CONCISE REPORT

Expression of Ia-Like and HLA-A,B Antigens on Human Multipotential Hematopoietic Progenitor Cells

By John H. Fitchen, Carol Le Fèvre, Soldano Ferrone, and Martin J. Cline

Human multipotential hematopoietic progenitor cells can be assayed by their ability to form colonies of mixed cell lineages in vitro. These cells display Ia-like antigens and HLA-A,B,C antigens as evidenced by inhibition of colony formation by specific monoclonal antibodies and complement.

CELLULAR INTERACTIONS are believed to play an important role in the regulation of hematopoiesis. Characterization of the antigenic profile of hematopoietic stem cells might help to elucidate the molecular basis of these controlling influences. Several laboratories have reported that human granulocyte-macrophage progenitor cells (colony-forming unit-granulocyte, macrophage; CFU-GM) express Ia-like antigens. These antigens resemble murine I-E antigens in tissue distribution, biologic properties, and molecular structure and have been implicated in the regulation of the immune response. Ia-like antigens have also been detected on the more primitive progenitor cells that form granulocyte-macrophage colonies in diffusion chambers (colony-forming unit-diffusion chamber; CFU-D) implanted in the peritoneal cavity of neutropenic mice. Robinson et al. have suggested that a proportion of primitive erythroid progenitors (burst-forming unit-erythroid; BFU-E) also express Ia-like antigens. However, it has been reported that murine pluripotent hematopoietic stem cells (colony-forming unit-spleen; CFU-S) lack Ia antigens. These findings have raised questions about the expression of Ia-like antigens on primitive human hematopoietic stem cells. Recently, an assay has been developed for human progenitor cells that form colonies of mixed cellular composition in vitro. These human multipotential progenitors (colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte; CFU-GEMM) may be closely related to murine CFU-S in that they represent primitive precursors of multiple lines of hematopoietic differentiation. In this study, we have tested whether monoclonal antibodies against nonpolymorphic determinants of Ia-like antigens can mediate complement-dependent inhibition of human CFU-GEMM.

MATERIALS AND METHODS

CFU-GEMM Assay

Multipotential stem cells were assayed as described by Fauser and Messner. Briefly, bone marrow was drawn in preservative-free heparin from informed and consenting healthy adult volunteers and a single cell suspension of light-density marrow cells obtained on a Ficoll-Hypaque density gradient. The cells were then plated at 2 x 10⁶ cells/ml in Dulbecco's modified Eagle's medium/1.0% methylcellulose supplemented with sodium selenite, 30% fetal bovine serum, and 5% conditioned medium from peripheral blood leukocytes stimulated with phytohemagglutinin (PHA). Colonies were scored after 12-14 day incubation at 37°C in humidified atmosphere of 5% CO₂ in air. Putative mixed colonies were identified in the plates with an inverted microscope, then individually transferred onto glass slides and forcefully air-blown to spread the cells. Slides were stained with Wright's stain and cell types verified under 100× magnification.

Antibodies

The preparation and serologic and immunochemical characterization of monoclonal antibodies to Ia-like antigens and to HLA-A,B,C antigens have been described elsewhere. The anti-human β₂-microglobulin (μ) antiserum was from a cow immunized with purified human urinary β₂m.

Cytotoxicity Assay

A quantity of 6 x 10⁴ light-density bone marrow cells was incubated with monoclonal antibody for 30 min at room temperature. Normal rabbit serum (complement source) was added, and incubation continued for an additional 60 min. The cells were then washed twice, and 3 ml of medium/methylcellulose mixture was added and cells plated at 2 x 10⁵/ml. Plates were scored for total colonies (granulocyte-macrophage, erythroid, and mixed) and mixed colonies after 12-14 day incubation at 37°C in a humidified atmosphere of 5% CO₂ in air.
Table 1. Effect of Antibodies Against la-Like and HLA Antigens on Human Multipotential Stem Cells

<table>
<thead>
<tr>
<th>Antibody Specificity</th>
<th>Monoclonal Antibody No.</th>
<th>C' Added</th>
<th>Total Colonies per 2 x 10^6</th>
<th>Mixed Colonies per 2 x 10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>None added</td>
<td>-</td>
<td></td>
<td>328 ± 26</td>
<td>9 ± 1</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
<td>331 ± 25</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Anti-la-like*</td>
<td>Q2/47</td>
<td>-</td>
<td>336 ± 97</td>
<td>7 ± 1</td>
</tr>
<tr>
<td></td>
<td>Q2/47</td>
<td>+</td>
<td>6 ± 3</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>Q5/13</td>
<td>-</td>
<td>359 ± 10</td>
<td>8 ± 2</td>
</tr>
<tr>
<td></td>
<td>Q5/13</td>
<td>+</td>
<td>18 ± 7</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Anti-HLA-A,B</td>
<td>Q1/28</td>
<td>+</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Anti-β,μ heteroantibodies</td>
<td>Q1/28</td>
<td>-</td>
<td>365 ± 51</td>
<td>11 ± 2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

RESULTS

Incubation of marrow cells with each of the monoclonal antibodies to la-like antigens prior to plating in methylcellulouse produced potent complement-dependent inhibition of mixed colony formation (Table 1). In addition, the granulocyte-macrophage and erythroid colonies that are also seen in this culture system were markedly reduced (Table 1). In contrast, treatment of marrow cells with complement alone did not alter the number of total or mixed colonies formed. Similarly, anti-HLA-A,B antibody and an anti-β,μ xenoantiserum, in conjunction with complement, completely inhibited colony formation (Table 1).

DISCUSSION

The findings that monoclonal antibodies against la-like and HLA-A,B,C antigens produce marked complement-dependent inhibition of mixed colony formation suggest that human multipotential stem cells express la-like and HLA-A,B,C antigens. The growth of mixed colonies requires factors from mitogen-stimulated mononuclear cells, and since la-positive mononuclear cells are required for mitogen responses, it could be argued that monoclonal antibodies against la-like antigens reduced mixed colony formation by killing critical auxiliary cells rather than acting directly on CFU-GEMM. This explanation for our results seems unlikely, however, since conditioned medium from PHA-stimulated mononuclear cells was added to the cultures after antibody-treated marrow cells were washed free of antibody and complement.

Our results are at variance with previous reports suggesting that human and murine pluripotent stem cells lack la antigens. Based on indirect experiments, Moore et al. have suggested that human pluripotent stem cells lack la-like antigens. They used a long-term (Dexter) culture system in which an initial inoculum of bone marrow cells is allowed to form an adherent layer and is then “recharged” with a second inoculum of marrow cells. Proliferation of CFU-GM can be maintained in such cultures for weeks to months and is believed to reflect feed-in from more primitive stem cells. Moore and co-workers treated the “recharging” cells with complement and heteroantiserum to la-like antigens and monitored cultures for the presence of CFU-GM. This treatment markedly reduced the number of CFU-GM in the “recharging” cell suspension, but by 2 wk, the number of CFU-GM detected among the nonadherent cells was the same as in control cultures. This observation suggested that a primitive precursor of CFU-GM was unharmed by treatment with anti-la-like antiserum and that this precursor therefore lacks la-like antigens. However, the possibility that CFU-GM were generated in the adherent layer (which was not treated with antiserum) was not excluded. Active stem cell proliferation in the adherent layer has been reported in a similar culture system using mouse bone marrow.

Basch et al. have reported that a heteroantiserum against la antigens was not reactive with murine CFU-S in cytotoxicity or fluorescence-activated cell-sorting studies. They concluded that CFU-S lack la antigens. Observations in our laboratory suggest that CFU-S lack la antigens. Our findings suggest that la-dependent regulatory influences could occur at the level of very primitive hematopoietic progenitors.
REFERENCES

12. Pellegrino MA, Belvedere M, Pellegrino AG, Ferrone S: B peripheral lymphocytes express more HLA antigens than T peripheral lymphocytes. Transplantation 25:93, 1978
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