HTLV-III Infection After Bone Marrow Transplantation

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We prospectively documented the development of a fatal, secondarily acquired severe immunodeficiency in a 19-year-old man who underwent uncomplicated bone marrow transplantation. He had no graft versus host disease (GVHD) and had normal recovery of his immune system as determined by lymphocyte phenotyping, mitogenic responses of his peripheral blood lymphocytes, and his ability to secrete immunoglobulin. This alteration in immunity was associated with the acquisition of antibody to HTLV-III. His only risk factor for the development of HTLV-III infection was the transfusions he had received during the transplant and recovery period. Two of his 54 transfusions were from an asymptomatic individual at high risk for acquired immunodeficiency syndrome (AIDS), who was subsequently found to be seropositive for anti-HTLV-III and from whom HTLV-III was isolated. The loss of immunocompetence in patients without chronic GVHD disease is unusual, and our data support the view that this patient’s immunodeficiency was due to HTLV-III. When bone marrow transplant recipients without chronic GVHD develop late opportunistic infections, consideration should be given to transfusion-associated AIDS.

Phenotype analysis. Peripheral blood mononuclear cells were obtained by Ficoll-Paque (Pharmacia, Piscataway, NJ) density gradient centrifugation of heparinized peripheral blood. Monocytes were depleted by incubation with carbonyl-iron (Technicon, Tarrytown, NY), Ficoll-Paque centrifugation, and plastic adherence. The surface phenotypes were established by incubating the cells for 30 minutes at 4 °C with the fluoresceinated monoclonal antibodies Leu-4, Leu-2, and Leu-3 (Becton Dickinson, Mountain View, Calif) and were washed three times with phosphate-buffered saline (PBS). They were analyzed on a FACS Analyzer (Becton Dickinson, Sunnyvale, Calif).

Lectin-stimulated proliferation. Peripheral blood was collected in preservative-free heparin (100 U/mL) and the mononuclear cells were separated on a Ficoll-Paque gradient. The mononuclear cells were washed twice in Hanks’ balanced salt solution (HBSS) (Grand Island Biological Co, Grand Island, NY) and resuspended in RPMI 1640 (GIBCO) with 5% heated inactivated human AB serum, L-glutamine, penicillin, streptomycin, and mycostatin. Blastogenic responses to phytohemagglutinin (PHA) and concanavalin A (Con A) (Difco Laboratories, Detroit) were determined in triplicate in a microtiter system with 10 cells per well. Optimal concentrations of mitogens were determined in preliminary experiments on normal donors for each batch of mitogen and, in the case of PHA, three concentrations of PHA over a tenfold range were assessed for each separate patient determination to assure study at maximum response. Median concentrations required were 2 μg/mL of PHA and 3 μg/mL of Con A. Cells were incubated at 37 °C in a 5% CO2 incubator and were pulsed on day 3 for 18 hours with 1.0 mCi/mL 3H-thymidine (New England Nuclear, Boston, Mass). Cultures were harvested on an automatic microtiter harvester (MASH II; M.A. Bioproducts, Bethesda, Md), and incorporated radioactivity was determined in a scintillation counter. PHA and Con A stimulation indices are determined by dividing the observed counts per minute (cpm) by the background control values; in our laboratory, normal stimulation indices are >20.

Screening of blood donors. Blood donation numbers of all blood products transfused to the patient were provided to the regional blood collecting facility. Donors were contacted by the collecting facility and 15 agreed to complete an extensive questionnaire and to be screened with complete blood counts, lymphocyte phenotyping, and anti-HTLV III serology (performed at the Centers for Disease Control).

RESULTS

Case report. A 19-year-old, white man without prior history of homosexuality, drug abuse, bleeding disorder, travel to Haiti, or transfusion was admitted to the Brigham and Women’s Hospital in September, 1981 with acute
monocytic leukemia (FAB M5a). Complete remission was achieved with daunorubicin and cytosine arabinoside (ara-C), and he received four cycles of consolidation chemotherapy (daunorubicin, ara-C, and 6-thioguanine). During the period of anti-leukemia chemotherapy, he received 10 units of washed red blood cells (RBC), 3 units of single donor platelets, but no fresh frozen plasma or granulocyte transfusions.

In April 1982, he underwent allogeneic bone marrow transplantation from his HLA-matched, MLR-nonreactive brother. The patient was prepared for transplantation with ara-C 500 mg/m²/d for seven days, cyclophosphamide 60 mg/kg/day for two days, and 850 rad single-fraction total body irradiation at a midline dose rate of 5 rad per minute. After transplantation, he received four doses of methotrexate (10 mg/m²) on days 1, 3, 6, and 11. His course was uncomplicated; he had >500 granulocytes per microliter at day 34, and developed grade I acute GVHD, which did not require therapy. No chronic GVHD was observed clinically or on skin biopsy. Following his transplant, he received 7 units of washed RBC as well as platelet transfusions from 18 different donors, either as platelet pheresis products or as single donor platelet concentrate (prepared from whole blood donors). He never required granulocyte transfusions or fresh frozen plasma. An arteriovenous shunt (which had been placed prior to transplant for vascular access) was removed on day 240 (month 8, October 1982); during the surgery, he received an additional 2 units of washed RBC and two bags of pooled platelet concentrates from a total of 13 donors. The next year he was spent primarily at home, with limited contact with nonhousehold members. He had mild persistent thrombocytopenia after transplant, with platelet counts ranging from 70,000 to 100,000/µL. During this time in vitro lymphocyte function returned to normal (Fig 1) as did immunoglobulin levels, but his absolute lymphocyte count had started to decline. He denied homosexual activity or intravenous drug use.

On day 680 (month 23, February 1984) he developed a low-grade fever and cough. Over the ensuing two to three weeks, he had slowly progressive shortness of breath; he became hypoxic, and his chest X-ray demonstrated a diffuse interstitial pneumonitis. Open lung biopsy established the diagnosis of P. carinii pneumonia. Immediate treatment was complicated by intravenous thrombocytopenia after transplant, with platelet counts ranging from 70,000 to 100,000/µL. During this time in vitro lymphocyte function returned to normal (Fig 1) as did immunoglobulin levels, but his absolute lymphocyte count had started to decline. He denied homosexual activity or intravenous drug use.

After recovery from the pneumonia, he continued to have daily fevers to 101 °F, although no source of infection could be determined. The thrombocytopenia never resolved (range 10,000 to 50,000/µL), although bone marrow aspiration demonstrated adequate numbers of megakaryocytes and showed no evidence of recurrent leukemia. He became severely cachectic, and lost >20% of his body weight; he did not respond to parenteral nutrition therapy. Over the next three months, he developed recurrent bilateral pneumothoraces, which required virtually constant chest tube drainage. There were no skin lesions consistent with Kaposi's sarcoma. Just prior to his death, he became obtunded and had focal seizures. CAT scan revealed an enhancing lesion in the left frontal cortex, which was thought to be consistent with toxoplasmosis, lymphoma, or abscess. Brain biopsy was refused. He died 850 days after transplantation. Postmortem examination was refused.

**HTLV-III.** Frozen serum specimens were obtained from March 1982 (immediately prior to the transplant), and day 75 (May 1982, immediately following the transplant) as well as a fresh serum specimen on day 780 (month 25, May 1984). His serum was examined for anti-HTLV-III antibodies by indirect immunofluorescence on an HTLV-III-infected H9 cell line by Essex and an established technique, and was found to be negative before transplant on day 75.
but to be positive on day 780 (Fig 1). The positive test was confirmed by radioimmunoprecipitation (Western blot analysis). The marrow donor had no risk factors for AIDS. He was also seronegative for HBsAg and cytomegalovirus (CMV), and had normal responses to lectin stimulation of his peripheral blood lymphocytes immediately prior to the marrow harvest. Although the marrow donor could not be tested for anti-HTLV-III antibodies, the survey of blood product donors revealed one male, homosexual subject who donated two pheresis platelet products in October and November 1981, both of which were transfused into this patient. At the times of donation, the donor met all of the criteria for normal voluntary blood and platelet pheresis donors. In January 1985, this donor was contacted, and blood was subsequently found to be anti-HTLV-III positive by the Centers for Disease Control; HTLV-III virus was recovered. His complete blood count (CBC) (including absolute lymphocyte count and platelet count) and immunoglobulin levels were normal, but his helper/suppressor ratio was 0.37 (normal 0.9 to 3.5). At the time of writing, he remains asymptomatic.

**DISCUSSION**

Although the recovery of immune function after bone marrow transplantation may be prolonged, most patients who do not have chronic GVHD have relatively normal in vitro parameters of immune function by 1 year. Once in vitro immune reactivity recovers, it generally persists. Furthermore, patients without chronic GVHD rarely develop *P. carinii*, or other opportunistic infections as late (> 1 year) complications of transplantation. At a time when immune function is usually stable, this patient developed severe lymphopenia, loss of Leu-3+ lymphocytes, cutaneous anergy, loss of his in vitro mitogen responsiveness, opportunistic infection, diffuse lymphadenopathy, severe thrombocytopenia, and polyclonal hypergammaglobulinemia (IgA). This discrepancy from the usual course of immunologic reconstitution after marrow grafting suggested that the illness was not due to a late complication of transplantation. The clinical syndrome was consistent with the diagnosis of HTLV-III-associated immunodeficiency or AIDS. Because we perform lymphocyte function studies on our transplant patients during and after transplantation, we were able to document prospectively the timing and course of immunologic recovery and subsequent immunoincompetence which led to his death. It is the loss of his in vitro cellular immune reactivity following recovery that led us to suspect that he had AIDS, rather than the persistent defects in cellular immunity that have been reported after marrow grafting. All of the above observations are consistent with the selective infection of Leu-3+ cells with HTLV-III and the consequent disproportionate loss of helper/inducer cells.

His only known risk factor for the development of HTLV-III infection was the transfusion of 54 blood products that he had received during the leukemia treatment, transplant, and recovery period, 2.5 to 3 years prior to his illness and, in particular, 2 units from an anti-HTLV-III seropositive, homosexual individual transfused 16 months prior to the first demonstrable reduction in Leu3+ cells, and 34 months prior to death. The latency between this transfusion and the development of opportunistic infections is consistent with the incubation period observed in transfusion-associated AIDS. It is unusual to be able to document the probable date of HTLV-III exposure and complement that dating with serial immune function studies. It is notable that the patient was seronegative immediately prior to his transplant. The subsequent development of HTLV-III antibodies suggests continued viremia followed by antibody production when his humoral immune response recovered. On the other hand, viremic but antibody-negative patients have been reported, and it is unknown to what extent his underlying leukemia and chemotherapy might have delayed or prevented the development of antibody. However, it is also possible that the blood product that resulted in the HTLV-III transmission was administered either during the transplant or shunt removal from a donor who was not contacted.

The appearance of anti-HTLV-III antibody after transplantation demonstrates that the patient was capable of mounting a humoral immune response to new antigens, and supports the hypothesis that he had actually developed AIDS. Antibodies to HTLV-III are uncommon in the general population, and he received plasma only in association with platelet transfusions; thus, it is unlikely that the antibody titer reflects passive transfer of immunoglobulin.

In patients who have undergone marrow grafting and in other patients with immunodeficiencies, abnormalities of the helper/suppressor (Leu-3/Leu-2) T cell ratio are difficult to interpret. Evidence from multiparameter flow cytometric analysis of posttransplant lymphocytes shows that in many patients the Leu-2+ cells may not be mature suppressor/cytotoxic T cells by conventional criteria (ie, simultaneously expressing the surface antigens Leu-2 and Leu-4). Leu-2 also appears to be on a subset of natural killer (NK) cells (Leu-2+, Leu-11+, Leu-4—, and cytolytic against K-562). In many transplant patients, these Leu-2+ NK cells predominate during lymphoid regeneration. The presence of large numbers of Leu-2+ NK cells would tend to reduce the “helper/suppressor” ratio as measured by single parameter phenotyping. Therefore, the use of “helper/suppressor” T cell ratios may be misleading in this group of patients. Since his cells were not studied by multiparameter analysis, it is uncertain whether in this case the anti-Leu-2 labelled cells are NK cells or suppressor T cells. However, in this case there was clearly a reduction in the absolute number of Leu-3+ cells. These observations support the suggestion that the quantitation of subsets of T lymphocytes is a more appropriate indicator of HTLV-III infection than subset ratios.

Although patients who undergo bone marrow transplantation are exposed to blood products from many donors, the risk of transmission of HTLV-III is small. It can be inferred from the recent reduction in the number of 17- to 35-year-old, male blood donors and HBsAg-positive blood donors that publicity and FDA guidelines have reduced the numbers of blood donors at high risk for transmitting HTLV-III. The recent general availability of anti-HTLV-III screening of all blood donors is expected to reduce this risk even further. However, due to the variable and long incuba-
tion period, some patients transplanted in the last one to five years may still be at risk. When patients without chronic GVHD develop opportunistic infections or lose immune reactivity more than one year after transplantation, consideration should be given to transfusion-associated AIDS.

REFERENCES


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JH Antin, BR Smith, BM Ewenstein, RJ Arceci, JM Lipton, PL Page and JM Rappeport