Donor Cell Leukemia Developing Six Years After Marrow Grafting for Acute Leukemia

By Robert P. Witherspoon, William Schubach, Paul Neiman, Paul Martin, and E. Donnall Thomas

A patient who developed recurrent leukemia more than six years after marrow grafting from an HLA-identical same-sex sibling is reported. Differences in DNA restriction fragment length polymorphisms between donor and host demonstrated that the DNA in the recurrent leukemia sample was probably of donor origin. Possible mechanisms that could explain the long latent period between transplantation and expression of leukemic transformation are discussed. We conclude that future cases of late leukemic recurrence after marrow grafting should be studied to determine whether, in contrast to early relapses, late relapses occur in donor cells in most or all instances.

© 1985 by Grune & Stratton, Inc.

PATIENTS TREATED with allogeneic marrow transplantation for acute leukemia can have long-term disease-free survival without maintenance chemotherapy. Some of these patients are now approaching 14 years from grafting. However, recurrence of leukemia after grafting is observed in 20% to 60% of patients, depending on the type and stage of disease. Recurrences of leukemia have appeared between one and 36 (median six) months postgrafting. In most cases the origin of the recurrent leukemia was in host-type cells, as shown by cytogenetic studies of sex differences, banding markers, or other chromosomal abnormalities. In a minority of patients, however, leukemia and other lymphoproliferative malignancies have been reported to occur in donor cells.

We report here a case of recurrence of leukemia more than six years after marrow grafting. The donor and recipient were of the same sex, and there were no other cytogenetic differences between the donor and the host. However, by using DNA restriction fragment length polymorphisms, we were able to show that the leukemia was probably in cells of donor origin.

MATERIALS AND METHODS

Case Report

In May 1976 a 25-year-old woman (unique patient No. 679) sought medical attention after a two- to three-month history of ecchymoses. She was pale and had ankle and mucosal petechiae. Peripheral blood and bone marrow examination disclosed the presence of acute leukemia with morphological features of the lymphoid type. She was treated with vincristine and prednisone weekly for six weeks without response. She subsequently received two courses of intravenous chemotherapy, each consisting of daunorubicin 75 mg/m² daily for three days, and cytosine arabinoside and 6-thioguanine in October and December 1976. She subsequently received two courses of maintenance chemotherapy with cytosine arabinoside and 6-thioguanine in October and December 1976. She continued in remission until March 1977, when a bone marrow examination revealed 7% blast forms.

She underwent conditioning for marrow grafting with 12 mg intrathecal methotrexate, 120 mg/kg cyclophosphamide intravenously (iv), and 10 Gy total body irradiation as previously described. and received 13.1 x 10⁹ nucleated bone marrow cells from her HLA-A, -B, and -D-identical sister in April 1977. Engraftment was demonstrated by marrow examination seven days after marrow infusion. In order to prevent graft-vs-host disease (GVHD), methotrexate was given IV after the marrow infusion on days 1, 3, 6, and 11 and weekly thereafter until day 102 as described. Horse antihemocyte globulin (ATG) 7 mg/kg was given for six doses every other day between days 20 and 31. Despite these measures the patient developed acute GVHD involving skin and liver on day 35. She was treated with prednisone 2 mg/kg for ten days, during which time the skin rash disappeared and liver function abnormalities improved. She did well until 121 days postgrafting, when she developed diffuse interstitial pneumonia. An open lung biopsy specimen failed to reveal an etiologic agent, and she recovered after receiving prednisone 1.5 mg/kg for two weeks.

In May 1982 she was observed to have diffuse fibrotic changes in both breasts. One year later she noted telangiectasis around the right areola, as well as more extensive fibrotic changes. Bilateral subcutaneous mastectomies were carried out, and on histologic examination, the tissue removed showed a leukemic infiltrate. A marrow examination showed that 50% of the marrow space was occupied by leukemic blasts similar in appearance to those in the breast tissue. The cytochemical stains and immunologic markers of the blast cells favored acute myelogenous leukemia FAB M-1 classification (myeloperoxidase and alpha-naphthlyesterase positive, PAS and terminal transferase negative; surface receptors for sheep erythrocytes, complement, IgG, and common acute lymphocytic leukemia antigen negative). Cytogenetic analysis of the leukemic cells showed multiple chromosomal abnormalities in six of ten metaphases examined (five metaphases with 47,XX, 5p-, 7p+, -8, 14q+, -16, -16, -22, -22 plus six unidentifiable chromosomes; one metaphase with 47,XX, 5p-, 8, 14q+, -16, -16, -20, -22, -22 plus seven unidentifiable chromosomes; four metaphases with 46XX). The patient and donor did not have different fluorescent cytogenetic polymorphisms before transplantation. The donor was examined and had no evidence of leukemia before or after marrow grafting.

DNA Restriction Fragment Length Analysis

Because there were no cytogenetic or other markers that could distinguish whether the malignant clone in the recurrent leukemia...
originated from cells of the marrow donor or represented reemergence of the patient's original leukemia, we exploited a restriction fragment length polymorphism that distinguishes the two genotypes. The clone pAW101, which originates from a highly polymorphic immunoglobulin heavy chain switch region was used in this analysis. High-molecular-weight DNA was prepared from the patient's normal skin fibroblasts, from the patient's marrow tumor cell sample, and from peripheral blood mononuclear cells of the donor. These were digested with EcoRI, electrophoresed on a 0.5% neutral agarose gel, transferred to nitrocellulose, and probed with the pAW101 probe as previously described.

RESULTS

Figure 1 shows that the analysis resulted in two bands of 19 and 16 kilobases (kb) when the patient's normal fibroblast DNA was used (lane A). However, DNA from both the tumor and marrow donor's mononuclear cells yielded bands of 16 and 12 kb (lanes B and C). The sensitivity of this analysis is such that we can estimate that at least 80% of the DNA in the tumor sample was of donor origin. The results of this analysis strongly suggest that all of the tumor DNA arose from donor cells, a conclusion that could best be supported by performing a similar analysis using different probes derived from different genomic fragments. Unfortunately, we lacked adequate amounts of DNA to repeat the analysis.

DISCUSSION

In 1976 this patient's leukemia was thought to be lymphocytic on the basis of morphological criteria. However, its failure to respond to treatment with vincristine and prednisone, commonly used for acute lymphocytic leukemia, and its subsequent response to daunomycin, cytosine arabinoside, and 6-thioguanine suggested that the disease may have been something other than acute lymphocytic leukemia at that time. Whatever the type of the original leukemia, the leukemia developing six years after transplant arose from a cell that was different from that of the original leukemia and probably arose in a donor cell.

Several possible mechanisms may exist, none of which can be proved at present, for the cellular or molecular events that led to this second malignancy. Previous occurrences of donor cell leukemia reported from this and other centers have all been observed within 36 months of transplantation. These early occurrences are compatible with relatively acute mechanisms of transformation. These mechanisms, which have been previously discussed, include transfer to the transplanted marrow cells of a dominant oncogene residing in the DNA of either an oncogenic virus or in chromosomes of degenerating irradiation-damaged host leukemic cells. In the present case, however, the six-year latent period between transplantation and expression of the leukemic transformation makes it more difficult to envision how such "single-hit" mechanisms might operate. A possible explanation for the long latent period may be found from recent studies in tissue culture and animal tumor systems. These studies suggest that in some cases more than one oncogene may be necessary for the full expression of neoplastic transformation. For example, this patient's leukemia may have resulted from transfer of one required oncogene at the time of transplantation and the activation of an additional required oncogene(s), which occurred after a long latent period.

These concepts are highly speculative and, of course, other factors may have contributed to expression of the leukemia. These factors include exposure of donor cells to methotrexate, impairment of immune surveillance of malignant clones by antithymocyte globulin and prednisone treatment, or chronic antigen stimulation of donor cells by minor histoincompatibilities with the host. The notion that these mechanisms played a role in inducing leukemia is perhaps less attractive because they are also operating in patients transplanted for aplastic anemia. We have successfully transplanted more than 200 aplastic anemia patients after conditioning with cyclophosphamide alone and methotrexate prophylaxis for acute GVHD and have not seen a case of leukemia developing in donor cells. Whatever the underlying mechanism, this case has significant implications for the interpretation of late relapses of leukemia in marrow graft recipients. The analysis suggests that the original leukemic cell population was eliminated by the transplantation procedure. It will be important to analyze additional cases in order to determine, in contrast to early relapse, whether most or all of late relapses occur in donor cells.
ACKNOWLEDGMENT

We thank Dr. Mang-So Tsoi for growing the patient's skin fibroblasts in culture to obtain original host DNA, and Bart Steiner for technical help.

REFERENCES

17. Balazs I, Purrello M, Rubenstein P, Alhadeff B, Siniscalco M: Highly polymorphic DNA site D1452 maps to the region of Burkitt lymphoma translocation and is closely linked to the heavy chain γ1 immunoglobulin locus. Proc Natl Acad Sci USA 79:7395, 1982
Donor cell leukemia developing six years after marrow grafting for acute leukemia

RP Witherspoon, W Schubach, P Neiman, P Martin and ED Thomas