Heterogeneity of B Cell Involvement in Acute Nonlymphocytic Leukemia

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In order to study the pattern of B cell involvement in acute nonlymphocytic leukemia (ANLL), multiple B lymphoid cell lines were established by Epstein-Barr virus transformation of peripheral blood mononuclear cells from two patients with the disease who were heterozygous for the X chromosome-linked glucose-6-phosphate dehydrogenase (G6PD). In one patient, the progenitor cells involved by the leukemia exhibited multipotent differentiative expression, whereas in the other patient the cells showed differentiative expression restricted to the granulocytic pathway. In the patient whose abnormal clone showed multipotent expression, the ratio of B-A G6PD in B lymphoid cell lines was skewed in the direction of type B (the enzyme characteristic of the leukemia clone) and significantly different from the 1:1 ratio expected. It is, therefore, likely that the neoplastic event occurred in a stem cell common to the lymphoid series as well as to the myeloid series. In contrast, evidence for B cell involvement was not detected in the patient whose ANLL progenitor cells exhibited restricted differentiative expression. These findings underscore the heterogeneity of ANLL. Clinically and morphologically similar malignancies in these two patients originated in progenitors with different patterns of stem cell differentiative expression. This difference may reflect differences in cause and pathogenesis.

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A CUTE nonlymphocytic leukemia (ANLL) is a clonal proliferation of primitive hemopoietic cells that are heterogeneous with respect to the pattern of differentiative expression: In some patients the disease is expressed in progenitor cells with differentiative expression restricted to the granulocytic pathway, whereas in others it involves stem cells that differentiate to granulocytes, erythrocytes, and platelets. Remission also may be heterogeneous, with restoration of normal nonclonal hemopoiesis in some patients or return to an apparently normal, but nonetheless clonal, putatively preleukemic state in others.

Evidence has been provided that in chronic myelocytic leukemia (CML) and refractory anemia, clonally derived pluripotent stem cells are capable of B lymphoid differentiation. To study hierarchical stem cell relationships in ANLL, we investigated the derivation of B cells susceptible to transformation by Epstein-Barr virus (EBV) in two patients with the disease, one with restricted and the other with multipotent differentiative expression of the involved stem cells.

The genetic marker we used is the X chromosome-linked enzyme glucose-6-phosphate dehydrogenase (G6PD). Because in somatic cells of females, portions of one X chromosome are genetically inactive, women heterozygous at the G6PD locus for the common gene (Gd^B) and the variant Gd^A have two populations of cells, one producing B-type G6PD and the other synthesizing A-type enzyme. Consequently, neoplasms with a unicellular development in these subjects manifest either B or A G6PD, whereas normal tissues express both enzymes.

Multiple B lymphoid cell lines were established in vitro by exposure of the patients' mononuclear blood cells to EBV. B lymphocytes infected with EBV may become transformed, allowing growth as permanent cell lines that ultimately become homogeneous with respect to the expression of cytoplasmic immunoglobulin (clg) and G6PD type. Analysis of the cell lines from these two patients with differing types of ANLL provides further evidence for heterogeneity of stem cell differentiative expression in ANLL.

CASE REPORTS

Case 1. A 67-year-old black woman was diagnosed as having ANLL (FAB M2) in March 1983. The karyotype was abnormal with complex chromosomal rearrangements but did not contain the Philadelphia chromosome. She was treated with combination chemotherapy and entered a complete clinical and hematologic remission. In December 1983 the leukemia relapsed; the patient failed to respond to treatment and died in January 1984. Detailed clinical and cytogenetic data have been reported.

Case 2. An 18-year-old black woman was referred in November 1983 for evaluation of pancytopenia. The blood counts were hematocrit 20%, white blood cell count 3,000/μL, and platelet count 50,000/μL. A marrow aspirate was hypercellular with 80% blast cells. The modal karyotype in marrow cells was 46,XX, t(8;21)(q23;q22) (the chromosome studies were done in the laboratory of Drs A. Milunsky and D. Minka at the Boston University School of Medicine). A diagnosis of ANLL FAB M2 type was made, and the patient was given remission induction chemotherapy. She achieved a complete remission in four weeks, was given one cycle of consolidation therapy, and received no further treatment until she relapsed in June 1984.

MATERIALS AND METHODS

Specimens were sent to Seattle for G6PD assays and other studies. Techniques used for cell separation, hemopoietic colony growth, preparation of extracts for G6PD assay, and electrophoresis have been described.

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B lymphoid cell lines. Mononuclear cells were isolated from peripheral blood by centrifugation over a Ficoll-Hypaque gradient. Interface cells were collected and rosetted with neuraminidase-treated sheep erythrocytes. Nonrosetting cells were resuspended in culture medium (RPMI 1640 supplemented with 10% fetal calf serum), diluted with an equal volume of filtered three-day supernatant from the EBV-producing cell line MRC-5, and plated into 96-well microculture trays. Cells were plated at concentrations of 1 to 8 x 10^4 cells in 0.2 mL per well. Cultures were fed twice weekly and expanded by transfer to larger wells as required. Assays of cell lines for G6PD, clg, and chromosomes were performed as described.9

RESULTS

Both patients were heterozygous for G6PD with 1:1 ratios of B-A-type enzyme in skin and cultured skin fibroblasts.

Case 1. At diagnosis, G6PD tests of blood and marrow cells tested directly and after growth in vitro showed that granulocytes, erythrocytes, and platelets exhibited almost exclusively type B G6PD.4 When the patient entered a complete clinical remission with an M1 marrow and normal peripheral blood counts, the marrow progenitors and blood cells were karyotypically normal but still expressed essentially only a single G6PD type.4

B lymphoid cell lines were established while the patient was in clinical remission. Seven hundred microculture wells were plated with cells from four specimens of peripheral blood. The transformation efficiency was very low (<5%), as has been observed in CML.4 and refractory anemia.4 Sufficient numbers of cells for G6PD assays were obtained from 27 lines, all of which displayed a single G6PD phenotype. Twenty-one lines expressed B-type G6PD and six, A-type G6PD, a ratio significantly different from the expected 1:1 (P = .007, two-tailed binomial test of proportions). Cytoplasmic Ig was found in 18% to 90% of cells in each of the 22 tested lines. Of the ten lines that expressed G6PD type B and a single Ig light chain, seven produced kappa and three synthesized lambda, not different from the expected ratio of kappa-lambda. Two of 16 analyzable lines displaying type B G6PD were cytogenetically abnormal, one with a modal karyotype of 47,XXX and the other with 46,XX,t(10:18)(q21 or 22;q23). The four lines producing A-type G6PD that could be studied in detail had normal karyotypes.

Case 2. Peripheral blood and marrow cells were assayed before chemotherapy, but several hours after the patient had received 2 units of packed red cells from Caucasian blood donors. Erythrocytes showed approximately 35% G6PD A and 65% G6PD B. Each of 38 granulocytic colonies grown from marrow exhibited G6PD type B. Attempts to grow erythroid colonies and to prepare homogenous populations of blast cells, platelets, and lymphocytes were unsuccessful. However, two weeks later, after one cycle of combination chemotherapy, when blast cells were still present in peripheral blood, essentially only type B G6PD was found in marrow-nucleated cells. Peripheral blood and marrow cells were reasayed during remission, and equal amounts of B- and A-type G6PD were found in red cells, platelets, granulocytes, and mononuclear cells.

B lymphoid cell lines were established four weeks after therapy similar to that given to Case 1 had been instituted and at a time when the patient was in remission. Fourteen lines were recovered from 120 microculture wells plated. Two lines that expressed both G6PD enzymes were excluded from further analysis. Of the remaining 12 lines, six displayed B-type G6PD and six, A-type G6PD, the same B-A ratio found in nonhemopoietic tissues. The ratio of lines producing only kappa to those synthesizing only lambda light chains was 5:3.

DISCUSSION

Both patients with ANLL were heterozygous for G6PD with 1:1 ratios of B-A-type enzyme in nonhemopoietic tissues. In Case 1, the finding of a single-enzyme G6PD phenotype in erythroid cells, granulocytes, and platelets indicates that her disease was clonal and involved a stem cell with multipotent differentiative expression.4 Evidence for clonality of the second patient's leukemia is provided by the finding of only B-type G6PD in granulocytic colonies before treatment and in marrow-nucleated cells two weeks after initiation of therapy. G6PD tests of erythrocytes were performed after the patient had been transfused with 2 units of packed red blood cells from Caucasian blood donors. Because the electrophoretic A-type G6PD variants are essentially confined to black populations, it can be assumed that the enzyme in the transfused cells was G6PD type B. The finding, therefore, of 35% G6PD A in red blood cells a few hours after the patient received 2 units of presumably G6PD type B red blood cells strongly suggests that at the time of initial study there were approximately equal amounts of G6PD A and B activities in the patient's red cells and that the progenitors involved by the leukemia did not differentiate along the erythroid pathway. Thus it is likely that in this patient, differentiative expression of the progenitors involved by the leukemia was restricted to the granulocytic pathway.

The pattern of remission was also different in the two subjects. In the second patient, the finding of a normal double-enzyme G6PD phenotype in all hemopoietic cell populations during remission indicates that nonclonal, presumably normal hematopoiesis was restored. In contrast, in the first patient, the single-enzyme G6PD phenotype in blood and marrow cells persisted during remission.4 Although formally possible for reasons stated elsewhere,17 we think it unlikely that selection for cells expressing B-type G6PD explains the single-enzyme G6PD phenotype. It is also possible that hemopoietic reconstitution after chemotherapy occurred from only a few normal stem cells that by chance were producers of G6PD type B. However, the remission ratios of B-A G6PD in hemopoietic cells were similar to those of skin in each of eight studied patients with restricted differentiation-type ANLL (including Case 2), even after successive cycles of intensive treatment (data not shown). Thus we believe that the most likely explanation for the persistence of the single G6PD type during remission in Case 1 is that chemotherapy destroyed a leukemic subclone and restored a morphologically and karyotypically normal, but nevertheless clonal, preleukemic state.

In the first case, the ratio of B-A G6PD in B lymphoid cell lines was skewed in the direction of type B (the enzyme
characteristic of the leukemic clone) and significantly different from the 1:1 ratio expected. It is likely, therefore, that the neoplastic event occurred in a stem cell common to the lymphoid series as well as to the myeloid series. Because Ig light chain expression in some lines was kappa and in others, lambda, it can be inferred that, as in CML, Ig light chain gene rearrangement had not occurred in the pluripotent stem cell involved by this acute myeloid leukemia.

In the second case, the ratio of EBV-transformed lymphoid lines displaying B- and A-type G6PD was 1:1 as predicted, notably different from the ratio found in Case 1 with the multipotent expression-type of ANLL. Thus, in the second patient, there was no evidence for involvement of B lymphoid cells at the time of study after one cycle of induction chemotherapy. It is unlikely that if clonal B lymphoid cells were present before treatment, they would have been selectively affected so soon by chemotherapy. Similar chemotherapy treatment in Case 1 did not cause selective loss of the clonal B lymphoid cells.

Studies with the G6PD marker in all investigated chronic myeloproliferative disorders, including CML, myelodysplastic refractory anemia, polycythemia vera, and essential thrombocythemia, have demonstrated that clonally derived pluripotent stem cells are capable of B lymphoid differentiation.8,9,10 Apparent heavy chain Ig gene rearrangements were recently reported in blast cells from two of 14 children with ANLL, suggesting that the disease involved stem cells capable of B lymphoid differentiation in some patients.19 However, the presence of heavy chain Ig gene rearrangements in leukemic cells may not necessarily imply that a neoplasm is of B cell lineage.19 The data presented here for B cell involvement in the patient whose abnormal clone showed multipotent myeloid differentiative expression provide strong evidence that ANLL can develop in a stem cell capable of B lymphoid as well as myeloid differentiation.

The failure to detect B cell involvement in the second patient underscores the heterogeneity of ANLL. Clinically and morphologically similar malignancies in these two patients originated in progenitors with different patterns of stem cell differentiative expression. In the patient with the restricted expression-type of ANLL, the disease involved either relatively differentiated ("committed") granulocyte progenitors or multipotent stem cells in which differentiation to the erythrocytic and lymphocytic lines was blocked. The future availability of probes to distinguish myeloid cells at various levels of differentiation (available now for the lymphoid lineage) may make it possible to determine which of these alternatives is correct.

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REFERENCES

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