Need for an accurate molecular diagnosis to assess the donor origin of leukemia relapse after allogeneic stem cell transplantation

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ABSTRACT

The occurrence of leukemic transformation of donor cells in vivo after allogeneic transplantation has been reported in several cases. To date the pathogenic mechanisms, which most likely take place in the donor after engraftment, still remain to be elucidated and different hypotheses have been proposed. Possible environmental factors, transfer of oncogenic material from host to donor and viral infections, such as that caused by Epstein-Barr virus (EBV), have all been suggested to play a crucial role in generating the new transformation event. Interestingly, recent data have provided new evidence for a direct link between viral genes and specific chromosome translocations as in the case of adenovirus E1A for EWS-FLI1 fusion transcript characteristic of Ewing's tumor. However, at least in some cases the donor origin of the leukemia relapse could be misinterpreted because of the lack of adequate molecular analysis. The case we describe here underlines this possibility and strongly suggests that appropriate experiments and controls should be performed before such a diagnosis is made.

Design and Methods

Patient and donor samples

Peripheral blood or bone marrow samples were taken from the patient and the donor after informed consent, using forms approved by the Institutional Review Board, had been obtained.
Immunophenotyping

The immunophenotyping was performed by flow cytometry on freshly isolated leukemic cells using the following fluorochrome isothiocyanate (FITC)-conjugated or phycoerythrin (PE)-conjugated monoclonal antibodies: anti-CD45, CD13, CD33, CD3, CD5, CD7, CD19, CD20, CD10, CD34, HLA-DR, CD11b and FACScan analyzer (Becton Dickinson, San José, CA, USA).

Karyotyping

Conventional karyotyping of leukemic blasts was performed according to standard methods of G-banded chromosome preparations on freshly isolated cells and after in vitro incubation for 48 hours with and without mitogens. Fluorescence in situ hybridization (FISH) was performed using commercially available BCR-ABL double color labeled probes (Oncor, Gaithersburg, MD, USA).

Molecular findings

Total cellular RNA and high molecular weight DNA were extracted according to standard methods. Reverse transcription-polymerase chain reaction (RT-PCR) of the BCR-ABL chimeric transcript and amplification of variable number of tandem repeat (VNTR) loci including ApoB, ApoC2, YNZ-22, MCT118, 33.6, D11S533 or the Y chromosome specific DYS14 sequence were performed using a Thermal Cycler 9600 apparatus (Perkin Elmer Cetus, Norwalk, CT, USA) as described elsewhere. PCR amplification, cloning and sequencing of the CDRIII region of the BCR gene on chromosome 22. Therefore analysis of t(9;22) chromosome translocation had revealed the same BCR-ABL (e1a2) chimeric transcript. Two months later a bone marrow aspirate revealed an overt leukemia relapse (more than 50% leukemic blasts) with an immunophenotype similar but not identical to that documented at diagnosis (CD19+, CD10+, CD20+, CD34+, HLA-DR+, CD33+, CD11b+). Conventional cytogenetic analysis 47, XX, del(9) (p21), +i(17) (q10), del(20) (q12), ider(22) (qter), t(9;22) (q34;q11) revealed the presence of multiple karyotypic abnormalities including the Ph1+ chromosome and surprisingly, an apparent female karyotype in all the 25 metaphases analyzed (Figure 1, panel A). FISH analysis and RT-PCR confirmed the presence of the Ph1+ chromosome and that of a BCR-ABL (e1a2) chimeric gene (Figure 1, Panel B and Figure 2, panel A). The patient did not respond to a further chemotherapy program and died soon after.

Results

Case report

In October 1996 a 49-year old man was admitted to our Department with a diagnosis of acute lymphoblastic leukemia (ALL). His hemoglobin concentration was 10.7 g/dL, white cell count 8·10⁹/L, platelet count 25·10⁹/L and his bone marrow was aspirated and showed an immunophenotype consistent with a diagnosis of B-precursor ALL (CD19+, CD10+, CD20+, CD34+, HLA-DR+, CD33+, CD11b+). While conventional cytogenetic analysis showed a normal karyotype, the molecular RT-PCR analysis of t(9;22) chromosome translocation documented a BCR-ABL gene rearrangement (e1a2 breakpoint) coding for the p190 chimeric oncoprotein. The patient was treated according to the institutional protocol and enrolled into an allogeneic stem cell transplantation program. In January 1997, after a conditioning regimen based on busulfan (16 mg/kg) and melphalan (110 mg/m²) he received granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells (5·10⁹ CD34+ cells/kg) collected from an HLA-identical female sibling donor. Cyclosporin A (CyA) and methotrexate were given for graft-versus-host disease (GVHD) prophylaxis. With this treatment, the patient achieved a complete hematologic and molecular remission as documented by repeated negative results of nested RT-PCR analysis of the BCR-ABL gene. Twenty-four months after transplantation, because of fever, myalgia and low back pain, bone marrow was aspirated and despite the lack of morphologically identifiable leukemic blasts, RT-PCR analysis documented the reappearance of the BCR-ABL (e1a2) chimeric transcript. Two months later a bone marrow aspirate revealed an overt leukemia relapse (more than 50% leukemic blasts) with an immunophenotype similar but not identical to that documented at diagnosis (CD19+, CD10+, CD20+, CD34+, HLA-DR+, CD33+, CD11b+). Conventional cytogenetic analysis 47, XX, del(9) (p21), +i(17) (q10), del(20) (q12), ider(22) (qter), t(9;22) (q34;q11) revealed the presence of multiple karyotypic abnormalities including the Ph1+ chromosome and surprisingly, an apparent female karyotype in all the 25 metaphases analyzed (Figure 1, panel A). FISH analysis and RT-PCR confirmed the presence of the Ph1+ chromosome and that of a BCR-ABL (e1a2) chimeric gene (Figure 1, Panel B and Figure 2, panel A). The patient did not respond to a further chemotherapy program and died soon after.

Molecular evaluation of the origin of the leukemic relapse

The discrepancy between a normal karyotype at diagnosis and the presence of a Ph1+ chromosome at relapse could reflect the well known inability of cytogenetic analysis to detect the neoplastic clone in some cases of ALL. Most striking however, was the presence of an apparently female karyotype in leukemic blasts isolated at the moment of relapse which prompted us to confirm at the molecular level the donor origin of the leukemia relapse. Although PCR analysis had revealed the same BCR-ABL (e1a2) chimeric transcript in leukemic blasts at diagnosis and relapse, the huge length (>55 Kb) of the minor breakpoint cluster region (m-bcr) did not allow us to perform a simple Southern blot analysis to investigate the DNA breakpoints of the BCR gene on chromosome 22. Therefore we performed PCR amplification of the Y chromosome-specific DYS14 sequence in DNA samples isolated at diagnosis and relapse. As shown in Figure 2, panel B, while the male origin of the leukemic clone at diagnosis was evident, no
amplification of the DYS14 sequence occurred at relapse. To identify other, more informative probes, the molecular analysis of several VNTRs including Apo B, Apo C2, MCT118, and 33.6, D11S533 was performed but none of these discriminated between patient and donor cells (data not shown). The analysis performed with the YNZ-22 probe, while suggesting that at the time of relapse a relevant proportion of hematopoietic cells were of donor origin, could not formally rule out the presence of patient’s leukemic cells because of co-migration of two alleles (Figure 2, panel C). Therefore a further set of experiments was performed in order to identify an unequivocal marker of clonality. We then decided to clone and sequence the IgH gene rearrangements detected in leukemic blasts isolated at diagnosis and relapse. This set of experiments was performed by PCR amplification, cloning and sequencing of the CDRIII region of the IgH gene rearrangements. As shown in Figure 3, the DNA sequencing demonstrated the identity of the two CDRIII regions thus formally proving the patient origin of the leukemia relapse.

Discussion
Here we report a leukemia relapse, occurring in a man after allogeneic transplantation from an HLA-identical sister, in which cytogenetic analysis strongly suggested the donor origin of the leukemic cells. This diagnosis was further suggested by the PCR results obtained using a sensitive Y-chromosome specific molecular probe and by the molecular analysis performed with the YNZ-22 VNTR. However, the possibility of generating a leukemia-specific probe based on the unique DNA rearrangement of the IgH gene allowed unequivocal demonstration of the patient origin of the leukemic relapse. Because many additional, secondary genetic defects can occur after a myeloablative conditioning regimen, the simplest interpretation of the apparent female karyotype at relapse is the consequence of
the loss of the Y chromosome which could have eventually taken place along with duplication of an X-chromosome. Leukemia transformation of engrafted human bone marrow cells was first described more than thirty years ago when the lack of adequate molecular tools at that time cut down the chances of making a precise molecular diagnosis of the leukemic clone. Therefore, in the majority of cases, conventional cytogenetics has been used to confirm the donor origin of the leukemic relapse and several technical pitfalls may have been encountered. More recently, sophisticated, FISH-based cytogenetic techniques have also been employed but usually they have been equally applied to investigate sex-related chromosomes. Interestingly, the use of molecular probes for the evaluation of highly polymorphic DNA sequences (VNTRs) and restriction fragment length polymorphisms (RFLPs) in some cases corroborated, with more robust evidence, the diagnosis of leukemia recurrence in donor cells. In other cases, however, this molecular analysis allowed correction of the erroneous assignment of a donor origin to a secondary leukemia thus confirming the inadequacy of a diagnosis based only on sex-related cytogenetic markers. However, in some cases, it was shown that even the molecular analysis of VNTR could not definitively assign the origin of the leukemic clone because deletion or amplification of chromosomal segments carrying marker loci may lead to a misinterpretation of results. Overall, only a few cases of those so far reported in the literature could provide an unequivocal and leukemia-specific molecular marker demonstrating the donor origin of the leukemia relapse after allogeneic transplantation. Our results strongly emphasize the need to identify leukemia-specific sequences, which may represent the only definitive tool to identify the origin of a malignant clone during its natural history in a patient despite possible further genetic evolution.

Potential implications for clinical practice

Our results indicate that a diagnosis of leukemia relapse in donor cells cannot be supported only by cytogenetic evidence and must be demonstrated by an accurate molecular characterization of the leukemic cells using unequivocal, clone-specific molecular probes. In the absence of such a characterization, the diagnosis of leukemia relapse in donor cells should not be accepted.

Contributions and Acknowledgments

OS contributed to the design of the study and carried out the molecular biology experiments. US and AM performed the cytogenetic analysis. GM B and ML helped in doing the molecular biology experiments. GD followed the patient clinically. TB is the head of the department and critically revised the manuscript. AR designed the study and wrote the manuscript.

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Disclosures

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References


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