CONCISE REPORT

Human Myeloid Precursors Forming Colonies in Diffusion Chambers Expresses the Ia-Like Antigen

By H. P. Koeffler, E. Niskanen, M. Cline, R. Billing, and D. Golde

Normal human bone marrow contains cells capable of forming myeloid colonies (CFU-DG) in fibrin clots in diffusion chambers placed in the peritoneal cavity of cyclophosphamide-treated mice. Evidence has accumulated indicating that these colony-forming cells represent an earlier stem cell than the granulocyte-monocyte precursor cell (CFU-C) assayed in soft agar. We provide data showing that these stem cells express the "Ia-like" or DR antigen. In the presence of rabbit Ia antiserum at a titer of 1:300, all CFU-DG were inhibited. Cytotoxicity was complement-dependent. Data are also presented that suggest that the megakaryocyte stem cell also expresses the Ia antigen.

THE HUMAN Ia-LIKE (DR) antigen is a cell membrane polypeptide dimer of approximately 27,000 and 33,000 daltons.1,2 This glycoprotein is structurally similar to murine Ia antigens. It has been suggested that the Ia antigen represents a normal differentiation marker on hematopoietic precursors.3,5 The antigen is expressed on the early erythroid precursor cells (BFU-E, burst-forming unit-erythroid; CFU-E, colony-forming unit-erythroid),4 on granulocyte-monocyte precursor cells (CFU-C),3,5,7 and on the null and B-lymphocyte.3,8,9 The antigen is not expressed on mature granulocytes, erythrocytes, or plasma cells.3,5,8,9 The murine pluripotent stem cell (CFU-S) does not react with Ia antiserum.10

Several investigators have provided evidence that the cells that form human myeloid colonies in fibrin clot diffusion chambers (CFU-DG) implanted in the peritoneal cavity of mice represent an earlier precursor cell than the CFU-C.3,11-13 In this article we show that this cell also expressed the Ia antigen.

MATERIALS AND METHODS

Cells

Heparinized aspirates of bone marrow were obtained from informed normal volunteers. The mononuclear cells were isolated by Ficoll-Hypaque centrifugation, washed twice, and counted for use in the cytotoxicity assay.
Ia ANTIGEN ON HUMAN CFU-DG

Antiserum

The expression of the “Ia-like” antigen was studied using rabbit anti-human B antiserum. The antiserum was produced by subcutaneous immunization of rabbits with papain-solubilized membranes from human spleens containing B-lymphoblastic lymphoma. The antiserum has been studied extensively and has been shown to react with B lymphocytes, early granulocytic precursors, and the majority of acute myelogenous and lymphocytic leukemia cells, but not with T lymphocytes, granulocytes, erythrocytes, or platelets.

Cytotoxicity Testing

The CFU-DG cytotoxicity assay was performed with $2 \times 10^6$/ml normal human mononuclear bone marrow cells in 100 $\mu$l alpha-medium containing 20% heat-inactivated fetal calf serum with either 100 $\mu$l heat-inactivated Ia antiserum or heat-inactivated normal rabbit serum (as control). The cells were incubated for 30 min at 37°C in an atmosphere of 5% CO$_2$ in air. Normal rabbit serum (100 $\mu$l) was then added as a source of complement to the cells for an additional 60 min. The normal rabbit serum was not in itself cytotoxic. The cells were washed 3 times with phosphate-buffered saline and placed in quadruplicate diffusion chambers, as previously described, at 4 x 10$^5$ cells in 100 $\mu$l alpha-medium and 20% fetal calf serum. The diffusion chambers were implanted in mice pretreated 4 hr before with cyclophosphamide (300 mg/kg). Two chambers were placed in the peritoneal cavity of each mouse. After 7 days, the chambers were removed and placed into another cyclophosphamide-treated mouse. The plasma clot was removed after 7 days, fixed, stained with hematoxylin, dried between 2 wire meshes, made transparent in immersion oil, and the number of CFU-DG containing 20 cells or more were enumerated.

RESULTS

The Ia antiserum inhibited the growth of the CFU-DG (Fig. 1). Normal human bone marrow formed 82 ± 11 (mean ± SE) colonies when 4 x 10$^5$ cells were placed in diffusion chambers in cyclophosphamide-treated mice (Table 1). In the presence of Ia antiserum at a titer of 1:300, all (100%) of the colony-forming cells were inactivated. At titers of 1:3000, about 83% of the CFU-DG were inhibited. Cytotoxicity was complement-dependent, and those cells that were exposed to
Table 1. Ia Antiserum Inhibition of Human Bone Marrow Diffusion Chamber Colonies

<table>
<thead>
<tr>
<th>Treatment of Bone Marrow Cells</th>
<th>Colonies/4 x 10^3 Cells/Chamber (± SE)</th>
<th>Percent Inhibition of CFU-DG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>82 ± 11</td>
<td>0</td>
</tr>
<tr>
<td>Complement</td>
<td>75 ± 6</td>
<td>—</td>
</tr>
<tr>
<td>Anti-la serum 1:30 + complement</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Anti-la serum 1:300 + complement</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Anti-la serum 1:3,000 + complement</td>
<td>13 ± 5</td>
<td>83</td>
</tr>
<tr>
<td>Anti-la serum 1:30,000 + complement</td>
<td>69 ± 12</td>
<td>8</td>
</tr>
<tr>
<td>Anti-la serum 1:300 absorbed with B lymphocytes + complement</td>
<td>78 ± 9</td>
<td>0</td>
</tr>
<tr>
<td>Anti-la serum 1:300 absorbed with T lymphocytes + complement</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

either the antiserum or complement alone had normal colony formation. Absorption with B lymphocytes abrogated the antiserum effect on the CFU-DG. The antiserum did inhibit colony formation when absorbed with T lymphocytes.

In the control chambers there were 10 ± 2 colonies that were composed of cells with the morphological characteristics of megakaryocytes. The colonies contained 2–6 cells. These cells were large (greater than 20 μ), with irregular cytoplasmic contour and multilobulated nuclei containing occasional mitotic figures. These colonies were totally inhibited by 1:300 titer of Ia antiserum. They were present in normal numbers at an Ia serum titer of 1:30,000.

DISCUSSION

The Ia or DR 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 are thought to play a role in T- and B-cell cooperation and stimulation in mixed lymphocyte culture. The Ia antigen is also expressed on certain nonlymphoid cells of hematopoietic origin. Accumulated evidence suggests that the Ia antigen may represent an early myeloid differentiation antigen in man.

The Ia antigen is expressed on the human CFU-C, myeloblast, and some promyelocytes, but the antigen is lost during maturation to myelocytes, metamyelocytes, and mature granulocytes. In contrast, the macrophage that develops from the CFU-C retains the Ia antigen.

In the erythroid line, the BFU-E, CFU-E, and proerythroblasts are reported to express Ia determinants on their cell surface membranes. The antigen disappears on the orthochromic normoblasts and mature red cells. The antigen is present on null and B lymphocytes, but is absent on most plasma cells.

In the mouse, the pluripotent stem cell (CFU-S) does not express Ia determinants. An assay for CFU-S is not available in man. However, recent evidence suggests that the human myeloid colonies (CFU-DG) that form in plasma clots within diffusion chamber cultures in the peritoneal cavity of mice represent a more primitive myeloid precursor than the CFU-C. These cells differ from CFU-C in velocity sedimentation, cell cycle characteristics, and sensitivity to hypotonic water lysis. Our data clearly show that these early myeloid progenitors have the Ia determinant on their cell membranes. A summary of Ia expression on cells of the granulocyte-monocyte series is presented in Fig. 1.
Several authors have suggested that committed megakaryocytic stem cells can grow in plasma clot diffusion chambers. We observed megakaryocytic colonies in our control plasma clots. In the presence of high titers of Ia antiserum, these colonies were inhibited, suggesting that the committed megakaryocytic stem cell also expresses the Ia antigen on its cell membrane.

ACKNOWLEDGMENT
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