CONCISE REPORT

Cell Surface Phenotyping of Megakaryoblasts

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Surface phenotypic characterization of megakaryoblasts, identified by platelet peroxidase activity, was investigated in four patients who showed increased proliferation of megakaryoblasts: one patient with typical features of acute leukemia, one presenting with acute myelofibrosis, and two with Down’s syndrome in whom blasts disappeared spontaneously (transient abnormal myelopoiesis, TAM). MY10 and/or MY9 antigens were expressed on the surface of some megakaryoblasts, but MY7, and MY4, antigens specific to granulocytic or monocytic cells, were not. Some megakaryoblasts were positive for only anti-HLA-DR antibodies. It was speculated that, during the differentiation of the megakaryocytic lineage, MY9 antigen appears transiently on the surface of megakaryoblasts that have lost HLA-DR antigens and have gained the glycoprotein Ib/IIa antigen. This study also demonstrated that the proliferating blasts in some patients with TAM were mainly megakaryoblasts and suggested that the target cells in TAM are CFU-GEMM.

The use of specific monoclonal antibodies has provided important insights into leukocyte differentiation and cellular origin in leukemia. These antibodies were found to be very useful in investigating the stages of differentiation in leukemic blasts. However, little is known about the cell surface phenotypes of megakaryoblasts because of the rarity of megakaryoblastic leukemia.

Platelet peroxidase (PPO), a specific enzymatic marker of megakaryocytic cell lineage, is detected in early megakaryocytes and appears earlier than platelet-specific glycoproteins (GP), such as the GP Ib/IIa complex or GP Ib, or storage proteins in platelets, such as von Willebrand factor antigens. In this study, cell surface phenotypes were examined in patients with megakaryoblastic leukemia in whom the proportions of blast cells were more than 45% and PPO activity was detected in more than 65% of the blasts.

MATERIALS AND METHODS

Patients. Clinical data on all patients are shown in Table I. Patient 1 was an infant without Down’s syndrome. Ascension of bone marrow was easy, and the presence of myelofibrosis was unlikely. Patient 2 presented with acute myelofibrosis. Circulating blasts increased gradually. Patients 3 and 4 had Down’s syndrome with a spontaneous disappearance of proliferating blasts. Regarding the aspiration and biopsy of bone marrow, all patients were advised of procedures and attendant risks, in accordance with institutional guidelines, and gave informed consent.

Blood smears and cytochemical studies. Blood smears were stained with May-Grünwald-Giemsa. Cytochemical reactions for myeloperoxidase (MPO), Sudan black B, α-naphthyl acetate esterase, α-naphthyl butyrate esterase, acid phosphatase, and periodic acid–Schiff (PAS) were performed by conventional methods.

Electron microscopic studies. PPO was detected by the method of Anderson et al or Breton-Gorius et al with a slight modification as described elsewhere. More than 200 blasts were examined, and the blasts positive only in perinuclear space or endoplasmic reticulum were regarded as PPO-positive. Positive blasts in granules or Golgi cisternae were regarded as MPO-positive.

Cell surface phenotyping. For the detection of surface antigenic differences, samples were analyzed using a Spectrum III (Ortho Diagnostics, Westwood MA). A population of blast cells was clearly distinguished from the lymphocytes, monocytes, and granulocytes on the basis of light-scattering properties. Monoclonal antibodies used in this study were as follows: OKT3 (CD3), OKT11 (CD2), OKT4 (CD4), and OKT8 (CD8) purchased from Ortho Pharmaceutical Corp, Raritan, NJ; J5 (CD10), B4 (CD19), B1 (CD20), MY7 (CD13), MY4 (CDw14), MY9, and MY10 purchased from Coulter Immunology, Hialeah, FL; HLA-DR and LeuM3 purchased from Becton Dickinson, Mountain View, CA; and TP80 (IgGl), which detects GP Ib/IIa antigen, and another monoclonal anti-GPIIb/IIIa antibody, PLT-2, purchased from Cappel Laboratories, Cochranville, PA. The positivity for the monoclonal anti-GPIIb/IIIa antibodies was also examined by an indirect immunofluorescence method. Fc binding of these antibodies to the stained cells was excluded by the absence of reactivity with monoclonal antibodies directed to unrelated antigens of the same immunoglobulin subclasses and by the findings that the saturation of Fc receptors by preincubation with normal rabbit serum had no effect on the APAAP staining results obtained using monoclonal antibodies to GP Ib/IIa. The results of indirect immunofluorescence staining conjugated F(ab')2, immunoglobulin were almost the same as those of APAAP staining.

Terminal deoxynucleotidyl transferase (TdT) activity was detected by immunofluorescence using a Bethesda kit (Bethesda Research Laboratories Rockville, MD).

RESULTS

Light microscopic cytochemistry. Almost all of the patients’ blasts were negative for peroxidase, Sudan black B, and α-naphthyl butyrate staining but were positive for acid phosphatase. Blasts in patient 1 stained strongly for α-naphthyl acetate, whereas blasts in the other patients stained weakly for this enzyme. Some blasts from all patients were positive for PAS.
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MY 10 and MY9 in patients 1, 3, and 4, but their blasts were rarely positive for MY7 and MY4. In patient 2, the blasts were negative for MY9 and TP80 but positive for HLA-DR, whereas in patient 1, they were positive for both MY9 and TP80 but negative for HLA-DR.

DISCUSSION

We encountered four patients in whom the proliferating blasts were recognized as megakaryoblasts, although the patients’ clinical features varied. For identification of megakaryoblasts, detection of PPO activity is necessary when the proportion of blasts positive for antiplatelet antibodies is small because PPO activity appears earlier than platelet-specific glycoproteins such as GP Iib/IIa antigens during the process of megakaryocyte maturation.3,4 In this study, we examined whether other markers, especially myeloid markers, are expressed on megakaryoblasts with PPO activity. A considerable proportion of blasts was positive for MY10 and/or MY9 in patients 1, 3, and 4, whose blasts were rarely positive for MY7 or MY4. It is noteworthy that most of the blasts in patient 1 were positive for MY10 and MY9 in addition to high proportions of cells positive for anti-GP Iib/IIa and PPO activity. MY10 antigen is a cell surface protein with an apparent molecular weight of 115 kilodaltons and was produced after immunization with KG-1a human leukemia cells.11 Cell sorting and panning experiments demonstrated that CFU-GM and BFU-E were MY10-positive. The MY9 monoclonal antibody was produced by Griffin et al12 following immunization with the cells of a patient in the blast crisis of chronic granulocytic leukemia. MY9 antigen is expressed on CFU-GM, myeloblasts, promyelocytes, myelocytes, and monocytes. Approximately 50% of BFU-E and some of CFU-GEMM are also MY9-positive, although more mature erythrocytic cells are negative for this antigen.12,13 The present results suggest that megakaryoblasts, which are megakaryocyte precursors, express MY10 and MY9, although mature megakaryocytes and platelets are negative for these antibodies. The blasts of our patients were rarely positive for MY7 and MY4, which are markers of more myeloid development. MY7 antigen is specific to granulocytic and monocytic cells.14 MY4 antigen is expressed

Table 1. Clinical and Cytological Findings of Four Patients

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Percentages of cells positive for ultrastructural cytochemistry and immune markers refer to the percentage of blasts as assessed by their characteristic morphology and by size gating (see Materials and Methods).

Abbreviations: BM, bone marrow; POI (-), blasts negative for peroxidase activity; TP80 and PLT-2, antiplatelet GP Iib/IIa antibodies; NT, not tested.

Electron microscopic cytochemistry. In all patients more than 65% of the blasts were positive for PPO in perinuclear space and endoplasmic reticulum (Table 1, Fig 1). Less than 5% of the blasts were positive for peroxidase in granules and Golgi apparatus.

Cell surface phenotyping. The immunologic markers of the blasts are shown in Table 1. Most of the blasts from patient 1 reacted to TP80 (Fig 2), whereas most of the blasts from patient 2 were negative for this antibody. The proportions of cells positive for TP80 were similar to those of PPO-positive cells in patients 1 and 3, whereas in patients 2 and 4, the proportions of TP80-positive cells were rather low compared with those of PPO-positive blasts. In all patients, the blasts were negative for lymphoid markers such as B2, B4, OKT3, OKT11, OKT4, OKT8, J5, and TdT. In contrast, a considerable proportion of the blasts were positive for

Fig 1. Electron micrographs of megakaryoblasts from the blood of patients 2 (left) and 4 (right). Reaction products for platelet peroxidase are present in the nuclear envelope and endoplasmic reticulum in megakaryoblasts. Unstained sections; original magnifications ×6000 (A) and ×5000 (B).
marker expression should be confirmed in megakaryocyte colony-forming assays using normal bone marrow cells treated with monoclonal antibodies and complements.

A self-limited blastic proliferation, indistinguishable from acute leukemia except for its disappearance without therapy, has been noted in infants with Down’s syndrome. This phenomenon is designated as transient abnormal myelopoesis (TAM). In addition to previous reports that proliferating blasts in TAM were usually myeloblasts, the results found in patients 3 and 4, ie, mainly the megakaryocytic nature of proliferating blasts, suggest that the target cells in TAM are CFU-GEMM.

REFERENCES


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T Koike, S Aoki, S Maruyama, M Narita, T Ishizuka, H Imanaka, T Adachi, H Maeda and A Shibata