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Potential Role of a Mismatched HLA-Specific CTL Clone Developed Pre-Transplant in Graft Rejection following Cord Blood Transplantation

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ABSTRACT

Graft rejection is a serious complication in cord blood transplantation (CBT), but little is known about the mechanism of rejection. To investigate the potential role of T lymphocytes in graft rejection, we isolated a CD8$^+$ cytotoxic T lymphocyte (CTL) clone of recipient origin from blood obtained from a patient with graft rejection after CBT from an HLA-mismatched unrelated donor. The isolated CTL clone specifically recognized an HLA-B*1501 molecule as an alloantigen, which was expressed in donor cells but not in recipient cells. The results of a microchimerism analysis specific for HLA-B*1501 and a polymerase chain reaction assay specific for the T cell receptor on DNA from pretransplant peripheral blood mononuclear cells revealed that the patient was exposed to HLA-B*1501 prior to CBT, and that the CTL clone was in the patient's blood prior to transplantation. The present study demonstrates a potential role for pretransplant CTL in graft rejection following CBT.

KEY WORDS

Graft failure • HLA class I antigen • T lymphocyte • Microchimerism • Reduced-intensity stem cell transplantation

INTRODUCTION

Although cord blood transplantation (CBT) from unrelated donors is an attractive treatment for patients with hematologic disease [1-6], graft rejection is a serious complication and is associated with a high rate of mortality [3,4,7,8]. The mechanisms of graft rejection have been extensively studied in bone marrow transplantation (BMT). One mechanism proposed by the studies in human and animal models is an immunologic response in which the host-derived T lymphocytes recognize donor-specific antigens [9-14]. For CBT, however, the role of the host-derived T lymphocytes in graft rejection has never been demonstrated in humans.

Here, we demonstrate a potential role of the host-derived cytotoxic T lymphocytes (CTLs) for graft rejection after CBT.

MATERIALS AND METHODS

Study Patient

A 59-year-old woman with acute myelomonocytic leukemia received CBT from an unrelated male donor. The details of the CBT procedure and the clinical course of the patient were reported previously [15]. Briefly, the preparative regimen consisted of 125 mg/m^2 fludarabine and 180 mg/m^2 melphalan, and 2.9 × 10^7/kg nuclear cells were infused. White blood cell (WBC) and neutrophil counts increased to 100/μL and 60/μL, respectively, with 65% of donor chimerism on day 16, but both counts subsequently decreased to less than the detection limit of the autohemocytometer on day 26. Graft rejection was diagnosed based on severe marrow hypoplasia and a complete loss of donor chimerism in bone marrow cells on day 28. A second

CBT from an unrelated female donor was conducted on day 35. The infused nuclear cell dose was 2.9×10^7/kg. DNA typing of the HLA-A, -B, and -DRB1 loci demonstrated that the recipient was A*1101/A*2402, B*4404/B*5603, and DRB1*1201/DRB1*1302, the first CBT donor was A*1101/A*2402, B*1501/B*5603, and DRB1*0901/DRB1*1201, and the second CBT donor was A*2402/A*3303, B*4403/B*5101, and DRB1*1201/DRB1*1302. The patient had HLA antibodies, including those against HLA-A33, and broad HLA-DR antigens prior to transplantation, whereas HLA antibody against HLA-B*1501 was not detected. The patient has one 19-year-old daughter. DNA typing of the HLA-A, -B, and -DRB1 loci demonstrated that her daughter was A*1101/A*2601, B*1501/B*5603, and DRB1*0405/DRB1*1201.

Cell Culture

CTL clones were isolated from a blood sample as described previously [16]. Briefly, peripheral blood mononuclear cells (PBMCs) obtained from the patient on day 20 were cultured in interleukin-2-containing media without stimulator cells for 14 days, and T lymphocyte clones were isolated by limiting dilution.

Transfection and CTL Stimulation Assays

The patient's B-LCL were transfected by electroporation with the pEAK10 plasmid (Edge BioSystems, Gaithersburg, MD) encoding *HLA-B*1501* cDNA, selected for 3 days with 0.8 μg/mL of puromycin, and assayed as a target for N19D8 CTL. Cytotoxicity was determined using a chromium release assay [16].

Interferon-γ release assays were conducted as previously described [16]. Briefly, COS cells were transfected with a plasmid encoding *HLA-B*1501*, *B*5603* (negative control), or *B*4403* (negative control) cDNA using the FuGENE transfection reagent (Roche, Indianapolis, IN). COS transfectants were cocultured with N19D8 CTL, and interferon-γ production was measured in the supernatant after 24 hours using an enzyme-linked immunosorbent assay (Endogen, Pierce, Rockford, IL).

Polymerase Chain Reaction (PCR) Specific for the T Cell Receptor

The T cell receptor Vβ repertoire was determined by flow cytometry using an IOTest Beta Mark Kit (Beckman Coulter, Fullerton, CA). The nucleotide sequences of the CTL clone's uniquely rearranged T cell receptor Vβ chain gene were determined by direct DNA sequencing of the amplified PCR product [17]. To determine the presence of the N19D8 clone-specific T cell receptor rearrangement, nested PCR was performed on genomic DNA extracted from a CTL clone N19D8 and PBMCs using a T cell receptor Vβ17-specific primer set for the first PCR: 5′-TTTCAGAAAGGAGATATAGCT-3′ (sense) and 5′-TTCTGATGGCTCAAACAC-3′ (antisense) followed by a second primer set specific for the N19D8 clone T cell receptor: 5′-GGAGATATAGCTGAAG GGTA-3′ (sense) and 5′-CCCCGCAAAGCTCTCA-3′ (antisense). PCR products were sequenced and confirmed to be identical in sequence to the N19D8-specific T cell receptor rearrangement. The PCR was performed with thermalcycler (Model 9600; Perkin-Elmer, Boston, MA) for 35 cycles under the following conditions: denaturation at 95°C for 1 minute, primer annealing at 55°C for 1 minute, and primer extension at 72°C for 1 minute in the first step, and denaturation at 95°C for 1 minute, primer annealing at 67°C for 15 seconds, and primer extension at 72°C for 1 minute in the second step. Each reaction contained 0.4 mL of Advantage 2 Polymerase Mix (Clontech Laboratories Inc., Palo Alto, CA).

Microchimerism Analysis

The presence of the microchimerism was determined using a nested PCR approach on genomic DNA extracted from a fingernail sample and PBMCs as previously described [18]. Briefly, nested PCR was performed on genomic DNA using an HLA-B-specific primer set for the first PCR: 5′-GGCGGGGGCG CAGGACCTGA-3′ and 5′-GGCGGGGGCGCAG GACCCGG-3′ (1:1 mixture; sense) and 5′-GAGGC CATCCCCGGCGACCTAT-3′ (antisense) followed by a second primer set specific for HLA-B*1501: 5′-A CCGGGAGACACAGATCTC-3′ (sense) and 5′-CT TGCCGTCGTAGGCGG-3′ (antisense). The touchdown procedure [19] was performed as first-step PCR under the following conditions: (1) denaturation at 96°C for 20 seconds and primer annealing at 72°C for 2 minutes for 5 cycles, (2) denaturation at 96°C for 20 seconds and primer annealing at 70°C for 2 minutes for 5 cycles, (3) denaturation at 96°C for 20 seconds and primer annealing at 68°C for 2 mintues for 4 cycles, and (4) denaturation at 96°C for 20 seconds and primer annealing at 72°C for 2.15 minutes for 15 cycles. The second-step PCR was performed for 28 cycles under the following conditions: denaturation at 94°C for 1 minute, primer annealing at 62°C for 1 minte, and primer extension at 72°C for 1 minute. FastStart Taq DNA Polymerase (Roche) and AmpliTaq Gold PCR Master Mix (Applied Byosistems, Foster City, CA) were used in the first- and the second-step PCR, respectively.

RESULTS

Isolation of CTL Clone N19D8

Two CTL clones were isolated from the peripheral blood of the patient just after the onset of graft rejection. One clone lysed B-LCL from the patient but failed to lyse B-LCL from the donor of the first CBT. The other clone, designated N19D8, lysed