

## Brief Report

## TRANSMISSION OF A T-CELL LYMPHOMA BY ALLOGENEIC BONE MARROW TRANSPLANTATION

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**S**ECONDARY cancer is a well-established long-term complication of bone marrow transplantation.<sup>1-3</sup> These post-transplantation cancers are usually associated with exposure to radiation, other genotoxic agents, or Epstein-Barr virus; in rare cases, they result from the transfer of neoplastic cells in transplanted tissue.<sup>4</sup>

We describe two sisters, one the donor and the other the recipient of a bone marrow transplant, in whom subcutaneous panniculitic T-cell lymphoma<sup>5,6</sup> developed three years after the procedure. The finding of identical T-cell clones in the tumors of both sisters implicated the transfer of neoplastic T cells during bone marrow transplantation as the cause of the recipient's subcutaneous lymphoma.

## CASE REPORTS

A 19-year-old woman presented in 1993 with stage IVB, anaplastic large-cell lymphoma of T-cell lineage (Ki-1 lymphoma). She had a complete remission after treatment with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) followed by etoposide, vincristine, doxorubicin, cyclophosphamide, and prednisone, but lymphomatous meningitis later developed. After conditioning therapy, she received a T-cell-depleted bone marrow transplant from her HLA-identical sister, who was 25 years old, and again entered a remission. She died of an independent lymphoma eight years after the initial diagnosis and seven and a half years after the transplant.

Three and a half years after the transplantation, the marrow donor presented with eczematous dermatitis on her legs, a nodule on her left arm, and fever. Biopsy of the nodule revealed lobular lymphocytic panniculitis with perivascular lymphocytes. Biopsy of a persistent nodule on her leg revealed an ulcerated epidermis overlying

a dense lobular subcutaneous infiltrate composed of pleomorphic, atypical lymphocytes that surrounded adipocytes (Fig. 1). The atypical cells were positive for CD3, CD8, granzyme B, and TIA-1 (a granular protein of cytotoxic T cells) and negative for CD56 and CD20. The infiltrate contained a monoclonal population of T cells with rearranged T-cell receptor  $\gamma$  (*TCR $\gamma$* ) genes and was negative for Epstein-Barr virus on in situ hybridization.

Despite treatment, the donor's cutaneous lesions and constitutional symptoms progressed. A serologic test for human T-cell lymphotropic virus type I was negative, and the results of a serologic test for Epstein-Barr virus were consistent with a history of prior exposure. Evidence of the hemophagocytic syndrome and pancytopenia developed. After two cycles of CHOP chemotherapy, gross gastrointestinal hemorrhage occurred, and the patient died two years after presentation.

One month before her sister's death the recipient was seen for a one-year history of skin lesions on her legs. Physical examination showed eczematous plaques and nodules on the lower part of both legs. Histologic examination of a nodule revealed many similarities to the subcutaneous panniculitic T-cell lymphoma identified in her sister, including atypical T cells infiltrating the dermis and subcutis, with rimming of adipocytes. The subcutaneous tumor cells were positive for CD3, CD8, TIA-1, and granzyme B and negative for CD30 (Ki-1) and CD56. Polymerase-chain-reaction (PCR) assessment of this tumor revealed a monoclonal rearrangement of the *TCR $\gamma$*  gene. In situ hybridization was negative for Epstein-Barr virus. The findings in the subcutaneous nodule were consistent with a diagnosis of subcutaneous panniculitic T-cell lymphoma rather than a recurrence of the original lymphoma.

The similarities of the sisters' tumors raised the suspicion that the lymphoma had been transmitted from the donor to the recipient during bone marrow transplantation. Therefore, further tests were conducted.

## METHODS

## Specimen Collection, Histochemical Analysis, and Isolation of DNA

Peripheral-blood specimens were obtained from the donor and the recipient before transplantation and from the recipient periodically after transplantation. Tumor-biopsy specimens from the two sisters were prepared for histologic examination according to standard techniques. The specimens were stained with the following immunohistochemical stains: CD3 (dilution, 1:300) (Dako, Carpinteria, Calif.), CD4 (dilution, 1:10) (Vector, Burlingame, Calif.), CD8 (dilution, 1:20) (Dako), CD20 (dilution, 1:1000) (Dako), CD30 (dilution, 1:180) (Dako), and TIA-1 (dilution, 1:500) (Coulter, Miami, Fla.). DNA was isolated according to standard methods.<sup>7</sup>

PCR for *TCR $\gamma$*  Gene Rearrangements

After informed consent was obtained from the patient and her mother, a two-step PCR assay was used to identify rearrangements of the *TCR $\gamma$*  subunit gene.<sup>8-11</sup> The sizes of the PCR products were determined with the use of capillary electrophoresis (model 310, Applied Biosystems, Foster City, Calif.).

## Microsatellite Analysis

Peripheral-blood samples obtained from the donor and recipient before transplantation and specimens of subcutaneous panniculitic T-cell lymphoma from the two sisters were assessed for nine microsatellite loci by PCR with use of a forensics identity-testing kit (ABI Profiler, Applied Biosystems).

## Tumor-Specific PCR Analysis

Cycle sequencing of the *TCR $\gamma$*  PCR products was performed directly or after cloning into a TA cloning vector (One-Shot INV- $\alpha$ , Invitrogen, Carlsbad, Calif.) with use of a dye-terminator method (Big Dye, Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems). Initial linear amplification of *TCR $\gamma$*  was per-

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formed as previously described.<sup>9</sup> The second, tumor-specific PCR was performed with the use of TV- $\gamma$ , a standard forward primer, and TJ-N(R) (5'GAGTTTCTTATGGAGGCGGG3'), a tumor-specific reverse primer, for 45 cycles. The TJ-N(R) primer is specific for the unique N-region and joining-region sequences identified in the lymphomas from the donor and recipient; N regions are sites of insertion or deletion of bases during the rearrangement of T-cell receptor genes.

## RESULTS

### The Size of the Rearranged *TCR $\gamma$* Genes

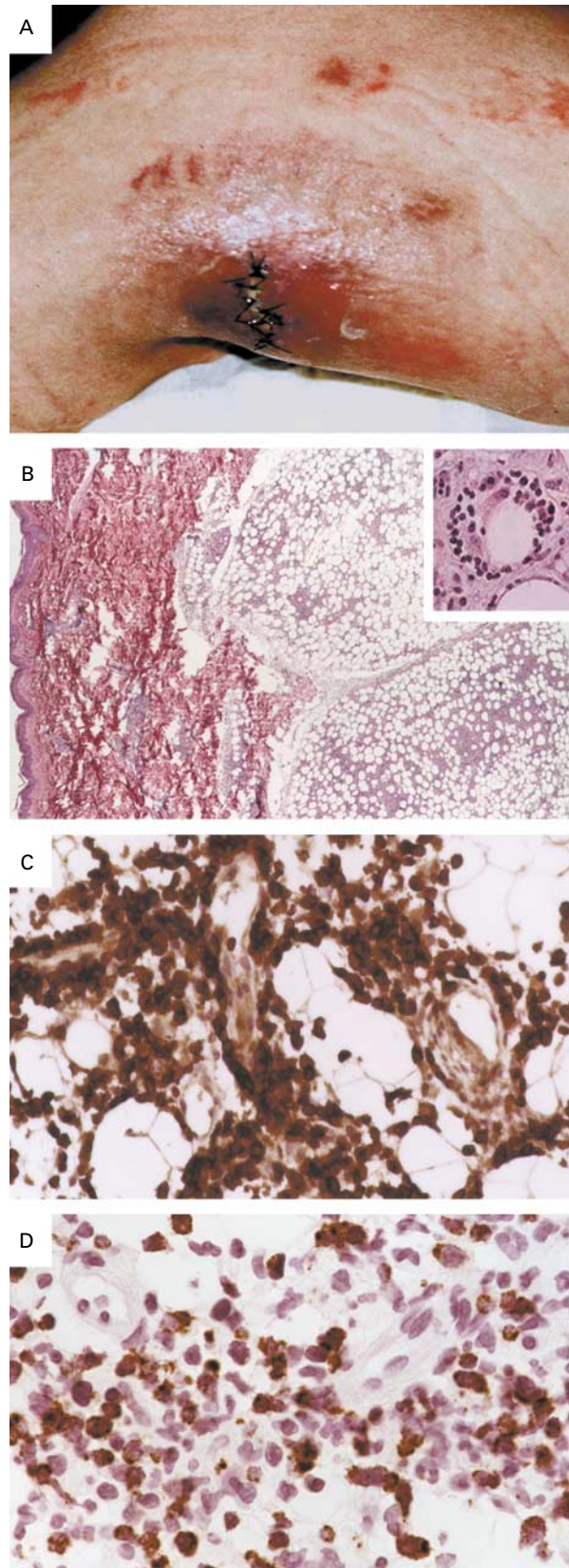
The DNA products (amplicons) generated by PCR analysis of randomly rearranged *TCR $\gamma$*  alleles vary in size and base sequence (Fig. 2A). PCR analysis of subcutaneous panniculitic T-cell lymphomas from the donor (Fig. 2B) and the recipient (Fig. 2C) produced amplicons of the same size (181 bases), findings consistent with the presence of identical T-cell clones in the two tumors. Similar analysis of the recipient's original Ki-1 lymphoma did not reveal this T-cell clone (data not shown).

### Microsatellite Analysis

We assessed whether the recipient's post-transplantation lymphoma was derived from donor or recipient cells using an identity-testing PCR kit that amplifies nine microsatellite loci. Figure 3 illustrates the result at one locus. The germ-line patterns of the donor's cells (Fig. 3A) and recipient's cells (Fig. 3B) differ. The patterns in the donor's lymphoma (Fig. 3C) and the recipient's lymphoma (Fig. 3D) were identical; both matched the donor's germ-line pattern. At other loci tested (data not shown), donor alleles were also the dominant pattern in the recipient's subcutaneous panniculitic T-cell lymphoma.

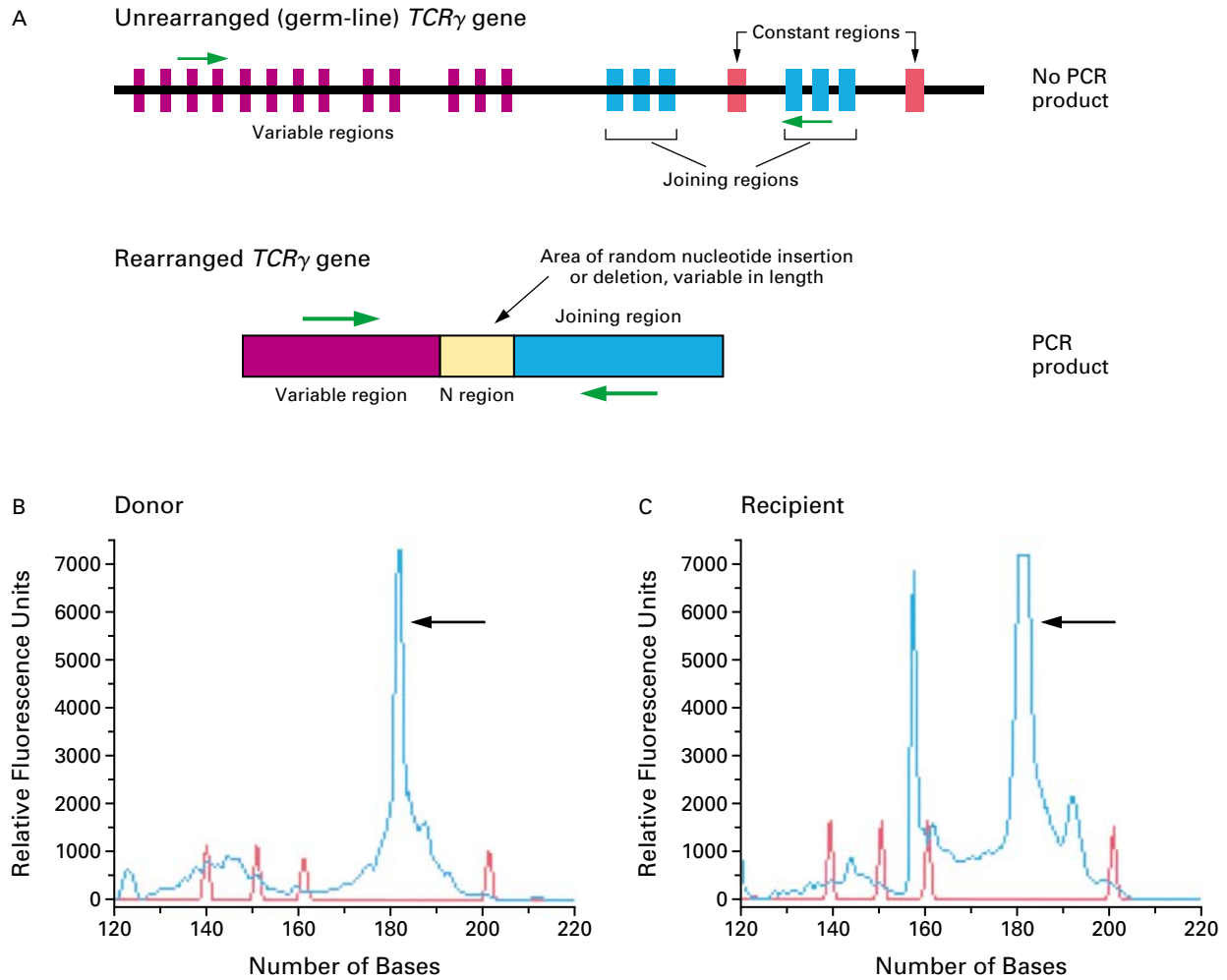
### Sequences of the Rearranged *TCR $\gamma$* Genes

We sequenced the *TCR $\gamma$*  gene PCR products from the subcutaneous-lymphoma specimens from the donor and recipient to identify the *TCR $\gamma$*  gene rearrangements. To do so, PCR amplicons were cloned, and the resulting sequences of the cloned products were identical in both tumors (Fig. 4). Particularly relevant were the identical N regions in the two tumors (Fig. 4). N regions are unique clonal markers because they are sites of random insertions and deletions of bases during the rearrangement of T-cell receptor genes.



**Figure 1.** The Donor's Lymphoma.

Panel A shows the nodular lesion on the donor's leg. Panel B shows the atypical lobular pattern of subcutaneous panniculitic T-cell lymphoma (hematoxylin and eosin,  $\times 100$ ) with pleomorphic lymphoid cells surrounding an adipocyte (inset; hematoxylin and eosin,  $\times 600$ ). Immunostaining showed that the neoplastic cells were positive for CD3, a pan-T-cell marker (Panel C), and TIA-1, a granular protein of cytotoxic T cells (Panel D) (Panels C and D: peroxidase and hematoxylin counterstain,  $\times 1600$ ).



**Figure 2.** Germ-Line and Rearranged T-Cell Receptor  $\gamma$  ( $TCR\gamma$ ) Gene (Panel A) and Results of T-Cell Receptor  $\gamma$  PCR Assay of Biopsy Samples of Subcutaneous Panniculitic T-Cell Lymphoma from the Donor (Panel B) and the Recipient (Panel C).

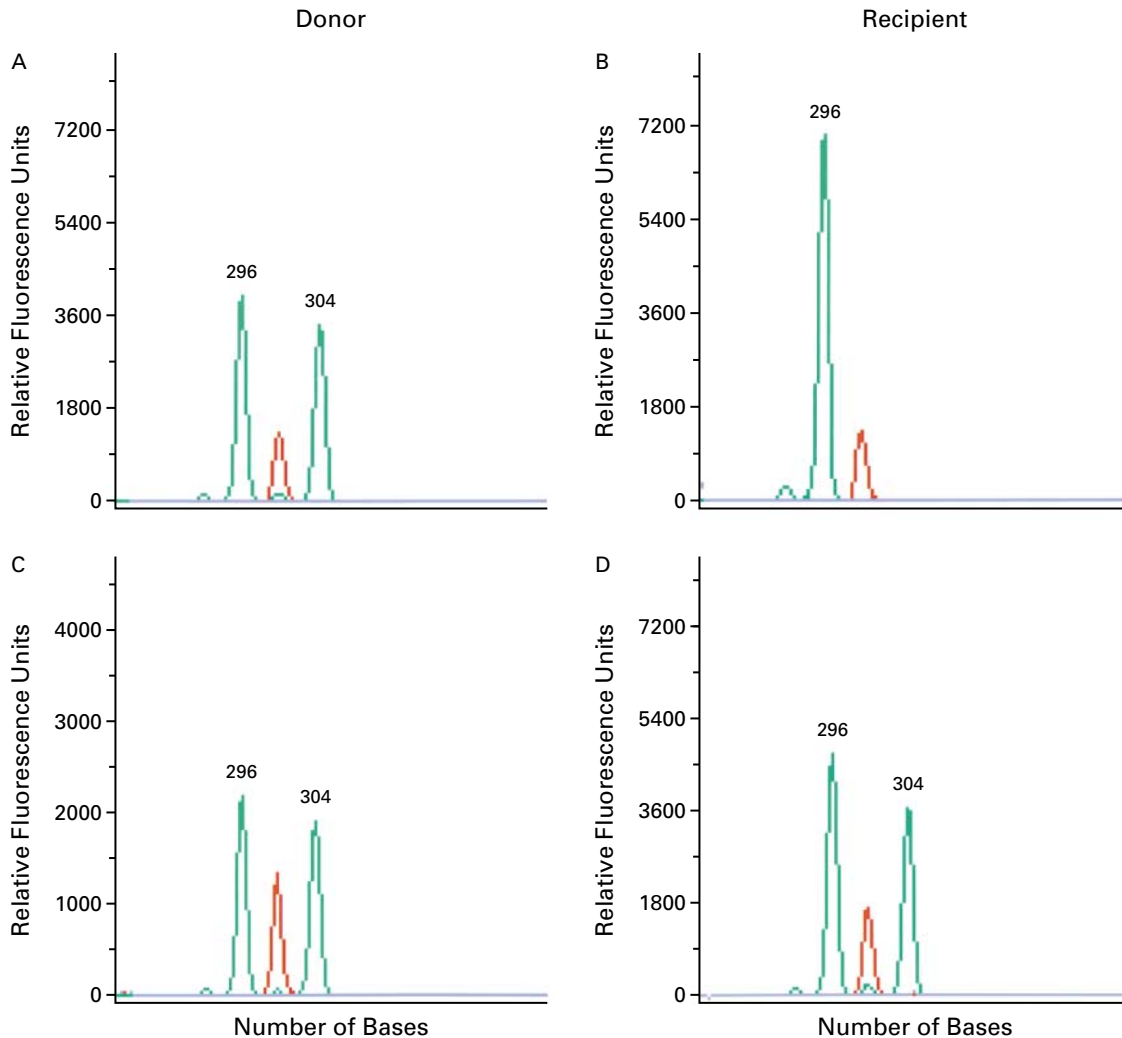
In Panel A, unrearranged (germ-line)  $TCR\gamma$  alleles yield no PCR product, whereas rearranged  $TCR\gamma$  alleles yield a PCR product because of the juxtaposition of the variable and joining segments. PCR primers are indicated by green arrows. A dominant peak at 181 bases (arrows) representing a monoclonal  $TCR\gamma$  gene rearrangement is present in tumor specimens from both the donor (Panel B) and the recipient (Panel C). The other peak, seen at position 157, in the recipient's tumor probably represents a subgroup of neoplastic T cells that have clonally evolved or that have rearranged their second T-cell receptor allele. Controls consisted of water and monoclonal and polyclonal specimens from the unrelated patients. The red peaks represent the internal size standard.

**PCR Analysis of Blood Cells before and after Transplantation**

On the basis of the above sequencing data, we designed a tumor-specific assay using a PCR primer complementary to the unique joining-region and N-region sequences of the neoplastic clone (Fig. 4). We validated the results of the assay using a tumor sample from the recipient (Fig. 5A). Controls, consisting of three polyclonal and five unrelated monoclonal samples, were not amplified by the tumor-specific primer (data not shown).

Examination of peripheral-blood cells obtained

from the donor before bone marrow transplantation with use of the standard T-cell receptor PCR assay did not reveal the malignant 181-base clone (Fig. 5B). This assay can detect approximately 1 clonal T cell among 100 polyclonal T cells.<sup>9</sup> The tumor-specific PCR of peripheral-blood cells obtained from the donor before transplantation revealed a  $TCR\gamma$  clone identical in size to that in her tumor (Fig. 5C), demonstrating that her blood contained clonal T cells when her marrow cells were harvested. The tumor-specific PCR analysis of bone marrow obtained from the recipient before transplantation showed no evi-



**Figure 3.** Results of Microsatellite Analysis at One Locus from the Peripheral Blood and Tumors of the Donor (Panels A and C) and the Recipient (Panels B and D).

The profile of one of the nine microsatellite loci obtained before transplantation (CSF1PO) is shown for peripheral blood from the donor (Panel A) and the recipient (Panel B). The donor is heterozygous at this locus (296 and 304 bases), whereas the recipient is homozygous (296 bases). Analysis of the sample of the subcutaneous panniculitic T-cell lymphoma from the donor (Panel C) shows the same pattern identified in peripheral-blood samples (Panel A), as expected. The pattern identified in the sample of subcutaneous lymphoma from the bone marrow–transplant recipient (Panel D) does not match the germ-line pattern (Panel B), but it does match the donor's pattern (Panel A). The red peaks represent the internal size standard of 300 bases.

dence of the malignant clone, but multiple samples of bone marrow and peripheral blood obtained after transplantation (from 1994 to 1999) were positive for the donor's clonal *TCRγ* rearrangement. These data show that the donor's neoplastic T-cell clone persisted in the recipient for five years before clinically evident disease appeared.

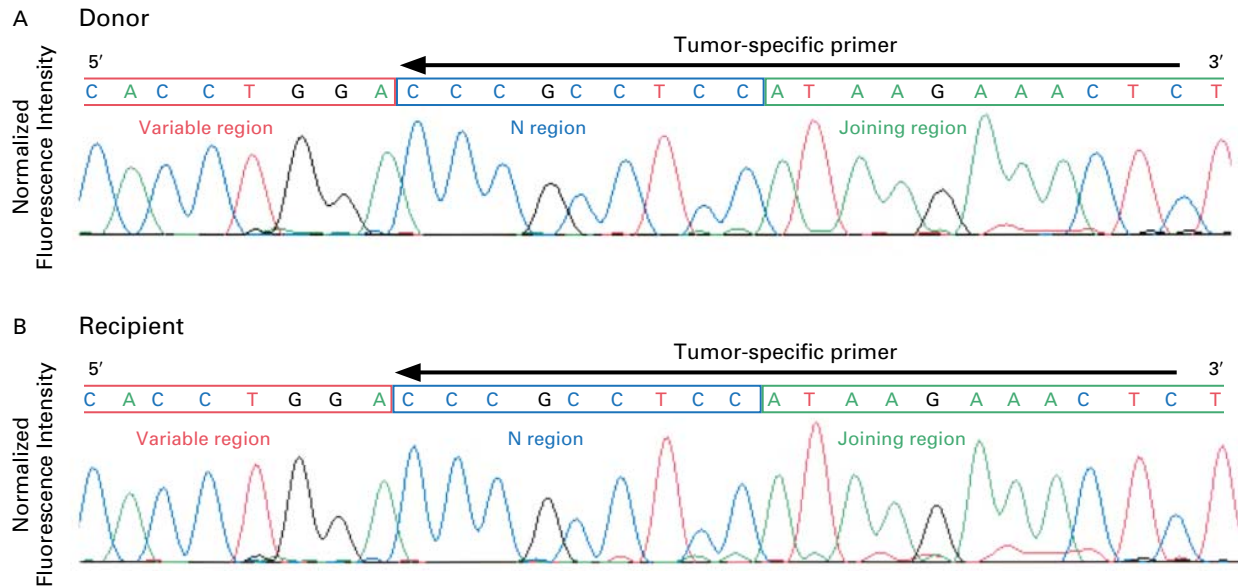
#### DISCUSSION

We describe the transmission of subcutaneous panniculitic T-cell lymphoma by bone marrow transplantation. This rare lymphoma, a proliferation of neo-

plastic T cells, is initially characterized by multiple subcutaneous nodules, especially on the arms and legs. Its behavior ranges from indolent to aggressive, and an adverse outcome is commonly associated with the hemophagocytic syndrome.<sup>5,12-15</sup>

The molecular fingerprint of this tumor, the rearrangement of its *TCRγ* gene, permitted us to document not only that the subcutaneous tumor in the recipient originated in the donor but also that there was a long period of latency (three years) before the neoplasm became clinically evident.

Patients who undergo bone marrow transplanta-



**Figure 4.** Sequencing of the Cloned Products of PCR Analysis of Specimens of Subcutaneous Panniculitic T-Cell Lymphomas from the Donor (Panel A) and the Recipient (Panel B).

The sequences of both clones were identical. The identical N regions (shown in blue) about identical variable regions (red) and joining regions (green). Only portions of the variable- and joining-segment sequences, close to the N region, are shown. The arrow over each chromatogram shows the position and direction of the tumor-specific primer used in the tumor-specific PCR assay.

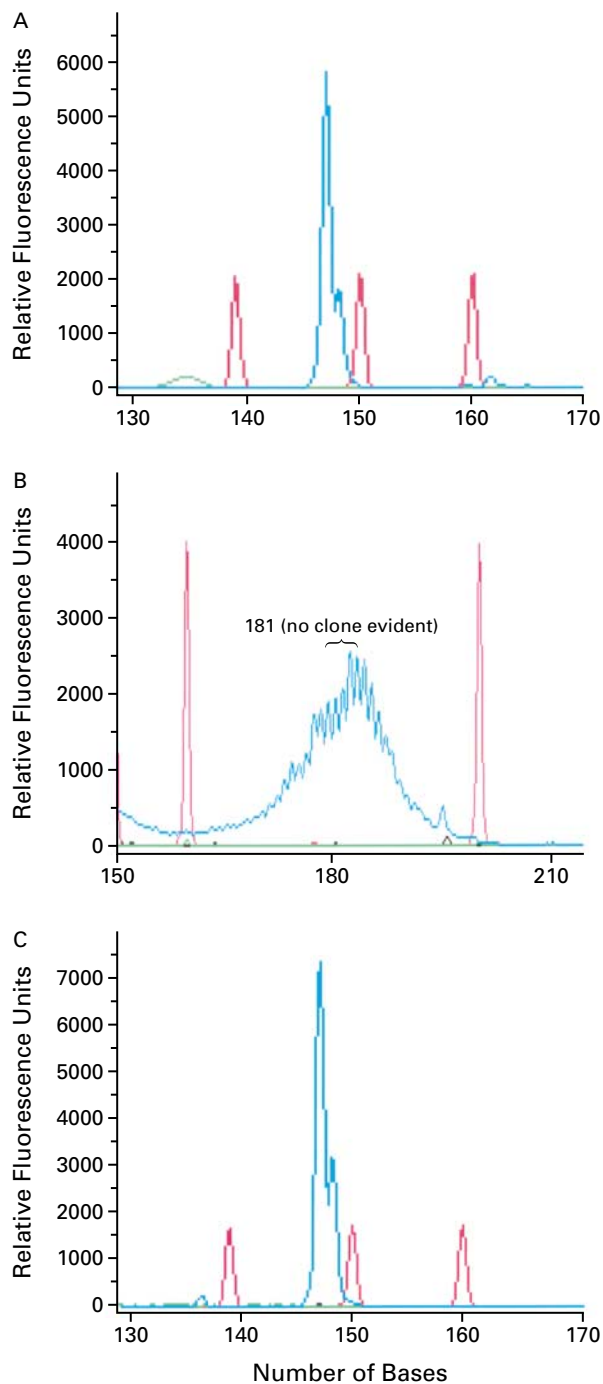
tion are at risk for the transmission of a variety of conditions.<sup>16,17</sup> Although the risk of a secondary hematologic cancer is increased by a factor of four to seven,<sup>3,18,19</sup> reflecting the mutagenicity of the preparative regimens for transplantation, the transmission of neoplastic tissue by bone marrow and solid-organ transplantation is uncommon. Acute promyelocytic leukemia has been transferred by the transplantation of a cadaveric liver allograft,<sup>20</sup> metastatic glioblastoma multiforme has been transferred in kidney and liver transplants,<sup>21,22</sup> and acute myeloid leukemia has been transmitted by the transplantation of donor bone marrow.<sup>4</sup>

Using molecular techniques, we have identified a case of transmission of subcutaneous T-cell lymphoma by a bone marrow transplantation. One important aspect of this case is that two independent T-cell lymphomas (the recipient's original anaplastic large-cell lymphoma and the donor's subcutaneous panniculitic T-cell lymphoma) arose in two sisters at young ages. Although it is tempting to postulate that there was a common genetic or infectious cause, these lymphomas appear to be clinically and histologically distinct from the familial lymphoma syndromes that arise in patients with Epstein-Barr virus or human T-cell lymphotropic virus type I infections.<sup>23-25</sup> Moreover, tests of the bone marrow recipient were negative for both these infectious agents. It is also interesting to con-

sider the possibility that with increasing age and a corresponding increase in the frequency of occult neoplasms in older donors, the likelihood of the transfer of a tumor during transplantation will increase. Methods for preventing such transfers may require

**Figure 5 (facing page).** Results of T-Cell Receptor  $\gamma$  PCR and Tumor-Specific PCR of Specimens of Subcutaneous Panniculitic T-Cell Lymphoma and Peripheral Blood from the Donor and the Recipient.

Tumor-specific PCR, performed with use of the tumor-specific reverse primer, reveals a strong peak at 148 bases in the recipient's tumor (Panel A). Serial DNA dilution experiments with DNA from the recipient's tumor demonstrated a limit of detection of approximately 1 tumor cell in 50,000 nontumor cells (data not shown). A standard T-cell-receptor PCR assay was used to examine the donor's peripheral blood at the time of transplantation (Panel B) and showed no evidence of the neoplastic clone, which would have been indicated by a peak at 181 bases. Assessment of the same peripheral-blood sample from the donor using tumor-specific PCR (Panel C) shows the 148-base tumor-specific rearrangement. The discrepancy in the length of the PCR product between the two assays (181 bases for the T-cell-receptor PCR, as compared with 148 bases for tumor-specific PCR) results from the shifting of the reverse primer to a more proximal (5') position in the tumor-specific PCR assay. The red peaks represent the internal size standard.



the transplantation of highly purified stem cells or the development of more sensitive screening assays for neoplastic cells.

We are indebted to Drs. William Baldwin, Steven Desidario, Frederick Racke, Noel Rose, and Johnathan Schneck for helpful discussions; to Dr. Terry Barrett for critical review of the manuscript;

to Cynthia Glaser, M.T., Karen Miller, M.T., Michael Hafez, M.T., and Lisa Cooper, M.T., for technical assistance; and to Anita Hawkins for cytogenetic analysis.

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