CONCISE REPORT

Phenotypic Characterization of Human Bone Marrow Granulocyte-Macrophage Forming Progenitor Cells

By Mark N. Levine, Joseph W. Fay, Nancy H. Jones, Richard S. Metzgar, and Barton F. Haynes

Cell surface antigens of the human bone marrow CFU-C have been studied. Human marrow cells were incubated with a variety of monoclonal antisera and complement prior to culture in semisolid media. By using indirect immunofluorescent studies, the percentage of bone marrow cells binding the antibodies was determined. The

IN VITRO colony-forming cells (CFU-C) are progenitors of granulocytes and macrophages and are identifiable by their capacity to form colonies of granulocytes and/or macrophages in culture. It has been demonstrated that the CFU-C bears Ia-like antigens. The evidence for the presence of Ia-like antigens on human myeloid progenitor cells is based on the demonstration of complement-dependent inhibition of granulocyte-macrophage colony formation in culture. Recently, using the technique of Köhler and Milstein for the production of myeloma lymphocyte hybrid cell lines, monoclonal antibodies reactive with a variety of human lymphocyte cell surface differentiation antigens have been produced. Recent studies from our laboratories have involved a search for tumor or normal lymphoid cell reactive monoclonal antibodies that might be used therapeutically as anti-tumor or immunosuppressive agents. In this article we examine the effects of a panel of antibodies reactive with subsets of either tumor or normal hematopoietic cells, in the presence and absence of complement, on CFU-C growth in human bone marrow culture.

MATERIALS AND METHODS

Antibodies

The properties of the antibodies used to characterize human bone marrow CFU-C (R4, L-243, 3A1, 4F2, 3F10, and DU-ALL-1) are summarized in Table I. Briefly, R4 is a rabbit heteroantisera with specific reactivity to the human Ia-like antigen. The remaining antisera are murine monoclonal antibodies. L-243 antibody (the generous gift of Dr. R. Levy) also reacts with a human Ia-like determinant. 3A1 binds to 85% of peripheral T lymphocytes and defines a major functional subset of peripheral T cells. Antibody 3F10 has specificity for a nonpolymorphic determinant of the human HLA-A, B, and C loci, while 4F2 antibody defines a surface glycoprotein found on peripheral blood monocytes and on a subset of activated lymphocytes. DU-ALL-1 defines a 24,000 molecular weight polypeptide antigen found on cells of most patients with acute lymphoblastic leukemia, cells of some patients with acute myelogenous leukemia, and on normal platelets. All of the antisera used fix complement and react in complement-dependent microcytotoxity assays.

CFU-C phenotype is HLA-, Ia-, 4F2-, 3A1-, and DU-ALL-1. This study provides information that is useful in the study of myeloid cell ontogeny and necessary for the use of some of these reagents in the treatment of bone marrow cells prior to human bone marrow transplantation in various clinical settings.

Fluorescent Labeling of Bone Marrow Cells

Cells were incubated with the various antisera for 30 min at 20°C, washed twice, and then incubated with FITC-conjugated goat anti-mouse IgG (Tago Inc., Burlingame, Calif.). After incubation for 30 min at 4°C, the cells were washed twice in phosphate-buffered saline, and the percentage of cells positive for fluorescence was determined on a Nikon fluorescent microscope.

Antibody and Complement Treatment of Bone Marrow Cells

Bone marrow from normal donors was aspirated from the iliac crest into a syringe and anticoagulated with preservative-free heparin. The marrow was layered on an equal volume of Ficoll-Hypaque lymphocyte separation media (LSM, Bionetics, Kensington, Md.) and centrifuged at 2000 rpm for 15 min. The cells at the interspace were resuspended and washed twice with Alphamedia (GIBCO, Grand Island, N.Y.) and the cell count adjusted to 2 x 10⁶/ml. A 1.0-ml cell suspension was pelleted and the supernatant discarded, and 0.2-ml of antisera was added. The combination was mixed and incubated for 30 min at room temperature. One milliliter of rabbit complement (pooled, 3 wk old) (Pel Freez) was diluted in 2 ml of Alpha-media, and 0.3 ml of this mixture was added to the antisera cell mixture, mixed, and incubated for 30 min at 37°C. Following this, a second incubation with antisera for 15 min at room temperature was performed, followed by a second complement incubation for 15 min at 37°C. Cells were then washed twice with Alpha-media and resuspended in 1 ml of media. Antisera were used in dilutions of 1/10, 1/100, and 1/1000.

CFU-C Assay

The CFU-C assay was performed according to the following procedure. The cells were plated in a mixture of methyl cellulose (69%), fetal calf serum (27%), and penicillin-streptomycin (4%) at

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Table 1. Antibodies Used to Characterize Human Bone Marrow CFU-C

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen Size</th>
<th>Specificity and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>R4*</td>
<td>29,000-34,000</td>
<td>Nonpolymorphic human la-like determinant*</td>
</tr>
<tr>
<td>L-243</td>
<td>29,000-34,000</td>
<td>Nonpolymorphic human la-like determinant*</td>
</tr>
<tr>
<td>3A1</td>
<td>40,000</td>
<td>T-cell specific; binds to 85% of peripheral T cells</td>
</tr>
<tr>
<td>4F2</td>
<td>40,000-80,000</td>
<td>Antigen of cell activation; binds to all peripheral blood monocytes and to a subset of alloantigen-activated lymphocytes</td>
</tr>
<tr>
<td>3F10</td>
<td>44,000-12,000</td>
<td>Nonpolymorphic determinant of human HLA-A,B, and C molecules</td>
</tr>
<tr>
<td>DU-ALL-1</td>
<td>24,000</td>
<td>Reacts with cells from most acute lymphoblastic leukemia and some acute myeloblastic leukemia patients</td>
</tr>
</tbody>
</table>

*Rabbit heteroantiserum. All other reagents in this chart are murine monoclonal reagents.
†Antigen size determined under nonreducing conditions using radioimmunoprecipitation and SDS-polyacrylamide gel electrophoresis.

The final concentration of 2 × 10^5 cells/ml. Placental conditioned media was used as the source of myeloid colony-stimulating factor. Each experiment was done in triplicate. All dishes were examined after culture for 7 days at 37°C, at 100% humidity in 5% CO₂, and aggregates of 50 or more cells were scored as colonies. Data are expressed as CFU-C colonies/2 × 10^5 cells in culture.

**Statistical Methods**

Comparisons between groups were made with the Wilcoxon matched-pairs significant rank test.

**RESULTS AND DISCUSSION**

By using fluorescein-conjugated anti-mouse IgG, the percentage of bone marrow cells binding antibody 3A1 was 8% ± 2.48% (SEM), 4F2, 45.33% ± 3.18%, and DU-ALL-1 13.74% ± 3.09%.

The number of CFU-C following treatment of bone marrow with various antisera and complement is shown in Table 2. The tabulated data are for antisera used at a dilution of 1:100. Similar results were obtained with dilutions of 1:10 and 1:1000 (not tabulated). When incubated with marrow alone without complement each antisera did not inhibit marrow growth (data not shown). Incubation of bone marrow with the monoclonal anti-la (L-243) and complement abolished all colony formation (data not shown).

As determined by complement-dependent cytotoxicity we reconfirm that the granulocyte-macrophage progenitor bears the la antigen. A monoclonal antibody, 4F2, which is on peripheral blood monocytes, inhibited CFU-C in the presence of complement and thus appears to be on the CFU-C. Human CFU-C are negative for the DU-ALL-1 antigen, as this antibody did not inhibit colony growth. Antibody 3A1 (anti-mature T-cell antibody) also did not inhibit CFU-C, while 3F10 (anti-HLA) inhibited colony growth completely (Table 2).

The phenotypic characterization of human bone marrow CFU-C as defined by antibodies used in our laboratory is summarized in Table 3.

It has previously been shown that the CFU-C bears antigens related to HLA-A,B as well as to the la determinant. The pre-CFU-C also appears to express the la antigen. However, the murine pluripotential stem cell (CFU-S) and the human pluripotential stem cell, as defined in continuous bone marrow culture, lack the la antigen.

Inhibition of CFU-C formation could result from cytotoxicity to CFU-C, from cytotoxicity to auxiliary cells involved in colony formation, and from cytotoxicity to maturing cells in colonies. However, in previous studies it has been shown that the mixing of bone marrow cells with peripheral blood mononuclear cells

Table 2. CFU-C From Normal Marrow After Incubation With Antisera

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control</th>
<th>Complement</th>
<th>R4 + C</th>
<th>DU-ALL-1</th>
<th>3A1 + C</th>
<th>4F2 + C1</th>
<th>3F10 + C</th>
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<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>20</td>
<td>0</td>
<td>15</td>
<td>NT†</td>
<td>NT</td>
<td>NT</td>
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<tr>
<td>2</td>
<td>9</td>
<td>10</td>
<td>1</td>
<td>16</td>
<td>17</td>
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<td>NT</td>
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<td>6</td>
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<td>NT</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>11</td>
<td>3</td>
<td>21</td>
<td>19</td>
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<td>NT</td>
</tr>
<tr>
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<td>11</td>
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<td>32</td>
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<td>NT</td>
</tr>
<tr>
<td>6</td>
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<td>0</td>
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<td>13</td>
<td>2</td>
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<td>0</td>
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<tr>
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<tr>
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<td>33</td>
<td>2</td>
<td>NT</td>
<td>NT</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Mean of three experiments.
†NT, not tested.
Control versus R4, p < 0.01.
Control versus 4F2, p < 0.02.
Control versus DU-ALL-1, not significant.
Table 3. Phenotypic Characterization of Human Bone Marrow CFU-C

<table>
<thead>
<tr>
<th>Antigen (Antibody)</th>
<th>Reactivity of CFU-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpolymorphic Ia (L-243, R4)</td>
<td>Positive</td>
</tr>
<tr>
<td>Nonpolymorphic HLA-A,B,C (3F 10)</td>
<td>Positive</td>
</tr>
<tr>
<td>3A1 (anti-T-cell)</td>
<td>Negative</td>
</tr>
<tr>
<td>4F2 (Anti-mature monocyte and activated hematopoietic cells)</td>
<td>Positive</td>
</tr>
<tr>
<td>DU-ALL-1</td>
<td>Negative</td>
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</table>

following treatment of marrow did not reverse the inhibition of growth.13

In vitro treatment of bone marrow with monoclonal antibodies may play a role in clinical bone marrow transplantation. In allogeneic transplantation, prior treatment of donor marrow cells with anti-T-cell antibody (e.g., 3A1) may eliminate the alloreactive immunocompetent cell, thus preventing or reducing graft-versus-host disease.16 In addition, in autologous bone marrow transplantation, serotreatment of bone marrow with monoclonal antibodies to tumor-associated antigens (e.g., DU-ALL-1) may be able to eliminate residual leukemic cells from remission bone marrow prior to cryopreservation without adverse effects on normal stem cells.17

Thus, our study has expanded the known phenotype of the human bone marrow CFU-C cell. This knowledge (regarding antibodies 4F2 and DU-ALL-1) should aid studies of human myeloid cell ontogeny, and may enhance prospects for future success in allogeneic and autologous bone marrow transplantation (antibodies 3A1, DU-ALL-1).

REFERENCES


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MN Levine, JW Fay, NH Jones, RS Metzgar and BF Haynes