Syngeneic Leukocytes Together With Suramin Failed to Improve Immunodeficiency in a Case of Transfusion-Associated AIDS After Syngeneic Bone Marrow Transplantation

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A 22-year-old man who underwent syngeneic bone marrow transplantation (BMT) for acute lymphoblastic leukemia acquired a human immunodeficiency virus (HIV) infection by transfusion of blood products from a donor at risk. The manifestations were acute encephalopathy together with thrombocytopenia in the early posttransplant period, and acquired immunodeficiency syndrome (AIDS) developed 20 months after BMT. Because he had a syngeneic donor, the possibility of reconstituting the immune system was investigated by repeated transfer of healthy syngeneic lymphocytes and by combining repeated transfer of syngeneic lymphocytes with the antiviral agent suramin to protect the infused leukocytes from being attacked by HIV. No improvement was observed clinically or in the patient’s immune functions by these efforts.

Immunologic function tests. In vitro cultures were performed in quadruplicate in round-bottomed microtiter plates. The culture medium consisted of RPMI 1640 medium containing bicarbonate (22 mmol/L), L-glutamine (2 mmol/L), penicillin (10,000 U/mL), streptomycin (100 µg/mL), and 20% heat-inactivated AB serum.

Mitogen- and antigen-induced proliferation. Cultures containing 1 × 10⁶ PBMC per well were stimulated with the mitogens phytohemagglutinin (PHA, 100 µg/mL; HA15; Wellcome Reagents, Beckenham, England), concanavalin A (Con A, 75 µg/mL; CALBiochem-Behring Corp, La Jolla, CA), and pokeweed mitogen (PWM, 50 µg/mL; GIBCO, Grand Island, NY), and the antigens tetanus toxoid (3 LF/mL; RIVM, Bilthoven, The Netherlands), diphtheria toxoid (3.3 LF/mL; RIVM), and Candida antigen (70 µg/mL; HAL, Haarlem, The Netherlands). On the last day of culture (day 4 for mitogens, day 6 for antigens), 1 µCi ³H-thymidine was added for eight hours, and the incorporated radioactivity was determined in a scintillation counter. The results (counts per minute) are expressed for mitogens as the percentage of a normal control tested on the same day and, for antigens, as stimulation indexes (SI): SI = mean counts per minute in cultures with antigens/mean counts per minute in cultures without antigens.

Helper and suppressor T cell activity and B cell differentiation capacity. These effector functions were determined as described previously. In brief, T and non-T cells from patient and control were separated, and mixtures of 2 × 10⁶ non-T cells and 2 × 10⁶ T cells (irradiated) were stimulated with PWM. B cell differentiation was studied after six days of culture by counting the number of cytoplasmic Ig-positive cells demonstrated by immunofluorescence. T cell effector functions and B cell differentiation capacity were expressed as the percentage of a normal control by means of the following formulas: (a) percent T cell helper function = (control non-T cell fraction + irradiated patient T cell fraction)/(control non-T cell fraction + irradiated control T cell fraction) × 100; (b) percent T cell suppressor function = [1 – (control non-T cell fraction + irradiated control T cell fraction + patient T cell fraction)] × 100; and (c) percent B cell differentiation = (patient non-T cell fraction + irradiated control T cell fraction)/(control non-T cell fraction + irradiated control T cell fraction) × 100.

Lymphocyte stimulation by herpesvirus-infected cells. Cell-mediated immunity to herpes simplex virus (HSV), cytomegalovirus (CMV), and varicella-zoster virus (VZV) was tested by culturing PBMC with fibroblasts infected with HSV (type 1, strain MS), CMV (strain AD 169), and VZV (strain ZI 63-1444). Control cultures included noninfected fibroblasts. Cultures were set up with 1 × 10⁶ PBMC and 1 × 10³ fibroblasts per well. On day 6 of culture 1 µCi ³H-thymidine was added, and the incorporated radioactivity was determined. Control cultures had low levels of radioactivity. The results are expressed as the SI.

MATERIALS AND METHODS

Phenotype analysis. Peripheral blood mononuclear cells (PBMC) were obtained by FicolIsopaque density gradient centrifugation. The surface phenotypes were determined in indirect immunofluorescence by reacting to Leu 4 (CD3, pan-T cell), Leu 2 (CD8, suppressor/cytotoxic T cell), Leu 7 (natural killer [NK] cell), and HLA/DR (clone L243) from Becton Dickinson, Rutherford, NJ, and B1 (CD20, pan-B) from Coulter Immunology, Hialeah, FL.

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SYNGENEIC LEUKOCYTES AND SURAMIN FOR AIDS

Delayed-type hypersensitivity. Intradermal injections were done with Multitest CM1 (Institut Méries, Lyon, France) containing 0.05 mL of the following antigens: Candida, 2,000 Mérieux U/mL; tuberculin, 300,000 IU/mL; Streptococcus, 2,000 Mérieux U/mL; diphtheria, 1,100,000 Mérieux U/mL; tetanus, 550,000 Mérieux U/mL; Proteus, 150 Mérieux U/mL; and Trichophyton, 150 Mérieux U/mL.

Antibodies to HIV. A commercially available enzyme-linked immunosorbent assay with purified HIV as the antigen (Vironostika, Organo, Oss, The Netherlands) was used to demonstrate the presence of antibodies to HIV. The positive test was confirmed by Western blot analysis.12

HIV antigen assay. Serum samples were assayed for HIV antigen by a solid-phase immunoassay (Abbott Laboratories, North Chicago, IL) as described previously.13

Consents. All procedures were carried out, with protocols approved by the local investigational review board. The donor and recipient gave informed consent for the study.

Case report. A 22-year-old man with an unremarkable medical history who did not fall within the well-established risk groups for AIDS was admitted to our hospital in April 1982 with acute lymphoblastic leukemia. Phenotyping of the blasts showed CD2, CD3, CD4, and CD38 positivity (mature helper T cells). He achieved a complete remission (May 1982) with daunorubicin, vincristine, and prednisone. CNS prophylaxis was given intrathecally with methotrexate. He received consolidation therapy with high-dose cytosine arabinoside and cyclophosphamide. Fourteen days after consolidation therapy an early relapse (June 1982) was observed, and aspiraginase was given, which resulted in a second complete remission. During the period of remission-induction chemotherapy he received 4 units of platelet concentrates (one unit contains platelets from six random donors) and 14 units of RBCs (one unit contains red cells from one random donor).

In July 1982 a bone marrow transplant from his identical twin was given. The conditioning regimen consisted of cyclophosphamide (120 mg/kg) and total-body irradiation (8 Gy; single fraction, 16 cGy/min). The early posttransplant period was uneventful, and he left the hospital 19 days posttransplant in good clinical condition with 8,300 leukocytes/μL and 70,000 platelets/μL. A bone marrow biopsy (and aspirate) showed 30% cellularity with a fair number of megakaryocytes. In this early posttransplant period he received platelet concentrates from his marrow donor (four times) and 14 units of RBCs (after BMT all allogeic blood cell transfusions were irradiated with 20 Gy).

He was readmitted to the hospital 20 days after discharge (39 days after BMT) because of grand mal seizures and lethargy. At admission, thrombocytopenia (9,000 cells/μL) was observed, and antiplatelet antibodies were found (not present in his identical twin). A computed axial tomography (CAT) scan of the cerebrum was normal, and lumbar puncture revealed CSF at normal pressure and without abnormalities. An EEG showed diffuse encephalopathy. He was treated with carbamazepine (Tegretol), prednisone, and platelet concentrates. He regained full consciousness within 48 hours. At discharge (September 1982) his (self-sustaining) platelet count was 30,000/μL, and a bone marrow aspirate showed hypercellularity without abnormalities. One month after discharge his platelet count increased to >100,000/μL. Prednisone treatment was tapered and stopped 4 months after initiation; carbamazepine therapy was continued. In this thrombocytopenic period he received 7 units of platelet concentrates and 2 units of RBCs.

In total he received blood cell transfusions from 84 random donors. The following 2 years were characterized by recurrent infections of the upper respiratory tract without other abnormalities. Carbamazepine therapy was stopped 2 years after initiation, and repeated EEGs revealed an improvement of the diffuse encephalopa-thy. No neurological abnormalities were found during follow-up. The only remarkable findings during these 2 years posttransplant were the (laboratory) data of ongoing active Epstein-Barr virus (EBV) and CMV infections together with a markedly inverted CD4/CD8 ratio (see Results). In November 1984 (28 months after BMT) he developed malaise, low-grade fever, vomiting, a decreased appetite, and diarrhea. Over the ensuing 4 weeks he had lost 14 kg of body weight. Recurrent infections of the upper respiratory tract together with otitis media and externa occurred. An extensive esophagitis caused by Candida albicans was found. At that time antibodies to HIV were found to be positive, and AIDS was diagnosed. In retrospect, frozen serum specimens obtained before and at regular intervals after BMT were analyzed for antibodies to HIV. He was found to be seronegative before BMT, and the first serum proved to be positive occurred 33 days after BMT. All serum specimens since proved to be positive for HIV antibodies. The marrow donor had no antibodies to HIV (at the time of BMT and when retested in January 1985).

In April 1985 a trial of a 6-week course of weekly transfusions of leukocytes from his identical twin was initiated and was repeated in October 1986 together with a 6-week course of weekly suramin, 1 g/wk, each dose being given IV over 20 minutes. This did not result in an improvement in his clinical status or immune dysfunction (see Results).

Over the next 28 months, after AIDS was diagnosed, his clinical condition deteriorated gradually and was characterized mainly by malaise, cachexia, diarrhea, recurrent fever, infections of the gastrointestinal tract (with Yersinia enterocolitica, Campylobacter fetus, C albicans, Mycobacterium avium, Pseudomonas aeruginosa), infections of the upper and lower respiratory tract (with HSV Staphylococcus aureus, C albicans) and infections of the skin (HSV, VZV). Later, confusion accompanied by lethargy developed and progressed to dementia. A CAT scan of the cerebrum showed dilated ventricles and prominent cortical sulci indicative of cerebral atrophy. Examination of the CSF revealed a rise in protein concentration, a lowered glucose concentration, and mild pleocytosis. In the CSF one unique oligoclonal Ig band to HIV was found that was much more intense than in the serum (in the serum together with other Ig bands). He died in March 1987 of bilateral pneumonia 56 months after BMT and 28 months after AIDS was diagnosed. An autopsy was refused.

RESULTS

Number of syngeneic leukocytes transfused to the patient. Table 1 shows the mean number of leukocytes and subpopulations that were transfused weekly for 6 weeks to the patient in trials 1 and 2.

Phenotype analysis. Lymphocyte subsets before and after BMT, at the time of seroconversion to HIV, and at the time AIDS was diagnosed are shown in Table 2. The

| Table 1. Mean Number of Leukocytes Transfused Weekly in Trials 1 and 2 |
|---------------------------|---------------------|---------------------|
| Cell Count (x 10^9)       | Trial 1 (6 wk)      | Trial 2 (6 wk)      |
| Leukocytes                | 9.1                 | 8.9                 |
| Lymphocytes               | 3.3                 | 3.7                 |
| Monocytes                 | 0.2                 | 0.3                 |
| T cells                   | 2.4                 | 2.6                 |
| B cells                   | 0.3                 | 0.3                 |
| NK cells                  | 0.5                 | 0.6                 |
| CD4 + T cells             | 1.8                 | 1.8                 |
| CD8 + T cells             | 0.6                 | 0.8                 |
Test reactivity to tetanus and Candida; cyte transfer on lymphocyte proliferation to CMV, HSV, Candida; antigen-induced proliferation tests. These functions were not influenced by lymphocyte subsets and, especially, the number of CD4+ T cells by suramin therapy (Table 3). T cell suppressor activity, differentiation capacity, and stimulation by herpesvirus-induced proliferation were increased after BMT (Table 3). T cell suppressor activity was increased, which resulted in an inverted CD4/CD8 ratio.

At the time AIDS was diagnosed, the patient was lymphopenic, a condition that remained during further follow-up (Table 3). Table 3 shows that lymphocyte transfer with or without suramin had only a minimal effect on the lymphocyte subsets and, especially, the number of CD4+ T cells remained very low.

Lymphocyte functions. Lymphocyte functions measured by mitogen-induced proliferation, T helper activity, B cell differentiation capacity, and stimulation by herpesvirus-infected cells remained very depressed after BMT (Table 2), and these functions were not influenced by lymphocyte transfer/suramin therapy (Table 3). T cell suppressor activity was increased after BMT (Table 2) and remained increased during trials 1 and 2 (Table 3). Antigen-induced proliferation was measured during trials 1 and 2 (Table 3). In trial 1 a transient positive effect was induced by lymphocyte transfer on lymphocyte proliferation to CMV, HSV, tetanus, and Candida; however, these in vitro effects were not reflected in vivo (ongoing CMV infection, absence of skin test reactivity to tetanus and Candida antigens, and persistent oropharyngeal Candida infection). Lymphocyte transfer/suramin therapy (trial 2) had no positive effect on antigen- and herpesvirus-induced proliferation tests.

**Delayed-type hypersensitivity.** The patient was anergic to all antigens tested before and after lymphocyte transfer/suramin therapy. The marrow/leukocyte donor, in contrast, showed skin test reactivity to tetanus and Candida.

**Effects of suramin on HIV antigen.** No significant changes in HIV antigen levels were found between samples obtained at the start of, during, and at the end of suramin therapy. The levels were 147, 172, and 156 pg/mL, respectively.

**Side effects of suramine therapy.** Suramin was given IV at a dose of 1 g/wk for 6 weeks. Side effects associated with the administration of 6 g suramin were transient and included skin rash, burning sensations of the skin of the legs, urinary abnormalities, mild renal dysfunction, and hepatic enzyme elevations. Serum suramin levels were not determined.

**DISCUSSION**

The patient was treated with high-dose chemotherapy followed by syngeneic BMT for acute lymphoblastic leukemia (helper T cell) in second remission. After an uneventful early posttransplant period he was readmitted 39 days after BMT because of acute neurological disease together with autoimmune thrombocytopenia. He recovered from these complications after treatment with carbamazepine and prednisone. The following 2 years after BMT were uneventful except for recurrent minor infections of the upper respiratory tract. However, some laboratory features were remarkable (Table 2): CD4+ T cell (helper T) levels

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-BMT</th>
<th>+ 1 mo</th>
<th>+ 2 mo*</th>
<th>+ 6 mo</th>
<th>+ 12 mo</th>
<th>+ 30 mo†</th>
<th>Normal Values</th>
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<tr>
<td>Lymphocytes</td>
<td>1,298</td>
<td>2,665</td>
<td>2,376</td>
<td>2,432</td>
<td>1,836</td>
<td>638</td>
<td>1,500-4,000</td>
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<td>T cells</td>
<td>1,077</td>
<td>2,478</td>
<td>1,592</td>
<td>1,751</td>
<td>1,157</td>
<td>179</td>
<td>795-3,520</td>
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<tr>
<td>B cells</td>
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<td>&lt;1</td>
<td>143</td>
<td>170</td>
<td>386</td>
<td>19</td>
<td>45-480</td>
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<td>NK cells</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>729</td>
<td>624</td>
<td>179</td>
<td>60-920</td>
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<tr>
<td>CD4+ T cells</td>
<td>597</td>
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<td>428</td>
<td>170</td>
<td>257</td>
<td>26</td>
<td>375-2,400</td>
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<td>CD8+ T cells</td>
<td>740</td>
<td>2,185</td>
<td>1,734</td>
<td>2,067</td>
<td>1,083</td>
<td>102</td>
<td>285-1,400</td>
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<tr>
<td>CD4/CD8 ratio</td>
<td>0.81</td>
<td>0.21</td>
<td>0.25</td>
<td>0.08</td>
<td>0.24</td>
<td>0.25</td>
<td>1.8 ± 0.6</td>
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<td>HLA-DR+ T cells</td>
<td>398</td>
<td>1,710</td>
<td>971</td>
<td>1,418</td>
<td>312</td>
<td>9</td>
<td>&lt;70</td>
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<td>Lymphocyte functions (% of normal)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>PHA</td>
<td>94</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>12</td>
<td>9</td>
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<tr>
<td>Con A</td>
<td>34</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>10</td>
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<tr>
<td>PWM</td>
<td>77</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>&lt;1</td>
<td>3</td>
<td></td>
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<tr>
<td>T helper</td>
<td>20</td>
<td>7</td>
<td>11</td>
<td>7</td>
<td>7</td>
<td>&lt;1</td>
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<tr>
<td>T suppressor‡</td>
<td>37</td>
<td>81</td>
<td>91</td>
<td>57</td>
<td>98</td>
<td>85</td>
<td></td>
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<tr>
<td>B cell differentiation</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>2</td>
<td>7</td>
<td>9</td>
<td>&lt;1</td>
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<td>Lymphocyte stimulation by herpesvirus-infected fibroblasts§</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CMV</td>
<td>2.3</td>
<td>1.0</td>
<td>&lt;1</td>
<td>6.3</td>
<td>1.7</td>
<td>5.3</td>
<td>66.1</td>
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<td>HSV</td>
<td>2.9</td>
<td>2.0</td>
<td>1.3</td>
<td>5.4</td>
<td>5.3</td>
<td>&lt;1</td>
<td>76.5</td>
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<tr>
<td>VZV</td>
<td>1.7</td>
<td>1.2</td>
<td>1.4</td>
<td>1.0</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>62.1</td>
</tr>
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</table>

Abbreviation: NT, not tested.

*Between 1 and 2 months after BMT seroconversion to HIV occurred.
†AIDS was diagnosed.
‡Normal value of T suppressor function, 0% to 50%.
§Patient was seropositive for all herpesviruses.
¶Mean values in 40 seropositive (for each virus) controls.
The retrovirus HIV primarily infects helper/inducer T cells (CD4+ T), which play a central role in induction and regulation of the immune system, and the resulting profound cellular and humoral immunodeficiency is the cause of recurrent and life-threatening infections and neoplastic processes that characterize AIDS. This has resulted in studies designed to correct these defects in patients with AIDS by giving immunomodulators, eg, interferons, peripheral blood lymphocytes, or BMT. In vitro studies have shown that the production of interferon and interleukin 2 is impaired, but in vivo application of γ-interferon was not successful. Results of further clinical studies using lymphokines are awaited. In addition, experiments to reconstitute the immune system by giving patients allogeneic lymphocytes or syngeneic lymphocytes together with syngeneic BMT were also quite discouraging.

Antiviral drugs designed to suppress or eliminate HIV are currently being developed, and some drugs, eg, suramin, HPA 23, and AZT, that inhibit reverse transcriptase, the enzyme necessary for retroviral replication, have been used in the treatment of patients with AIDS. Inhibition of virus replication has been found in some patients treated with these drugs, and partial immunologic and clinical responses have also been observed.

The aim of this trial was to protect newly infused leukocytes (especially CD4+ T cells) from being attacked by HIV by giving suramin just before lymphocyte transfusion and allowing unaffected lymphocytes to reconstitute the immune system. Our study first included a 6-week trial of weekly syngeneic leukocyte transfer. This approach failed to...
improve the number of leukocytes and their subsets as well as, except some in vitro responses to HSV, CMV, tetanus, and *Candida*, in vitro and in vivo immune dysfunctions (Table 3). The second 6-week trial included weekly suramin in addition to syngeneic leukocyte transfer. Suramin was given at the dose that, in other studies, showed HIV suppression as detected by the ability to culture virus from lectin-stimulated PBMC.29,30 The use of retroviral isolation to evaluate antiviral therapy is, however, limited by the ability to culture virus from lectin-stimulated PBMC.29,30 The use of retroviral isolation to evaluate antiviral therapy is, however, limited by the ability to culture virus from lectin-stimulated PBMC.29,30

In conclusion, no effect could be observed by treating a patient with AIDS with syngeneic leukocyte transfer together with suramin. This approach may be more successful in the future when more effective antiviral drugs are available.37

REFERENCES


Syngeneic leukocytes together with suramin failed to improve immunodeficiency in a case of transfusion-associated AIDS after syngeneic bone marrow transplantation

LF Verdonck, GC de Gast, JM Lange, HJ Schuurman, AW Dekker and BJ Bast