Evidence that juvenile myelomonocytic leukemia can arise from a pluripotential stem cell

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Children with neurofibromatosis type 1 (NF1) carry germline mutations in one allele of the NF1 gene and are predisposed to myeloid malignancies, particularly juvenile myelomonocytic leukemia (JMML). Disruption of the remaining NF1 allele can be found in malignant cells. Flow cytometric cell sorting techniques to isolate the malignant cell populations and molecular genetic methods to assay for somatic loss of the normal NF1 allele were used to study an unusual child with NF1 and JMML who subsequently had T-cell lymphoma. The data show that malignant JMML and lymphoma cells share a common loss of genetic material involving the normal NF1 gene and approximately 50 Mb of flanking sequence, suggesting that the abnormal T-lymphoid and myeloid populations were derived from a common precursor cell. These data support the hypothesis that JMML can arise in a pluripotent hematopoietic cell. (Blood. 2000;96:2310-2313)

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Introduction

Juvenile myelomonocytic leukemia (JMML) is a relentless myeloproliferative disorder of children characterized by the monoclonal overproduction of myeloid cells. Up to 14% of cases occur in children with neurofibromatosis type 1 (NF1).1,2 An autosomal dominant disorder caused by germline inactivation of one allele of the NF1 gene on chromosome 17, JMML can involve more than the myeloid lineage2 because a malignant clonal expansion of erythroid cells has been inferred by cytogenetic data.3 X chromosome inactivation and microsatellite polymorphic marker studies,3 and a JMML patient has been reported whose disease evolved to pre-B–cell acute lymphoblastic leukemia (ALL).4 Here we describe a boy with NF1 who was brought for treatment for JMML and in whom a T-cell lymphoma later developed. Molecular genetic and flow cytometric analyses provided strong evidence that both malignant clones derived from a common precursor with pluripotent potential, suggesting that JMML is a stem cell disorder.

Study design

Case report

A 3½-year-old boy with NF1 inherited through the maternal lineage was brought for treatment for JMML. Physical examination showed numerous café au lait spots and enlarged tonsils but an absence of hepatosplenomegaly. His white blood cell count was 96 700/mm3, with 5% blast cells and a hemoglobin level of 10.1 g/dL, and a platelet count of 218 000/mm3. The bone marrow showed an overwhelming myeloid predominance with less than 5% blasts and a normal karyotype, 46,XY. His peripheral blood myeloid cells formed colony-forming unit granulocyte-macrophage colonies in methylcellulose cultures without exogenous growth factors.

During the next 4 months leukocytosis persisted and was complicated by a worsening anemia and thrombocytopenia with an enlarging spleen, failure to thrive, and airway obstruction caused by hypertrophied tonsils. There was no response to isotretinoin administered at 100-200 mg/m2 per day. Adenotonsillectomy and splenectomy were performed, and histopathologic examination of the adenoids and tonsils revealed a dense infiltration with myeloperoxidase-positive cells. Similarly, the enlarged spleen showed expansion of the red pulp by immature myeloid cells. New, diffuse adenopathy and hepatomegaly developed 6 weeks later. A lymph node biopsy revealed a T-cell expansion consistent with lymphoma. He received combination high-dose chemotherapy, but respiratory distress, anasarca, and renal failure ensued, which led to his death 8 months after the diagnosis of JMML.

Flow cytometry

Mononuclear cells were obtained from Becton Dickinson Immunocytometry Systems (San Jose, CA), DAKO (Carpinteria, CA), and Pharmingen (San Diego, CA). Flow cytometric analysis of the lymphomatous node and bone marrow aspirate was performed as previously described.1 Using a FACS Vantage (Becton Dickinson), viable cells from an enlarged lymph node were separated into CD4+ surface CD3− (CD4+sCD3−) lymphoma cells, and CD4+sCD3+ (phenotypically normal T cells) populations and viable bone marrow cells were purified as bright CD45+ with bright CD5+ (phenotypically normal T lymphocytes), bright CD45 with intermediate side scatter (monocytes), and intermediate CD45 with high side scatter (maturing granulocytes) populations. The cells were lysed immediately with DNA preparation buffer (Gentra Systems, Minneapolis, MN) and were snap-frozen in liquid nitrogen.

DNA extraction and analysis for loss of constitutional heterozygosity

DNA was isolated from unfractionated and sorted blood, bone marrow, spleen, and lymph node populations as described.11 To screen for loss of
heterozygosity (LOH) at NF1. 4 intragenic polymorphisms were assayed: EVI20,12 an Alu repeat,13 a dinucleotide repeat,14 and a complex repeat.15 The extent of the chromosome 17 LOH region was determined by assay of polymorphic loci UT17212 and by 9 loci defined in the Genome Database (http://gdbwww.gdb.org/) by their identification numbers—D17S926 (GDB, 199252), D17S805 (GDB, 188452), D17S1294 (GDB, 686175), D17S1800 (GDB, 607032), D17S250 (GDB, 177030), D17S836 (GDB, 1218969), D17S1806 (GDB, 607848), D17S1830 (GDB, 1218973), and D17S928 (GDB, 1218974). Each locus was assayed using the polymerase chain reaction (PCR) to amplify DNA segments that contained a variable number of short nucleotide repeats. Thermocycle parameters and procedures for genotyping each locus have been described.12,15 Radiolabeled PCR products were resolved by electrophoresis. Loss and retention of heterozygosity was determined by comparing the alleles detected in the blood of both parents with the allele(s) detected in the patient’s tissues.

Results and discussion

LOH for NF1 served as a marker of somatic inactivation of the normal allele in various hematopoietic compartments and cells showing LOH at NF1 are likely to be derived from a common precursor cell. Similarly, X chromosome inactivation has been used to demonstrate the clonality of mononuclear cells in girls with precursor cell. Similarly, X chromosome inactivation has been used was determined by comparing the alleles detected in the blood of both products were resolved by electrophoresis. Loss and retention of heterozygosity

LOH for NF1 was determined by comparing the alleles detected in the lymph node and bone marrow specimens obtained at the initial diagnosis of JMML (Figure 2A, lanes 3 and 4, respectively). A similar loss of the normal paternal NF1 allele was identified in the lymph node and in bone marrow cells obtained with the onset of diffuse adenopathy (lanes 5 and 7, respectively), in maturing monocyte and neutrophil fractions purified from the bone marrow (lanes 8 and 9, respectively), and in the immunophenotypically aberrant CD4+CD3− subpopulation of cells purified from the lymphomatous lymph node (lane 11). Unfractionated spleen cells (lane 6) showed a marked reduction in the signal derived from the paternal allele, a result that is consistent with an admixture of NF1+/− and NF1 +/− cells. In contrast, the intensities of the mutant maternal and normal paternal alleles were similar in the phenotypically normal CD4+CD3+ T cells isolated from the lymph node (lane 10) and from the phenotypically normal CD5+ T cells purified from bone marrow (data not shown), suggesting that these cells were not involved in the malignant process.

To further investigate whether JMML and lymphoma cells derived from a common progenitor, loci spanning the length of chromosome 17 were assayed for LOH. Although multiple loci showed LOH in JMML cells and lymphomatous lymph node cells (CD4−CD3−), representative data for the D17S805 locus, which retained heterozygosity, and D17S1294, which lost heterozygosity, are shown in Figure 2B. The loci that lost heterozygosity were identical and spanned the long arm of the chromosome, a large region greater than 50 Mb in length (Figure 2B). In contrast, the normal CD4+CD3+ T cells from the lymph node retained heterozygosity at all chromosome 17 loci tested. These data strongly implicate a single genetic event that resulted in loss of the normal paternal NF1 allele in a progenitor cell that gave rise to both the myeloid leukemia and the lymphoma clones.

The most likely genetic mechanism of LOH in this case is a recombination between D17S805 and D17S1294 of a maternal and a paternal chromatid during the S/G2 phase of the cell cycle of an ancestral cell. All possible recombinants would have 2 apparently normal chromosome 17 homologs, which is consistent with the results of the 2 independent cytogenetic normal analyses of bone marrow from our patient. One recombinant would carry the unaltered NF1 maternal chromosome and a paternal chromosome in which the 17q arm with the NF1+ allele had been replaced with
In conclusion, analysis of this unusual patient provides insights into the clonal origins of JMML and the proliferation of NF1-deficient hematopoietic cells, and our data support the hypothesis that at least some cases of JMML originate in a pluripotent hematopoietic stem cell.

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References

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