Expression of Ia-Like Antigens Defined by Monoclonal OKIal Antibody on Hemopoietic Progenitor Cells in Cord Blood: A Comparison With Human Bone Marrow

Shoichi Koizumi, Masahiko Yamagami, Masayoshi Miura, Seiki Horita, Mieko Sano, Norisada Ikuta, and Noboru Taniguchi

Expression of Ia antigens on granulocyte/macrophage colony-forming cells (CFU-GM) in human cord blood was compared with that in bone marrow with the use of monoclonal OKIal antibody. Mononuclear cells prepared from cord blood and bone marrow were pretreated with OKIal antibody plus complement, and, thereafter, the ability of cord blood and bone marrow cells to form colonies of CFU-GM was assayed in semisolid agar culture. Consistent reduction in the number of CFU-GM in cord blood to 58.8% ± 13.0% (mean ± SD) of controls treated with complement alone was shown after elimination of la-antigen-bearing CFU-GM, but was significantly less remarkable than that in bone marrow (18.0% ± 5.6%). Although the reduction of both granulocyte (CFC-G) and macrophage colony (CFC-M) types of cord blood, characterized by the double staining for esterase activity, was shown following treatment with OKIal antibody plus complement, the relative inhibition of CFC-G was significantly greater than that of CFC-M (p < 0.02). These results suggest some differences in the characteristics of Ia-antigen-bearing CFU-GM between cord blood and bone marrow cells. Furthermore, it is suggested that Ia-dependent regulatory mechanisms might participate in the differentiation of CFU-GM to CFC-G and CFC-M.

CHARACTERIZATION of the surface antigenic structure of hemopoietic stem cells might help to understand their differentiation to functionally mature cells during the maturation process. Human Ia-like antigens resemble mouse I-E antigens in tissue distribution, biologic properties, and molecular structure,1 and are believed to play an important role in the regulation of the immune response.2 Recently, Ia-like antigens have also been detected on human hemopoietic progenitor cells, such as granulocyte/macrophage progenitor cells, colony-forming units-granulocyte/macrophage (CFU-GM)3-7 and erythroid progenitor cells (burst-forming unit-erythroid; BFU-E).8-10 More primitive multipotential hemopoietic progenitors (colony-forming unit-granulocyte, erythroid, macrophage and megakaryocyte; CFU-GEMM) have also been suggested to express Ia-like antigens,11 though some observations to the contrary have been made.12

Our previous studies13 and those of others14-17 indicated that a large number of hemopoietic progenitor cells were recognized in human cord blood and the peripheral blood of neonates. Of interest, the predominance of monocyte/macrophage colonies (colony-forming cells-monocyte/macrophage; CFC-M), as characterized by the double staining for esterase activity, was demonstrated in cord blood, whereas the colonies formed by bone marrow cells were largely granulocyte colonies (colony-forming cells-granulocyte; CFC-G).13,17 In the present study, some properties of Ia-like antigen-bearing CFU-GM, CFC-G, and CFC-M in cord blood were compared with those in bone marrow on the basis of complement-mediated cytotoxicity using monoclonal OKIal antibody.

MATERIALS AND METHODS

Cell Preparation

Cord blood samples were collected from the placental end of the cord cut immediately after delivery of normal newborns. Bone marrow samples were obtained from informed and consenting healthy adult volunteers. Heparinized cord blood was mixed with 2.5% dextran-phosphate-buffered saline (PBS, pH 7.4) in a 1:1 ratio and allowed to settle at 37°C for 30 min. Mononuclear cells in cord blood were separated by means of Ficoll-Hypaque (Lymphoprep, Nyegaad & Co., Oslo, Norway) gradient centrifugation at 400 g for 30 min. After centrifugation, mononuclear cells at the interface were collected, washed, and suspended in McCoy’s 5A medium (Flow, Mclean, Va.). Mononuclear cells were similarly separated from bone marrow: samples were diluted 1:5 with PBS and layered over Ficoll-Hypaque as described previously.13,14 Nonadherent (NA) cells were obtained after a removal of adherent cells by incubating mononuclear cell suspension, 5 × 10⁷/ml, in McCoy’s 5A medium with 15% fetal bovine serum (FBS, Flow, Stanmore, NSW, Australia) in plastic culture dishes (Falcon 3002, B.D., Cockeysville, Md.) for 60 min.

Monoclonal Antibody

The monoclonal antibody OKIal was purchased from Ortho Pharmaceutical Corporation (Raritan, N.J.). It has been reported that OKIal antibody reacts with the human Ia antigen framework.18

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EXPRESSION OF Ia-LIKE ANTIGENS

Table 1. CFU-GM From Bone Marrow After Treatment With Monoclonal OKIaI Antibody Plus Complement*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No Treatment</th>
<th>OKIaI Antibody</th>
<th>Complement</th>
<th>OKIaI Antibody + Complement</th>
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| Percent     | (mean ± 1 SD) | 95.6 ± 15.6     | 91.5 ± 10.3 | 100                         | 18.0 ± 5.6 |

*Bone marrow mononuclear cells were treated with monoclonal OKIaI antibody alone, complement alone, and OKIaI antibody plus complement prior to CFU-GM cultures. Each value represents the mean of triplicate cultures per 10^6 plated bone marrow cells.

Treatment With OKIaI Antibody and Complement

NA-cells prepared from cord blood and bone marrow were resuspended at 5 × 10^6/ml in McCoy’s 5A medium, and OKIaI monoclonal antibody was added to NA-cell suspension at a final dilution of 1/100. The cells were then incubated for 30 min at room temperature and agitated every 10 min. After incubation, rabbit serum as a complement source, fresh or stored at -80°C, was added to the cell suspension at a final dilution of 1/10, and further incubation was performed at 37°C in a shaking water bath for 45 min. At the end of this time, the cells were spun down at 300 g for 10 min, washed extensively in McCoy’s 5A medium, and resuspended to starting volume. The resultant population contained less than 4% OKIaI* cells by cytotoxicity assay. Control samples were treated in parallel with medium alone, OKIaI antibody alone, or complement alone.

CFU-GM Assay

CFU-GM in cord blood and bone marrow NA cells were cultured in double-layer semisolid agar using the technique of Pike and Robinson29 with micro modifications as described previously.30 Peripheral blood leukocytes obtained from one normal donor were used to prepare feeder layers in 0.5% agar (Bacto-agar, Difco, Detroit, Mich.) with 15% FBS throughout this study. After 10 days of incubation, aggregates more than 40 cells were counted as colonies.

Characterization of CFC-M and CFC-G

Cytochemical examination of colonies grown in agar gel, consisting of monocytes/macrophages (CFC-M) and granulocytes (CFC-G), was performed by the method of the double staining for esterase activity described by Kubota et al.21

At the end of incubation, the agar gel was carefully transferred en bloc onto a slide glass. After absorbing liquid medium with filter papers, the preparations were double stained for esterase activity in response to both alpha-naphthyl acetate and naphthol AS-D chloroacetate as substrates.

RESULTS

The relative proportion of Ia-antigen-bearing cells in bone marrow (12.9% ± 4.2%, mean ± SD) measured by cytotoxicity assay was shown to be almost the same as that in cord blood (10.1% ± 3.5%). The results of the bone marrow CFU-GM assays obtained after pretreatment with monoclonal OKIaI antibody plus complement are summarized in Table 1. Although the treatment of marrow cells with OKIaI antibody alone or complement alone did not alter the number of total colonies, a remarkable reduction of the number of CFU-GM was observed after treatment with OKIaI antibody plus complement in all experiments (18% ± 5.6% of control treated with complement alone). The colonies grown from bone marrow cells were shown to belong largely to CFC-G (88.3% ± 9.4% of total CFU-GM) by double staining for esterase activity, and no significant changes were

Table 2. CFU-GM From Cord Blood After Treatment With Monoclonal OKIaI Antibody Plus Complement*

<table>
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<tr>
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<th>Complement</th>
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<td>117</td>
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</table>

| Percent     | (mean ± 1 SD) | 103.8 ± 18.0   | 102.9 ± 14.0 | 100                         | 58.8 ± 13.0 |

*Cord blood mononuclear cells were treated with monoclonal OKIaI antibody alone, complement alone, and OKIaI antibody plus complement prior to CFU-GM cultures. Each value represents the mean of triplicate cultures per 10^6 plated cord blood cells.
observed in the relative proportions of CFC-G and CFC-M after treatment with OKIa1 antibody plus complement.

In contrast, the number of CFU-GM in cord blood was also reduced following treatment with OKIa1 antibody plus complement, but to a lesser degree (58.8% ± 13.0% of control treated with complement alone) when compared with that in bone marrow (Table 2). The predominance of CFC-M was demonstrated in cord blood CFU-GM (60.6% ± 7.7% of total CFU-GM). After treatment of cord blood cells with OKIa1 antibody plus complement, the number of both CFC-M and CFC-G was reduced. However, the relative inhibition of CFC-G was significantly greater than that of CFC-M (p < 0.02), as shown in Fig. 1.

**DISCUSSION**

The present study revealed some different responses to monoclonal antibody against Ia antigen of hematopoietic stem cells in human cord blood when compared with those in bone marrow. The total number of CFU-GM circulating in cord blood was almost the same as that in bone marrow. However, the treatment with OKIa1 antibody plus complement resulted in less marked inhibition of colony formation in cord blood than bone marrow. Such a fact suggests that there are some differences either in quantity or in quality of expressing Ia antigen between cord blood CFU-GM and bone marrow CFU-GM. It is possible that Ia antigens are at a lower concentration on circulating progenitor cells in contrast to marrow cells, and this makes them relatively resistant to the effect of complement acting on a monoclonal antibody. Furthermore, the relative susceptibility could possibly even be due to other membrane alterations associated with circulation.

Our previous studies revealed that the greater part of colonies formed by cord blood belonged to CFC-M and a smaller part of colonies belonged to CFC-G on the basis of the cytochemical characterization by double staining for esterase activity. As shown in the present study, CFC-G in cord blood and bone marrow seemed to be much more sensitive, while CFC-M seemed to be less sensitive in response to monoclonal OKIa1 antibody. Two separate subpopulations of myeloid progenitor cells, CFC-G and CFC-M, were clearly identified in rats by the use of the fluorescence-activated cell sorter (FACS). Although both CFC-G and CFC-M were shown to have almost the same cell size, CFC-G appeared to be ancestral to CFC-M, namely to give rise to not only mature granulocytes but also CFC-M, which produce mature monocytes and macrophages in the in vitro culture system. The response to monoclonal OKIa1 antibody of CFU-GM in cord blood differed in some aspects from that in bone marrow. These differences seemed, at least in part, to result from the remarkable predominance of CFC-M in cord blood. Furthermore, from these observations, it is suggested that Ia-dependent regulatory mechanisms might participate in the differentiation of CFU-GM to CFC-G and CFC-M during the maturation process.

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**REFERENCES**


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