Immunocytochemical Identification of Cells of Megakaryocytic Lineage by Alkaline Phosphatase Anti-alkaline Phosphatase Method

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Leukemia of megakaryocytic lineage is now recognized as a distinct hematologic entity and is classified as M7 in the schema established by the French, American, British Study Group. This disorder is characterized by infiltration by megakaryocytic cells into many tissues including blood and bone marrow. The exact number of such cells in blood bone marrow required to establish this diagnosis, however, has not been clearly defined. Cells of megakaryocytic lineage also have been found in a variety of myeloproliferative disorders. It is necessary to accurately identify the megakaryocytic cells in order to diagnose megakaryocytic leukemia and to assess the significance of the presence of such cells in the circulating blood.

At present, megakaryocytes and their precursors can be best identified either by ultrastructural study of platelet peroxidase or by immunocytochemical studies using monoclonal antibodies specific for platelet glycoprotein (Gp) IIb/IIIa complex or for factor VIII. There is a need to develop simple, practical, methods to facilitate accurate identification of the megakaryocytes and their precursor cells. We have obtained a specific monoclonal antibody (HP1-1D) to platelet Gp (IIb/IIIa)2,3 from Drs. C. Y. Li and W. L. Nichols of the Mayo Clinic, USA and have successfully used this antibody and an alkaline phosphatase anti-alkaline phosphatase (APAAP) method to identify cells of megakaryocytic lineage.

Materials and Methods

Smears of blood and buffy coat and smears of marrow aspirate from 17 patients were studied. In these patients there were 12 males and 5 females; their ages ranged...
between 29 and 83 years. The diagnosis of these cases included 1 acute myeloid leukemia, 1 refractory anemia with an excess of blasts in transformation (RAEB-T), 1 unclassified acute leukemia, 1 acute myelofibrosis, 1 chronic myelofibrosis, 1 refractory anemia with an excess of blasts (RAEB), 1 refractory anemia, 2 chronic myeloid leukemia (CML), 1 CML with blastic cell crisis, 1 essential thrombocythemia, 2 thrombocytosis, 1 acute myeloid leukemia in remission, 1 autoimmune hemolytic anemia, 1 multiple myeloma and 1 thrombocytopenia. The number of blast cells in blood ranged from 0 to 30% and in bone marrow from 0 to 38%.

The APAAP method was performed as follows: 1. Fix the smear in cold buffered formal acetone for 30 sec., wash in water and air dry. 2. React with monoclonal antibody (HP1-1D) in moist chamber for 30 min. Control smears were allowed to react with diluted mouse ascites in the same manner. 3. React with goat anti-mouse IgG for 30 minutes. 4. React with APAAP complex (Zymad) for 30 min. 5. Stain for alkaline phosphatase for 10 to 30 mins. in an incubation mixture containing naphthol AS phosphate and fast red violet LB salt. Endogenous alkaline-phosphatase is inhibited by 1 mM levamisole. Ultra-structural PPO reaction was carried out by the method of Breton-Gerius et al.4

Results

In smears stained with the monoclonal antibody HP1-1D, both platelets and megakaryocytes were stained intensely red. Such staining reaction was not seen in cells in the control smears. Cells other than platelets and megakaryocytes, such as segmented neutrophils, monocytes, lymphocytes, erythroblasts and plasma cells did not show any positive staining reaction with HP1-1D. Intense staining with HP1-1D also has been found in blast cells which otherwise would not be recognized as megakaryocytes. These blasts have been found in cases of acute myeloid leukemia, RAEB-T, acute myelofibrosis, chronic myelocytic leukemia and chronic myeloid leukemia in blastic cell crisis. The number of these cells in either blood or marrow varied from case to case. The existence of cells of megakaryocytic lineage in these cases was confirmed by ultrastructural demonstration of cells with platelet peroxidase activity. In one patient with unclassified acute leukemia, the leukemic cells were not stained with HP1-1D suggesting that they were not megakaryocytic cells. This finding was also in agreement with the ultrastructural cytochemical study which failed to show platelet peroxidase in the leukemic cells.

In the cases of essential thrombocytemia, thrombocytosis of unknown etiology, acute myeloid leukemia in remission, autoimmune hemolytic anemia, multiple myeloma and thrombocytopenia, all the cells that were stained positively with HP1-1D could be morphologically identified as megakaryocytes. Cells other than the megakaryocytes were not stained.
Conclusion

The APAAP method is simple in principle and easy to perform. It is superior to fluorocytometry in recognizing cells of interest in cytologic preparations containing many types of cells. By using this immunochemical method and the platelet specific antibody HP1-1D, it is possible to identify megakaryocytes and their precursors. This method is useful for the diagnosis of megakaryocytic leukemia and for assessment of the clinical significance of blood borne megakaryocytes in other myeloproliferative disorders.

References