CSF in the suspension phase.

Altered Expression of Ia-Antigens on Granulocyte-Macrophage Colony-Forming Cells From Patients With Chronic Myeloid Leukemia

Granulocyte-macrophage colony-forming cells from patients with chronic myeloid leukemia are hyporesponsive to inhibition by prostaglandin E.13–15 We therefore investigated the relationship between expression of Ia-antigens on leukemic CFU-GM and their responsiveness to prostaglandin E. Throughout the anti-Ia antibody dilution range of 1/100–1/1000, a consistent percentage (46%–52%) of normal CFU-GM were sensitive to antibody plus complement-dependent cytolyis (Fig. 2). By contrast, at all anti-Ia antibody concentrations tested, CFU-GM from patients with CML were significantly less sensitive to

<table>
<thead>
<tr>
<th>Time</th>
<th>Temperature</th>
<th>Addition*</th>
<th>CFU-GM/10^6 Bone Marrow Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Complement (C)</td>
<td>Colony</td>
</tr>
<tr>
<td>3 hr</td>
<td>4°C</td>
<td>Media</td>
<td>10^-8 MPGE, 10^-6 MPGE</td>
</tr>
<tr>
<td></td>
<td>37°C</td>
<td>Media</td>
<td>10^-8 MPGE, 10^-6 MPGE</td>
</tr>
<tr>
<td>6 hr</td>
<td>4°C</td>
<td>Media</td>
<td>10^-8 MPGE, 10^-6 MPGE</td>
</tr>
<tr>
<td></td>
<td>37°C</td>
<td>Media</td>
<td>10^-8 MPGE, 10^-6 MPGE</td>
</tr>
<tr>
<td>24 hr</td>
<td>4°C</td>
<td>Media</td>
<td>10^-8 MPGE, 10^-6 MPGE</td>
</tr>
<tr>
<td></td>
<td>37°C</td>
<td>Media</td>
<td>10^-8 MPGE, 10^-6 MPGE</td>
</tr>
</tbody>
</table>

*Prostaglandin added at the initiation of agar culture.
†Bone marrow cells were treated with anti-la antibody (NEI-O11) plus C' following suspension culture.
§Mean ± standard error of quadruplicate cultures.
$Percentage la-antigen-positive CFU-GM.
cytolytic reduction, though considerable heterogeneity was observed. At the highest anti-Ia antibody concentration employed (1/100), little or no reduction in CFU-GM proliferation was observed in 8 of 13 patients, while the percentage of Ia-antigen-positive CFU-GM in 5 patients were within the lower range of normal. However, as the concentration of antibody was reduced, the number of detectable Ia-antigen-positive CFU-GM in these patients decreased rapidly, suggesting a lower surface density distribution of Ia-antigens on these cells. In two patients, K.P. and C.R. (identified in Fig. 2 as □ and ○, respectively), the ability to reduce CFU-GM proliferation by anti-Ia antibody plus complement treatment was still evident at an antibody dilution of 1/500.

Analysis of the prostaglandin responsiveness of CML CFU-GM indicated that in 11 of 13 patients in whom no Ia-antigen could be detected in the antibody plus complement-dependent cytotoxicity assay, or in whom the ability to detect Ia-antigens fell rapidly with antibody dilution, the responsiveness of CFU-GM to inhibition by prostaglandin E was likewise absent or greatly reduced (Table 5). Furthermore, the small degree of prostaglandin sensitivity observed in these patients was still evident after anti-Ia antibody plus complement treatment, indicating a probable pharmacologic effect on Ia-antigen-negative CFU-GM. In the two patients, K.P. and C.R., in whom the ability to detect Ia-antigen-positive CFU-GM by antibody plus complement cytolysis persisted over an extended titration range, considerable prostaglandin responsiveness could be observed albeit still abnormal in comparison to normal CFU-GM (Fig. 1). Of interest is the fact that in these two patients, as with normal CFU-GM,

---

**Table 5. Prostaglandin Responsiveness and Ia Expression in Patients With Chronic Myeloid Leukemia**

<table>
<thead>
<tr>
<th>Prostaglandin E Concentration</th>
<th>Molar</th>
<th>CML (n = 11)</th>
<th>K.P. (□)</th>
<th>C.R. (○)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C'</td>
<td>C'</td>
<td>C'</td>
</tr>
<tr>
<td>10^-8 M</td>
<td>16 ± 4</td>
<td>12 ± 4 (14 ± 5%)</td>
<td>54 ± 5</td>
<td>0 (46%)</td>
</tr>
<tr>
<td>10^-7 M</td>
<td>3 ± 2</td>
<td>1 ± 2</td>
<td>34 ± 1</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>10^-6 M</td>
<td>0 ± 3</td>
<td>0 ± 1</td>
<td>16 ± 4</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>10^-5 M</td>
<td></td>
<td></td>
<td>10 ± 4</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>10^-4 M</td>
<td></td>
<td></td>
<td>2 ± 3</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>10^-3 M</td>
<td></td>
<td></td>
<td>0 ± 1</td>
<td>0 ± 1</td>
</tr>
</tbody>
</table>

*Inhibition of CFU-GM (colonies and clusters) per 10^6 low density (<1.074 g/ml) bone marrow cells.
†The prostaglandin responsiveness of this patient was analyzed on three separate occasions over an 18-mo period. Quantitation of the number of Ia-antigen positive CFU-GM was performed only once.
‡The presence of Ia-antigens and prostaglandin responsiveness in this patient was analyzed on two separate occasions during a 6-mo period.
§Anti-Ia antibody (NEI-O11) was used at a 1/100 dilution.
‖Percentage of Ia-antigen-positive CFU-GM detected.
prostaglandin responsiveness was not observed in the population of colony-forming cells that survived treatment with anti-Ia antibody plus complement.

DISCUSSION

The expression of Ia-antigenic determinants on normal myeloid progenitor cells early during differentiation and their subsequent loss with progressive maturation suggests a relationship between Ia-antigens and the control of proliferative events that occur early during hematopoietic differentiation. The detection of Ia-antigens on leukemic cells which reflects a derivation from Ia-antigen-bearing progenitor cells, similarly indicates that these antigens could be markers for stages of hematopoietic cell differentiation and suggests a putative role in cellular proliferation. Our results extend this concept and indicate that a subpopulation of normal CFU-GM expresses an Ia-antigen that appears to be a cellular marker associated with responsiveness to growth regulation by prostaglandin E in vitro. The loss or acquired inability to detect this antigenic determinant on normal CFU-GM during liquid culture is directly associated with an equivalent loss of sensitivity to prostaglandin E. In patients with chronic myeloid leukemia (CML), Ia-antigens are absent or expressed to a lesser degree on CFU-GM and may be related to the abnormal response of the leukemic colony-forming cells to inhibition by prostaglandin E.

The present communication describes the detection by monoclonal antibodies of Ia-antigenic determinants (or epitopic regions) expressed on a subpopulation of committed myeloid progenitor cells, which is associated with the regulation of their clonal proliferation in vitro. These studies contrast, however, with several reports using both rabbit anti-human-Ia heteroantisera and monoclonal antibodies, in which total inhibition of CFU-GM proliferation was observed following antibody plus complement treatment. Analysis of Ia-antigen expression on normal CFU-GM by fluorescence-activated cell sorting has indicated heterogeneity of expression. However, this could relate to the lack of sensitivity of cell sorting in comparison to cytotoxic reduction. Alternate views recognize heterogeneity of Ia-antigen representation related either to the expression of multiple Ia-antigens, as observed in the murine system, to the recognition of different epitopic regions on the Ia molecule, or to changes in Ia-antigen expression governed by events linked to the DNA synthetic cell cycle. Evidence for these alternatives is provided by recent studies that define monoclonal anti-Ia antibodies that recognize subpopulations of human Ia molecules and distinct epitopic regions expressed on subpopulations of human Ia-like antigens. In addition, both human and murine studies demonstrate the differential expression of Ia-antigens relative to changes in the DNA synthetic cell cycle. Thus, the inability to detect the expression of HLA-DR antigens on all normal human CFU-GM by cytotoxic treatment with monoclonal antibodies may relate to any of these possibilities. The significance of the differential expression of Ia-antigens as detected with three monoclonal mouse anti-human HLA-DR antibodies, however, is the direct association with the functional regulation of granulocyte-macrophage progenitor cell proliferation by prostaglandin E.

The nature of the defect(s) in growth and proliferation that underlies the leukemic process is as yet unclear. Certain observations—the retention of dependence on granulocyte-macrophage colony-stimulating factors for growth in soft agar culture and the ability to induce differentiation in response to various agents in vitro—suggest normal regulatory mechanisms may continue to operate. Other studies indicate gross abnormalities in the response of leukemic progenitor cells to the physiologic regulation seen in normals, of which the insensitivity of CML CFU-GM to inhibition by prostaglandin E is an example. This effect is consistently observed despite the presence of adequate numbers of morphologically recognizable target monocytoid colonies and clusters. In this study, the absence or decreased expression of Ia-antigens on CML CFU-GM coincides with the abnormal regulatory response to prostaglandin E. Although the mechanism by which Ia-antigen expression and regulation by prostaglandin E are linked is unclear, the evidence in normals suggests that the expression of Ia-antigens is associated with the inhibitory effect of prostaglandin E. It is tempting to conclude, therefore, that the constitutive absence or diminution of Ia-antigen expression on CFU-GM is an important factor related to the abnormal response of CML. Of approximately 25 patients with CML studied thus far, only two have shown a residual, though subnormal, response to prostaglandin E. The striking observation is that these are the two patients with levels of Ia-antigen on their CFU-GM clearly higher than all the other leukemic patients assayed. In addition, since one of these patients (C.R.) was off therapy while the other (K.P.) was on maintenance therapy at the time of the study, no correlation to therapy status is evident.

The presence of specific membrane prostaglandin receptors has been a constant characteristic of all cells responsive to their effects. The inability of anti-Ia antibody to block the inhibitory effects of prostaglandin E in the absence of complement suggests that the
receptor for prostaglandin E on CFU-GM is distinct from surface Ia-antigenic determinants. However, the expression of Ia-antigens only on those CFU-GM sensitive to prostaglandin inhibition and the loss of responsiveness simultaneous with the loss of detection of Ia-antigens supports a close—perhaps regulatory—association between Ia-antigen expression and prostaglandin responsiveness.

The active turnover of plasma membrane antigens on nucleated cells occurs both in vivo and in vitro. Thus, the shedding of Ia-antigens and prostaglandin receptors may underlie the loss of cell responsiveness. Association between Ia-antigen expression and prostaglandin responsiveness observed with time in culture at 37°C and the lack of expression of Ia-antigens and insensitivity to regulation of some CFU-GM. Perhaps the lack or diminution of detectable Ia-antigens on the surface of CML CFU-GM may relate to abnormalities or changes in the turnover of membrane determinants.

At present, the inability to obtain sufficiently pure CFU-GM populations precludes the direct study of antigen/receptor synthesis and turnover; however, methods for modulating receptor expression may provide insight into the control of progenitor cell proliferation. Murine studies indicate that lymphokine preparations increase Ia-antigen expression on peritoneal macrophages in vitro and immunogenic stimuli induce Ia-antigen-rich peritoneal exudates in vivo and augment and stabilize Ia-antigen expression in vitro. It will be of interest to apply similar methods to the study of myeloid progenitor cells in order to maintain Ia-antigen expression and thus retain the prostaglandin E response in culture. The reexpression of Ia-antigens and reinduction of prostaglandin sensitivity on CFU-GM that have lost these characteristics in culture, on new populations of colony-forming cells, and on populations of CFU-GM not previously expressing Ia-antigens, may serve to further elucidate the nature of regulation of normal myelopoiesis and possibly to modify disordered leukemic regulation.

ACKNOWLEDGMENT

The expert technical assistance of K. Mallory and administrative efficiency of R. Lemon are gratefully acknowledged. We thank C. Beeghly for typing this manuscript.

REFERENCES

22. Schlossman SF, Chess L, Humphreys RE, Strominger JL:
Distribution of Ia-like molecules on the surface of normal and leukemic human cells. Proc Natl Acad Sci USA 73:1288, 1976


Expression of la-antigens on normal and chronic myeloid leukemic human granulocyte-macrophage colony-forming cells (CFU-GM) is associated with the regulation of cell proliferation by prostaglandin E

LM Pelus, S Saletan, RT Silver and MA Moore