Expression of Ia Antigens on Myeloid Progenitor Cells in Chronic Myeloid Leukemia: Direct Analysis Using Partially Purified Colony-Forming Cells

By Stephen A. Cannistra, John F. Daley, Peter Larcom, and James D. Griffin

The regulation of Ia (HLA-DR) antigen expression on myeloid progenitor cells may be closely related to the control of myelopoiesis in both normal individuals and chronic myeloid leukemia (CML) patients. In an effort to study directly the expression and behavior of Ia surface molecules on myeloid progenitor cells, we used an immunologic purification technique to enrich these cells approximately 100-fold from the peripheral blood of CML patients.

The majority of cells in this blast population expressed HLA-DR antigens. Thirty percent to 40% of cells could form a granulocyte or monocyte colony in agar, and these cells tended to express the highest levels of HLA-DR. The number of HLA-DR molecules per cell increased about twofold as the cells traversed the cell cycle from G0/G1 to G2/M. This was true for unstimulated cells or cells exposed to colony-stimulating factors. Some of this increase was related to a corresponding increase in cell size and is also seen with other cell surface antigens such as β-2-microglobulin. Ia antigen expression was not modified by culture with colony-stimulating factors, fetal calf serum, or serum-free, prostaglandin-free medium for periods of up to 24 hours. These results demonstrate that Ia antigens are expressed on the myeloid progenitor cells of CML.

In chronic myeloid leukemia (CML), CFU-GMs also express Ia antigens, although some studies have suggested that the fraction of CML CFU-GMs lysed by treatment with anti-Ia antibody and complement is lower than in normal bone marrow. In both normal and CML CFU-GMs, expression of Ia antigens has been associated with responsiveness to growth inhibition by prostaglandin E1 (PGE1). Furthermore, Ia antigen expression has been induced in Ia- cells by culture with PGE1 for 24 hours, and this is associated with reestablishment of sensitivity to the inhibitory effects of prostaglandin E1. Thus, the expression of Ia antigens has been closely linked to the regulation of proliferation of myeloid progenitor cells.

A direct study of progenitor cell Ia antigens, however, has not been possible because of a lack of purified populations of these uncommon cells. Past investigators using complement lysis have found that Ia antigens expressed in low density, not permitting quantitation of Ia antigen density, not taking into account possible differences in susceptibility to complement lysis by different cell populations, and not taking advantage of the numerous anti-Ia monoclonal antibodies currently available which are not lytic with rabbit complement. For example, the fact that some investigators using complement lysis have found that...
only 50% of CFU-GMs are I\(\alpha^+\), whereas other investigators using immunologic separation techniques have found that most CFU-GMs are I\(\alpha^+\) may be partly due to insensitivity of the complement lysis assay for antigens expressed in low density.

We have previously described an immunologic technique to purify myeloid progenitor cells (MPCs) 80- to 120-fold from the peripheral blood of stable phase CML patients. In view of the potential regulatory function of I\(\alpha\) surface molecules in hematopoiesis, we have investigated the expression of HLA-DR antigens on CML CFU-GMs in an attempt to better define some of the biologic properties of these molecules on MPCs. Using highly purified populations of CML MPCs, we have directly measured the expression of selected determinants of the DR system, investigated the relationship of DR expression to cell cycle phase, and evaluated the ability of several in vitro treatments to modulate DR expression.

**MATERIALS AND METHODS**

### Source of MPCs

Peripheral blood samples (5 to 10 mL) were obtained from stable phase CML patients undergoing initial diagnostic testing. After collection into sterile heparinized syringes, the mononuclear cell fraction was separated by Ficoll-Hypaque diatrizoate gradient sedimentation, washed twice in minimal essential media (MEM; Gibco, Grand Island, NY) containing 2.5% pooled human AB serum (MEM-AB wash), and cryopreserved in the vapor phase of liquid nitrogen (100 μg/mL, to minimize cell agglutination).

### Preparation of Monocytes

Peripheral blood samples (60 mL) from normal volunteers were collected in sterile heparinized syringes, and the mononuclear cell fraction was separated by Ficoll-diatrizoate gradient sedimentation. The mononuclear cells were suspended in MEM-AB wash at a concentration of 1 x 10⁶ cells/mL, and 10-μL aliquots were placed in plastic culture dishes (Falcon Plastics, Oxnard, Calif) for 60 minutes at 37 °C for adherence. After incubation, the nonadherent lymphoid cells were removed with three wash steps using MEM-AB wash at room temperature. Adherent monocytes were harvested by gentle scraping in 10 mL MEM-AB wash at 4 °C. The resultant cell population was typically 95% monocytes, 5% lymphocytes on Wright-Giemsa stain.

### Monoclonal Antibodies

Monoclonal antibodies used in the purification of colony-forming cells have been previously characterized and include anti-MY8 (pan-myeloid), anti-Mo1 (pan-myeloid and NK cell), anti-Mo2 (monocyte), anti-B1 (B cell), anti-T11 (T cell), and 31C6 (basophil and NK cells) (T. Hercend and J. Griffin, Dana-Farber Cancer Institute, unpublished observations, 1983). Anti-I\(\alpha\) monoclonal antibodies used in phenotyping and quantification studies were 9-49 (IgM, monomorphic DR determinant), 12 (IgG2a, monomorphic DR determinant), 7.2 (IgG2b, monomorphic DR determinant), and NEN-011 (IgG2b, monomorphic DR determinant; New England Nuclear, Boston). Antibody 12 was kindly provided by Dr Lee Nadler (Dana-Farber Cancer Institute), antibody 9-49 by Dr Robert F. Todd (Dana-Farber Cancer Institute), and antibody 7.2 by Dr Gerald T. Nepom (Genetic Systems Corp, Seattle).

### Partial Purification of Myeloid Progenitor Cells

The method of myeloblast purification from the peripheral blood of CML patients has been previously reported. Briefly, 1 x 10⁶ peripheral blood mononuclear cells were incubated with anti-MY8, anti-Mo1, anti-Mo2, anti-B1, anti-T11, and 31C6 for 30 minutes at 4 °C. After two wash steps, affinity-purified rabbit anti-mouse immunoglobulin coupled to sheep erythrocytes by chromic chloride was added (0.7 mL of 10% vol/vol erythrocytes in MEM-AB wash per 10⁶ cells). The erythrocyte-mononuclear cell mixture was pelleted (800 g, ten minutes) and incubated at 4 °C for 20 minutes for rosette formation. The rosetted cells were then separated from the nonrosetted cells (containing the progenitor cells) by density gradient sedimentation. Interface cells (unrosetted) were washed twice in MEM-AB. A cytocentrifuge preparation was examined to determine purity. This technique typically resulted in a preparation of 85% to 95% blast forms (80- to 120-fold enrichment).

### CFU-GM Assay

Cells were plated at 0.5 to 1 x 10⁶ cells per culture in Iscove's modified Dulbecco's MEM, 20% FCS, 0.3% agar over an underlayer of 0.5% agar with 20% GCT (Gibco). After 1, 3, 7, and 14 days of culture (37 °C, 5% CO₂), colonies (>40 cells), clusters (8 to 40 cells), and doublets were counted as previously described. Cloning efficiency was calculated as total number of colonies, clusters, and doublets divided by the initial number of cells plated x 100%.

### Immunofluorescence Analysis of HLA-DR

HLA-DR expression was measured by an indirect immunofluorescence assay. Briefly, 2 x 10⁶ cells were washed twice in MEM-AB wash, and the pellet was then suspended in 100 μL of diluted antibody. Ascites was generally diluted 1:250. NEN-011 antibody was used at a dilution of 1:50, since this concentration was found to be saturating. After incubation for 30 minutes at 4 °C, the cells were washed twice and stained with fluorescein-conjugated goat anti-mouse Ig (Tago, Burlington, Calif) for 30 minutes at 4 °C. After two additional wash steps, 10,000 cells per sample were analyzed by flow microfluorometry (fluorescence-activated cell sorter, FACS-I, Becton Dickinson, Oxnard, Calif). Background fluorescence was measured using isotype-identical control monoclonal antibodies and was subtracted in each case.

### Complement-Dependent Cytotoxicity Assay

HLA-DR expression on CML CFU-GMs was also assessed by complement-lysis assay. For this procedure, 2 x 10⁶ partially purified MPCs were resuspended in 100 μL of diluted antibody for 30 minutes at 4 °C. The cells were washed once in MEM-AB and resuspended in 100 μL of newborn rabbit complement (Pel-Freeze, Rogers, Ark) at a 1:6 dilution for 90 minutes at 37 °C. This complement dilution was previously determined to be nontoxic when incubated with CML MPCs in the absence of lytic antibody. After complement incubation, the cells were washed once in MEM-AB and plated in a standard CFU-GM assay as described.

### Anti-I\(\alpha\) Binding Site Quantitation

An estimate of anti-I\(\alpha\) binding sites on MPCs and monocytes was performed by comparing the peak fluorescence intensity of each
anti-la antibody with that of a known monoclonal antibody for which the exact number of binding sites per cell has been previously determined. Fluorescence intensity measured by flow microfluorometry is proportional to the number of fluorescein molecules bound per cell. Using a linear amplifier, fluorescence intensity is linearly related to channel number on the flow cytometer. A calibration curve relating linear and logarithmic fluorescence on the EPICS V cell sorter (Coulter Electronics, Hialeah, Fla) was used in this study and has been previously described. The EPICS V was calibrated so that peak fluorescence of peripheral blood lymphocytes stained with anti-T8 (an IgG1 known to bind to approximately 60,000 sites per cell) was at channel 140. An estimation of the number of binding sites per cell for anti-la antibodies I2 (IgG2a), 9-49 (IgM), and 7.2 (IgG2b) was then obtained by comparing relative fluorescence of anti-la stained MPCs with anti-T8 stained lymphocytes. This is an approximation because of possible variations in the number of fluorescein molecules that would bind to monoclonals of different immunoglobulin subclasses. However, this technique can be used to compare accurately the relative number of binding sites per cell when different cell preparations are stained with the same antibody.

Cell Cycle Analysis

Progenitor cells were prepared as described and stained with anti-la (I2, monomorphic DR), followed by fluorescein-conjugated goat anti-mouse Ig. After two washes in phosphate-buffered saline (PBS), the cells were resuspended in 1 mL of 1% formalin for fixation (24 hours). Propidium iodide staining was performed by an adaptation of the method of Crissman et al. Cells (10^6) were washed twice in PBS (to remove formalin) and resuspended in 1 mL PBS. Then 100 µL of RNase (1 mg/mL in dH2O; Sigma Chemical Co, St Louis) was added, and the cells were incubated at 37 °C for 30 minutes. After incubation, 0.5 mL of propidium iodide (1 mg in 20 mL of 1.12% sodium citrate, Sigma) was added, and the cells were incubated at 4 °C for 30 minutes. The cells were analyzed on an EPICS V cell sorter (Coulter), and analysis of DNA histograms was performed using the PARA-1 computer program (Coulter). Forward-angle light scatter, la fluorescence (green), and DNA fluorescence (red) were simultaneously recorded on 10,000 cells per sample. In some experiments, MPCs were incubated with CSF-containing medium (5% GCT) for 12 hours to increase the fraction of cells in S phase.

Suspension Culture of MPCs

Previous reports have indicated the possibility that DR antigen expression diminishes within three to 24 hours of in vitro culture. This was investigated by incubating 10^6 purified CML progenitor cells in 1 mL of RPMI 1640 supplemented with 10% FCS (Gibco) at either 4 °C or 37 °C for 6 or 18 hours, with or without the addition of 20% GCT medium (Gibco) as a source of colony-stimulating factors (CSF). For five patients, the suspension culture were performed in 12 x 75-mm sterile plastic test tubes (Falcon). An additional three experiments were performed by incubating 10^6 cells in 1 mL of the same medium in flat-bottom plastic tissue-culture plates (24-well Linbro plates, 2.0 cm^2 area per well; Flow Laboratories, McLean, Va) for 18 hours. After incubation, the cells were washed twice in MEM-AB wash and stained with either control antibody or anti-la (I2). In order to eliminate the possible effects of prostaglandins (endogenous or in the culture medium), indomethacin (Sigma) at a concentration of 10^-4 mol/L was added to the suspension cultures. In some experiments, cells were incubated in a serum-free medium (Iscove's modified DMEM containing 5 µg/mL insulin, 5 µg/mL transferrin, and 5 ng/mL selenium; Collaborative Research, Waltham, Mass) for 24 hours in order to investigate the possibility that small amounts of colony-stimulating factors or prostaglandins were included in the FCS used for most cultures. In some experiments, FCS (20%) or PGE1 (10^-7 mol/L; Sigma) were added to the serum-free culture medium.

RESULTS

Progenitor Cell Purification

The partial purification of CML MPCs resulted in a preparation of 85% to 95% blast cells, as shown in Fig 1. The remaining cells (5% to 15%) were more mature granulated cells of the myeloid series (usually small numbers of basophils, promyelocytes, and myelocytes). Reanalysis of the purified progenitor cells by indirect immunofluorescence using anti-Mo2, anti-B1, and anti-T11 consistently revealed less than 1% of cells positive for each antibody tested, indicating less than 1% "contamination" by monocytes, B cells, and mature T lymphocytes. As previously described, although these cell preparations are morphologically homogeneous, only 20% to 50% of cells proliferate in agar to form a colony or cluster of mature myeloid cells at day 7. An additional subpopulation of cells will form a cluster at two to five days that has lysed by day 7. Also, 6% to 10% of cells will form an erythroid colony.

Expression of HLA-DR Antigens on CML Progenitor Cells

When analyzed by indirect immunofluorescence, a mean of 72% of cells in the MPC preparation expressed monomorphic DR determinants (range, 55%
Table 1. HLA-DR Expression on Myeloid Progenitor Cells and Monocytes

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Cell Type</th>
<th>9-49</th>
<th>l2</th>
<th>7.2</th>
</tr>
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<tbody>
<tr>
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<td>MPC*</td>
<td>68</td>
<td>55</td>
<td>58</td>
</tr>
<tr>
<td>2</td>
<td>MPC</td>
<td>71</td>
<td>64</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>MPC</td>
<td>75</td>
<td>76</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>MPC</td>
<td>75</td>
<td>NT</td>
<td>73</td>
</tr>
<tr>
<td>5</td>
<td>MPC</td>
<td>84</td>
<td>80</td>
<td>82</td>
</tr>
<tr>
<td>6</td>
<td>MPC</td>
<td>75</td>
<td>66</td>
<td>70</td>
</tr>
<tr>
<td>7</td>
<td>MPC</td>
<td>NT</td>
<td>84</td>
<td>NT</td>
</tr>
<tr>
<td>8</td>
<td>MONO†</td>
<td>79</td>
<td>77</td>
<td>77</td>
</tr>
<tr>
<td>9</td>
<td>MONO</td>
<td>70</td>
<td>71</td>
<td>66</td>
</tr>
<tr>
<td>10</td>
<td>MONO</td>
<td>74</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>11</td>
<td>MONO</td>
<td>74</td>
<td>76</td>
<td>77</td>
</tr>
<tr>
<td>12</td>
<td>MONO</td>
<td>88</td>
<td>51</td>
<td>85</td>
</tr>
<tr>
<td>13</td>
<td>MONO</td>
<td>NT</td>
<td>73</td>
<td>NT</td>
</tr>
<tr>
<td>14</td>
<td>MONO</td>
<td>NT</td>
<td>89</td>
<td>NT</td>
</tr>
</tbody>
</table>

MPC, myeloid progenitor cell; NT, not tested; MONO, monocytes.

* MPCs were purified as described in text, using peripheral blood of stable phase CML patients.
† Percentage of positivity (after subtracting background fluorescence) was determined by indirect immunofluorescence using flow cytometry. Anti-HLA-DR monoclonal antibodies used were 9-49, l2, and 7.2.
‡ Monocytes were from normal volunteers.

The results of seven patients with CML evaluated with anti-DR antibodies 9-49, l2, and 7.2 are shown in Table 1. For comparison purposes, results from simultaneous analysis of normal monocytes from seven individuals are also shown. Representative FACS cytograms for both MPCs and normal monocytes are shown in Fig 2. Although the majority of CML CFU-GMs are heterogeneous with respect to Ia antigen fluorescence intensity, there did not appear to be a discrete population of Ia+ cells (Fig 2). The fact that the “percentage” of Ia cells is less than 100% is partly owing to overlap with background fluorescence.

In order to assess the relationship between DR antigen expression and myeloid colony-forming ability, MPCs were separated into a cell fraction with high DR antigen density (40% of DR+ cells with the brightest fluorescence) and a population with low DR density (40% of DR+ cells with the dimmest fluorescence) by fluorescence-activated cell sorting (Fig 3), and the cloning efficiency of each population was determined on days 1 (doublets), 3 (clusters), 7 (clusters and colonies), and 14 (colonies) in a standard agar system.

One of three representative experiments is shown in Fig 4, with cloning efficiency on days 1, 3, 7, and 14 being 35%, 34%, 38%, and 20%, respectively, for the most fluorescent (“brightest”) HLA-DR group. Lower cloning efficiencies were consistently observed for the dim (+) HLA-DR cells. Thus, colony-forming efficiency in the range of 30% to 40% was characteristic of the partially purified CML MPCs used in this study, with a greater proportion of these cells being in the brightly fluorescent HLA-DR fraction. There were too few HLA-DR+ cells to analyze CFU-GM in this group. However, the recovery of CFU-GMs in the HLA-DR+ fractions generally was greater than 80%, in agreement with our previous studies. These observations with immunofluorescence were supported by simultaneous complement-lysis experiments in which CML CFU-GM colony growth was inhibited by greater than 90% in each of three experiments after treatment with either anti-I2, 9-49, or 7.2 and complement (data not shown).

Quantitative Assessment of HLA-DR Molecules on CML Progenitor Cells and Normal Monocytes

An estimate of the number of antibody binding sites per cell was obtained by comparing mean cell fluorescence of anti-Ia stained MPC preparations with mean cell fluorescence of a known standard (anti-T8 stained normal peripheral blood T lymphocytes). By this method, values from three experiments were 133,000 ± 14,000 binding sites per cell for 9-49; 36,000 ± 3,000 for l2; and 80,000 ± 9,000 for 7.2, whereas on normal monocytes, similar calculations revealed 42,000 ± 10,000 for 9-49; 39,000 ± 8,000 for l2; and 38,000 ± 14,000 for 7.2. Thus, the monomorphic DR determinants appear to be expressed with...
relatively high antigen density on both CML MPCs and normal monocytes. These numbers are only approximations, and their accuracy may be affected by several factors, including variations in the avidity of the fluoresceinated polyclonal goat anti-mouse immunoglobulin for different anti-Ia monoclonal antibodies (I2 is an IgG2a, 9-49 is an IgM, and 7.2 is an IgG2b). However, because CFU-GMs tend to be concentrated in the highest fluorescent fraction, this method would tend to underestimate the actual number of antibody binding sites per CFU-GM cell.

The Relationship of DR Antigen to the Cell Cycle

Previous studies using complement lysis have shown that DR antigen expression by the normal CFU-GM may increase as the cell progresses from G0/G1 through S phase. In order to evaluate the relationship of DR antigen expression to cell cycle phase, DR fluorescence intensity and forward-angle light scatter (an indirect estimate of cross-sectional cell diameter) were determined separately for cells in G0/G1, S, or G2/M using two-color fluorescence analysis (Fig 5). Most experiments were performed after exposing MPC preparations to CSF-containing medium for 12 to 18 hours to increase the fraction of cells in S phase. In the absence of CSF exposure, approximately 5% ± 2% of cells were in S phase and 10% ± 4% were in G2/M; following CSF treatment, 21% ± 4% of cells were in S phase and 14% ± 3% of cells were in G2/M (mean of three experiments). CSF treatment over this time period did not affect the percentage of cells expressing HLA-DR antigens (see below).

As shown in Fig 5C, the relative Ia fluorescence increases from G0/G1 to G2/M. The forward-angle light scatter also increases from G0/G1 to G2/M, suggesting an increase in cell size as these cells traverse the cell cycle (Fig 5B). Interestingly, the β2-microglobulin fluorescence intensity increased through the cell cycle by an equivalent degree (Fig 5D). Using the mean peak fluorescence of each curve to estimate the relative number of antibody binding sites, the number of both HLA-DR and β2-microglobulin antigens per cell approximately double from G0/G1 to G2/M (Fig 6 shows the means of three experiments). Control (background) fluorescence also increases slightly from G0/G1 to G2/M. Identical results were obtained whether or not the cells were exposed to CSF before analysis. These results demonstrate that CML MPCs express HLA-DR antigens during all phases of the cell cycle, and that there is a significant increase in both cell size and the absolute number of DR antigens per cell in G2/M compared with G0/G1. This increase in DR antigens is not restricted to Ia-like antigens, since a comparable increase is also observed with β2-microglobulin antigens.

Modulation of HLA-DR

Partially purified MPCs were incubated for six or 18 hours at either 4 °C or 37 °C and subsequently stained with NEN-011 or I2 (monomorphic DR determinants) in an attempt to detect loss of Ia antigen density with in vitro culture. The anti-Ia monoclonal antibody NEN-011 was used in view of previous reports indicating that the DR determinant identified by this antibody was diminished on normal and CML CFU-GM after a...
EXPRESSION OF Ia ANTIGENS IN CML

The study of Ia antigens on human MPCs may provide insights into the function of these surface molecules in normal hematopoiesis. A direct study of Ia antigen expression, however, has been previously hampered by the inability to obtain a purified population of MPCs. Using immunologic purification techniques and a panel of well-characterized monoclonal antibodies, we have enriched MPCs from stable phase CML peripheral blood by a factor of 80- to 120-fold.

**DISCUSSION**

The effects of in vitro culture of CML progenitor cells on HLA-DR expression. Progenitor cells were incubated for 18 hours in either 10% FCS/RPMI 1640 at 41 °C (A) or 37 °C (B), or with the addition of 20% GCT as a source of colony-stimulating factors at 37 °C (C).

**Table 2.** Effect of In Vitro Incubation (18 Hours) on CML Progenitor Cell HLA-DR Expression

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Anti-HLA-DR Monoclonal AB*</th>
<th>Fluorescent Cells (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 °C</td>
<td>37 °C</td>
</tr>
<tr>
<td>1</td>
<td>I2</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>NEN-011</td>
<td>70</td>
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<tr>
<td>2</td>
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<td></td>
<td>NEN-011</td>
<td>75</td>
</tr>
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<td>3</td>
<td>I2</td>
<td>70</td>
</tr>
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<td></td>
<td>NEN-011</td>
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<tr>
<td>5</td>
<td>I2</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>NEN-011</td>
<td>NT</td>
</tr>
</tbody>
</table>

NT, not tested.

*Both anti-DR monoclonal antibodies I2 and NEN-011 define monoclonal DR determinants.

†Percentage of positive cells as determined by indirect immunofluorescence after 18-hour incubation in sterile plastic test tubes at 4 °C or 37 °C. Samples 1 and 2 were incubated with 20% GCT as a source of colony-stimulating factors. Samples 3, 4, and 5 were cultured without GCT.

**Table 3.** HLA-DR Expression Following In Vitro Culture of CML Progenitor Cells in Serum-Free Medium

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>HLA-DR (I2) Positive Cells (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>20% FCS 10⁻⁶ mol/L PGE,</td>
</tr>
<tr>
<td></td>
<td>4 °C    37 °C</td>
</tr>
<tr>
<td>1</td>
<td>–       –</td>
</tr>
<tr>
<td></td>
<td>–       –</td>
</tr>
<tr>
<td>2</td>
<td>–       –</td>
</tr>
<tr>
<td></td>
<td>+       –</td>
</tr>
<tr>
<td></td>
<td>NT     87</td>
</tr>
<tr>
<td>3</td>
<td>–       –</td>
</tr>
<tr>
<td></td>
<td>+       –</td>
</tr>
<tr>
<td></td>
<td>–       NT</td>
</tr>
</tbody>
</table>

FCS, fetal calf serum; PGE, prostaglandin E; NT, not tested.

*10⁸ partially purified CML progenitor cells were cultured for 24 hours in flat-bottom culture plates at 4 °C or 37 °C in serum-free medium (Materials and Methods) in the presence (+) or absence (−) of 20% FCS or 10⁻⁶ mol/L PGE.

†Determined by indirect immunofluorescence.
This purification procedure yields a morphologically homogeneous blast population (85% to 95%), with a substantial fraction of cells able to form a colony of mature granulocytes or monocytes in agar in response to CSF. In each of the 14 stable phase CML patients evaluated in this report (Tables 1 through 3), the majority of cells in the purified preparations express HLA-DR antigens when evaluated by indirect immunofluorescence analysis. Although partially purified progenitor cells were heterogeneous with respect to Ia antigen expression, there did not appear to be a discrete population of Ia + cells. Further, although previous studies have suggested that CML CFU-GMs express “low levels” of HLA-DR, we found that antigen quantitation of HLA-DR was equivalent to that of normal monocytes. Also, cell-sorting experiments demonstrated that CFU-GMs in the MPC preparation were enriched in the fraction with the highest HLA-DR antigen density, suggesting that immunofluorescence analysis of HLA-DR in the entire MPC preparation actually underestimated HLA-DR expression by CFU-GMs. Observations with immunofluorescence were supported by simultaneous complement-lysis experiments in which CML CFU-GM colony growth was inhibited by more than 90% after treatment with anti-I2, anti-9-49, or anti-7-2 and complement.

Previous studies using complement lysis have demonstrated variability of HLA-DR antigen expression in CML patients. The data reported here also show differences in the amount of Ia antigen expression among CML patients (Table 1), although to a lesser degree than described by Broxmeyer and Pelus et al. Ultimately, it will be important to evaluate additional patients by direct analysis of purified progenitor cells in order to further define the range of Ia antigen expression in stable phase CML.

Because of the potential role HLA-DR antigens may play in hematopoietic stem cell regulation, we investigated several factors that might influence the expression of HLA-DR on these cells. Previous reports have suggested a relationship between Ia antigen expression and cell cycle phase in both myeloid and nonmyeloid cells. Sarkar et al have noted an increase in HLA-DR antigen density during the G2/M phase of a synchronized human B lymphoid cell line (WI-L2), and Lanier has detected selective expression of the I-E antigen group during the G2/M phase of a mouse B cell lymphoma line (WEHI-231). On the basis of complement lysis and thymidine suicide studies, Broxmeyer has shown that Ia antigen expression (HLA-DR) is increased in S phase, and this is correlated with the ability of normal human CFU-GM to be inhibited by acidic isoferritin. We therefore examined the relationship between Ia antigen expression and cell cycle phase on partially purified CML MPCs using dual fluorescence. Myeloblast preparations were stained simultaneously for HLA-DR expression (fluorescein) and DNA content (propidium iodide). The relative DR antigen fluorescence per cell progressively increased from G0/G1 to G2/M. Identical results were obtained with either freshly isolated cells or cells exposed to CSF-containing medium for 12 to 18 hours to increase the fraction of cells in S/G2/M. However, the fluorescence of cells stained with anti-β2-microglobulin (an antigen that is not known to be selectively expressed during any specific phase of the myeloid cell cycle) also increased from G0/G1 to G2/M, suggesting that some of the apparent increase in cellular DR antigen in S/G2/M might be due to an increase in cell size. Using light scatter as an indirect measure of cell diameter, it was demonstrated that relative cell size progressively increased from G0/G1 to G2/M, as anticipated. The relative number of anti-Ia antibody binding sites increased by a factor of 1.4 from G0/G1 to S phase and by a factor of 1.9 from G0/G1 to G2/M (Fig 6). Sarkar et al have measured mean cell volume of the WI-L2 B lymphoid cell line for each cell cycle phase, calculating a volume of 623 μm3 for G0 cells, 1,092 μm3 for S-phase cells, and 1,248 μm3 for G2/M cells. If cells are assumed to be spheres, the corresponding surface area of these cells would increase by a factor of 1.4 in S phase and 1.6 in G2/M, in good agreement with the increase in the HLA-DR binding sites observed in this study. Although these data suggest that the antigen density (antigens per unit of surface area) of HLA-DR does not significantly change during the cell cycle of CML MPCs, it is not possible to conclude this with certainty since direct measurements of cell volume were not made. Further studies will be necessary to determine whether the increase in DR antigens observed in S phase can be wholly accounted for by the increase in cell size. Our results, however, do support the earlier observations of Broxmeyer that Ia antigens are increased in the S phase of CFU-GM.

In order to further characterize the behavior of Ia-like antigens on CML CFU-GMs, a series of factors that might modulate expression of these molecules was investigated. Loss of Ia antigens on the CFU-GMs after incubation for three to 24 hours at 37 °C has been detected by complement lysis. However, when we directly evaluated CML myeloblast Ia expression by immunofluorescence, no loss of HLA-DR molecules was detected after either six or 18 hours of incubation at 37 °C in either glass test tubes or flat-bottom culture dishes. Further, this was true in the presence or absence of CSF. Because PGE has been reported to induce the reexpression of Ia antigens on CFU-GMs, we also considered the possibility that prostaglandins...
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were present as a contaminant in the growth medium. However, MPCs that were incubated in defined, serum-free medium containing indomethacin to inhibit endogenous production of prostaglandins also maintained expression of HLA-DR antigens. These data are in agreement with the results of Fitchen and Burger, demonstrating no loss of Ia antigen on normal human CFU-GM after 24 hours in suspension culture. Furthermore, HLA-DR expression also has been shown to persist or slightly increase in normal human monocytes cultured at 37°C for 12 hours, although this may be related to the presence of contaminating T cells, which maintain monocyte Ia antigen expression by the production of gamma interferon. In the experiments presented here, T cells were removed in order to minimize this possibility, but the role of gamma interferon in the regulation of Ia antigen expression on MPCs deserves further study. Finally, the fact that Ia antigen expression persisted in this system despite suspension culture in either plastic test tubes or flat-bottom culture plates suggests that direct cell–cell contact may not be a necessary requirement for continued Ia antigen expression in vitro.

The data presented here on the effects of in vitro culture do not support data obtained on normal and CML CFU-GMs using complement lysis. The study of Ia antigen expression by complement lysis is complicated by the fact that sensitivity to complement lysis may be changed by in vitro culture conditions or by membrane alterations that accompany transition through the cell cycle, particularly when dilute complement is used. For example, Pellegrino et al. noted increased susceptibility of the human cell line RPMI 8866 to complement lysis with HLA alloantisera during the G0 and S phases of the cell cycle in the absence of increased antigen expression. Altered sensitivity to complement lysis, dependent on cell cycle phase, has also been observed in several murine tumor lines, including YAC, YAC, and L1210. It is possible, therefore, that results obtained by complement lysis may reflect changes in membrane sensitivity to lysis as well as changes in antigen expression. Further studies using antibodies other than anti-Ia will be necessary to determine whether variations in complement sensitivity can be detected in CFU-GMs.

As purification techniques improve, it should become possible to perform direct analyses of Ia antigen expression on populations of normal myeloblasts. The study of additional Ia antigen classes (DS, SB) also would become possible in a purified cell system, especially in view of the growing availability of well-characterized anti-Ia monoclonal antibodies. Differences in the pattern of Ia antigen expression between CML and normal CFU-GMs may serve to further define the nature of the proliferation abnormalities that accompany CML. More important, by studying progenitor cells, monocytes, and B cells directly in the same individuals, it should be possible to identify differences in the expression of various Ia antigen subtypes between different cell lineages and to examine the degree of Ia antigen expression at various stages of differentiation. Such an approach may provide useful information regarding the function of Ia surface molecules in the regulation of hematopoiesis.

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