Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation

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SUMMARY

Infection with the human immunodeficiency virus type 1 (HIV-1) requires the presence of a CD4 receptor and a chemokine receptor, principally chemokine receptor 5 (CCR5). Homozygosity for a 32-bp deletion in the CCR5 allele provides resistance against HIV-1 acquisition. We transplanted stem cells from a donor who was homozygous for CCR5 delta32 in a patient with acute myeloid leukemia and HIV-1 infection. The patient remained without viral rebound 20 months after transplantation and discontinuation of antiretroviral therapy. This outcome demonstrates the critical role CCR5 plays in maintaining HIV-1 infection.

**HIV-1** enters host cells by binding to a CD4 receptor and then interacting with either CCR5 or the CXC chemokine receptor (CXCR4). Homozygosity for a 32-bp deletion (delta32/delta32) in the CCR5 allele results in an inactive CCR5 gene product and consequently confers high resistance against HIV-1 acquisition.1

Allogeneic stem-cell transplantation from an HLA-matched donor is a feasible option for patients with hematologic neoplasms, but it has not been established as a therapeutic option for patients who are also infected with HIV.2 Survival of patients with HIV infection has improved considerably since the introduction of highly active antiretroviral therapy (HAART),3 and as a consequence, successful allogeneic stem-cell transplantation with ongoing HAART was performed in 2000.4

In this report, we describe the outcome of allogeneic stem-cell transplantation in a patient with HIV infection and acute myeloid leukemia, using a transplant from an HLA-matched, unrelated donor who was screened for homozygosity for the CCR5 delta32 deletion.

CASE REPORT

A 40-year-old white man with newly diagnosed acute myeloid leukemia (FAB M4 subtype, with normal cytogenetic features) presented to our hospital. HIV-1 infection had been diagnosed more than 10 years earlier, and the patient had been treated with HAART (600 mg of efavirenz, 200 mg of emtricitabine, and 300 mg of tenofovir per day) for the previous 4 years, during which no illnesses associated with the acquired immunodeficiency syndrome (AIDS) were observed. At the time that acute myeloid
leukemia was diagnosed, the patient’s CD4 T-cell count was 415 per cubic millimeter, and HIV-1 RNA was not detectable (stage A2 according to classification by the Centers for Disease Control and Prevention). Initial treatment of the acute myeloid leukemia consisted of two courses of induction chemotherapy and one course of consolidation chemotherapy. During the first induction course, severe hepatic toxic effects developed and renal failure occurred. Consequently, HAART was discontinued, leading to a viral rebound (6.9×10⁶ copies of HIV-1 RNA per milliliter). The therapy was resumed immediately, before a viral steady state was reached, and 3 months later, HIV-1 RNA was undetectable.

Seven months after presentation, acute myeloid leukemia relapsed, and the patient underwent allogeneic stem-cell transplantation with CD34+ peripheral-blood stem cells from an HLA-identical donor who had been screened for homozygosity for the CCR5 delta32 allele. The patient provided informed consent for this procedure, and the protocol was approved by the institutional review board. The HLA genotypes of the patient and the donor were identical at the following loci: A*0201; B*0702,3501; Cw*0401,0702; DRB1*0101,1501; and DQB1*0501,0602. The patient underwent a conditioning regimen and received a graft containing 2.3×10⁶ CD34+ cells per kilogram of body weight.⁵ Prophylaxis against graft-versus-host disease consisted of 0.5 mg of rabbit antithymocyte globulin per kilogram 3 days before transplantation, 2.5 mg per kilogram 2 days before, and 2.5 mg per kilogram 1 day before. The patient received two doses of 2.5 mg of cyclosporine per kilogram intravenously 1 day before the procedure and treatment with mycophenolate mofetil at a dose of 1 g three times per day was started 6 hours after transplantation. HAART was administered until the day before the procedure, and engraftment was achieved 13 days after the procedure. Except for the presence of grade I graft-versus-host disease of the skin, which was treated by adjusting the dosage of cyclosporine, there were no serious infections or toxic effects other than grade I during the first year of follow-up. Acute myeloid leukemia relapsed 332 days after transplantation, and chimerism transiently decreased to 15%. The patient underwent reinduction therapy with cytarabine and gemtuzumab and on day 391 received a second transplant, consisting of 2.1×10⁶ CD34+ cells per kilogram, from the same donor, after treatment with a single dose of whole-body irradiation (200 cGy). The second procedure led to a complete remission of the acute myeloid leukemia, which was still in remission at month 20 of follow-up.

**METHODS**

**CCR5 GENOTYPING**

Genomic DNA was extracted from heparinized peripheral-blood monocytes obtained from the patient and the prospective donor, with the use of the QIAamp Blood Midi Kit (Qiagen). Screening of donors for the CCR5 delta32 allele was performed with a genomic polymerase-chain-reaction (PCR) assay, with primers flanking the site of the deletion (forward, 5’CTCCCGAGAATCATCTTTTACC3’; reverse, 5’TCAATTCGACACCGAAGCAG3’), resulting in a PCR fragment of 200 bp for the CCR5 allele and 168 bp for a delta32 deletion. Results were confirmed by allele-specific PCR and by direct sequencing with the use of the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). Sequences were analyzed with the use of Vector NTI ContigExpress software (Invitrogen).

**VIRAL-ENVELOPE GENOTYPING**

Coreceptor use by HIV-1 was assessed through V3 amino acid sequences of the env region for both DNA and RNA. Bulk PCR products were subjected to direct sequencing and determined according to the 11/25 and net charge rules, as described by Delobel et al.⁶ For RNA, the HIV env region was sequenced from position 6538 to 6816 and Web position-specific scoring matrix (WebPSSM), and geno2pheno bioinformatic software was used to predict viral coreceptor use. In addition, an ultradeep PCR analysis with parallel sequencing (454-Life-Sciences, Roche) was performed.⁷

**CHEMOKINE RECEPTORS AND SURFACE ANTIGENS**

Mucosal cells were isolated from 10 rectal-biopsy specimens according to the method of Moos et al.⁸ CCR5 expression was stimulated by phytohemagglutinin (Sigma), and the cells were analyzed by means of flow cytometry with the use of antibodies against CD3, CD4, CD11c, CD163, and CCR5 (BD Biosciences).

**CHIMERISM**

Standard chimerism analyses were based on the discrimination between donor and recipient alleles.
Arising from secondary structures of PCR products.

produce both bands, plus an additional third band that may be an artifact changed to delta32); after transplantation, with ongoing engraftment, the genotype CCR5 (delta32/delta32) produces a single band of 168 bp. The sample that is homozygous for the CCR5 (+/+ results in a single band 200 bp. The sample that is homozygous for the CCR5 delta32 allele (CCR5 delta32/delta32) produces a single band of 168 bp. Before stem-cell transplantation (SCT), the patient had a heterozygous genotype (CCR5+/ delta32); after transplantation, with ongoing engraftment, the genotype changed to CCR5 delta32/delta32. Samples containing heterozygous alleles produce both bands, plus an additional third band that may be an artifact arising from secondary structures of PCR products.

**RESULTS**

**DISTRIBUTION OF CCR5 ALLELES**

Genomic DNA from 62 of 80 potential HLA-identical stem-cell donors registered at the German Bone Marrow Donor Center was sequenced in the CCR5 region. The frequencies of the delta32 allele and the wild-type allele were 0.21 and 0.79, respectively. Only one donor was homozygous for the CCR5 delta32 deletion in this cohort.

**ANALYSIS OF HIV-1 CORECEPTOR PHENOTYPE**

Sequence analysis of the patient’s viral variants revealed a glycine at position 11 and a glutamic acid at position 25 of the V3 region. The net charge of amino acids was +3. These results indicated CCR5 coreceptor use by the HIV-1 strain infecting the patient, a finding that was confirmed by sequencing RNA in the HIV *env* region. The ultradeep sequencing analysis revealed a proportion of 2.9% for the X4 and dual-tropic variants combined.

**RECIPIENT CHIMERISM**

With ongoing engraftment, the PCR patterns of CCR5 were transformed, indicating a shift from a heterozygous genotype to a homozygous delta32/delta32 genotype (Fig. 1). Complete chimerism, determined on the basis of allelic short tandem repeats, was obtained 61 days after allogeneic stem-cell transplantation.

**CELLULAR AND HUMORAL IMMUNE RESPONSES**

Secretion of interferon-γ by antigen-specific cells was induced according to the method of Ganepola et al. For measurement of T-cell-mediated immune responses, two HLA-A*0201-binding peptides were used: HIV-1 

**AMPLIFICATION OF HIV-1 RNA AND DNA**

HIV-1 RNA was isolated from plasma and amplified with the use of the Cobas AmpliPrep–TaqMan HIV assay system (Roche). Total DNA was isolated from peripheral-blood monocytes and rectal-biopsy specimens with the use of the QIAamp DNA Blood Mini Kit and the AllPrep DNA/RNA Mini Kit, respectively (both from Qiagen). The *env* and long-terminal-repeat regions were amplified according to the method of Cassol et al. and Drosten et al. The sensitivity of the RNA assay was 40 copies per milliliter, and the lower limit of detection for both complementary DNA (cDNA) PCR assays is 5 copies per reaction, with a positivity rate of more than 95%. Each assay contained $2 \times 10^4$ to $5 \times 10^4$ CD4+ T cells. The successful amplification of 1 μg of cellular DNA extracted from various housekeeping genes (GAPDH, CCR5, and CD4) extracted from 1 μg cellular DNA indicated the suitability of the DNA isolated from the mucosal specimens.

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transplantation, whereas levels of antibodies to soluble glycoprotein 120 and glycoprotein 41 remained detectable (Fig. 2B).

**Quantification of Viremia**

The HIV-1 load was measured with the use of RNA and DNA PCR assays (Fig. 3). Throughout the follow-up period, serum levels of HIV-1 RNA remained undetectable. Also during follow-up, the semiquantitative assay showed no detectable proviral DNA except on the 20th day after transplantation, for both the env and long-terminal-repeat loci, and on the 61st day after transplantation, for the env locus.

**Rectal-Biopsy Specimens**

In rectal-biopsy specimens obtained 159 days after transplantation, macrophages showed expression of CCR5, whereas a distinct CCR5-expressing population was not present in the mucosal CD4+ T lymphocytes (Fig. 4).

**Discussion**

To enter target cells, HIV-1 requires both CD4 and a coreceptor, predominantly CCR5. Blocking of the preferentially used CCR5 receptor by inhibitors or through gene knockdown conferred antiviral protection to R5-tropic variants.\(^{13,14}\) The homozygous CCR5 delta32 deletion, observed in approximately 1% of the white population, offers a natural resistance to HIV acquisition. We report a successful transplantation of allogeneic stem cells homozygous for the CCR5 delta32 allele to a patient with HIV.

Although discontinuation of antiretroviral therapy typically leads to a rapid rebound of HIV load within weeks, in this patient, no active, replicating HIV could be detected 20 months after HAART had been discontinued.\(^{15}\) This observation is remarkable because homozygosity for CCR5 delta32 is associated with high but not complete resistance to HIV-1. This outcome can be explained by the behavior of non-CCR5-tropic variants, such as CXCR4-tropic viruses (X4), which are able to use CXCR4 as a coreceptor. The switch occurs in the natural course of infection, and the proportion of X4 increases with ongoing HAART.\(^{16}\) Genotypic and phenotypic assays can be used to determine the nature and extent of coreceptor use, but the presence of heterogeneous viral populations in samples from patients limits the sensitivity of the assay.\(^{17}\) When genotypic analysis was performed in two laboratories applying WebPSSM and geno2pheno prediction algorithms, X4 vari-
ants were not detected in the plasma of our patient. To determine the proportion of minor variants in the plasma, we performed an ultradeep sequencing analysis, which revealed a small proportion of X4 variants before the allogeneic stem-cell transplantation.

Even after prolonged HAART, the persistence of HIV-1 populations in various anatomical compartments can be observed in patients without detectable viremia. Even after prolonged HAART, the persistence of HIV-1 populations in various anatomical compartments can be observed in patients without detectable viremia. In particular, the intestinal lamina propria represents an important reservoir of HIV-1, and genomic virus detection is possible in patients without viremia. In this patient, a rectal biopsy performed 159 days after transplantation revealed that CCR5-expressing macrophages were still present in the intestinal mucosa, indicating that they had not yet been replaced by the new immune system. Although these long-lasting cells from the host can represent viral reservoirs even after transplantation, HIV-1 DNA could not be detected in this patient's rectal mucosa.
It is likely that X4 variants remained in other anatomical reservoirs as potential sources for re-emerging viruses, but the number of X4-tropic infectious particles after transplantation could have been too low to allow reseeding of the patient's replaced immune system.

The loss of anti-HIV, virus-specific, interferon-\(\gamma\)-producing T-cells during follow-up suggests that HIV antigen stimulation was not present after transplantation. This disappearance of effector T cells was not associated with a deficient immune reconstitution, as shown by the absence of relevant infection or reactivation of other persistent viruses, such as CMV and Epstein–Barr virus. Thus, the absence of measurable HIV viremia in our patient probably represents the removal of the HIV immunologic stimulus.\(^{20}\) Antibodies against HIV-envelope antigens have remained detectable, but at continually decreasing levels. The sustained secretion of antibodies might be caused by long-lived plasma cells that are relatively resistant to common immunosuppressive therapies.\(^{21,22}\)

In the past, there were several attempts to control HIV-1 infection by means of allogeneic stem-cell transplantation without regard to the donor's CCR5 delta32 status, but these efforts were not successful.\(^{23}\) In our patient, transplantation led to complete chimerism, and the patient’s peripheral-blood monocytes changed from a heterozygous to a homozygous genotype regarding the CCR5 delta32 allele. Although the patient had non–CCR5-tropic X4 variants and HAART was discontinued...

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**Figure 4. Expression of CD Surface Antigen and Chemokine Coreceptor in the Patient’s Rectal Mucosa.**

Mucosal cells isolated from rectal-biopsy specimens obtained 159 days after stem-cell transplantation were activated by phytohemagglutinin and analyzed with the use of flow cytometry. Cells were gated for lymphocytes by their characteristic forward- and side-scatter profile and were analyzed for CCR5 expression within the CD4+ T-cell population (Panel A). Macrophages were identified as CD11c+ and CD163+ within the CD4+ cell gate and analyzed for CCR5 expression (Panel B). Whereas intestinal CD4+ T lymphocytes were negative (0.0%) for CCR5 expression, 14.6% of macrophages expressed CCR5 after engraftment, indicating a complete exchange of intestinal CD3+/CD4+ lymphocytes but not of intestinal CD3+/CD4+ macrophages.
for more than 20 months, HIV-1 virus could not be detected in peripheral blood, bone marrow, or rectal mucosa, as assessed with RNA and proviral DNA PCR assays. For as long as the viral load continues to be undetectable, this patient will not require antiretroviral therapy. Our findings underscore the central role of the CCR5 receptor during HIV-1 infection and disease progression and should encourage further investigation of the development of CCR5-targeted treatment options.

Supported by a grant from the German Research Foundation (DFG KFO grant 104/1).

Dr. Hofmann reports serving as a consultant or advisory-board member and on speakers’ bureaus for Celgene and Novartis. No other potential conflict of interest relevant to this article was reported.

We thank Alexander Schmidt, Petra Leuker, and Gerhard Ehninger (German Bone Marrow Center, Tübingen and Dresden, Germany) for their encouragement and cooperation regarding access of donor blood samples; Emil Morsch (Stefan Morsch Foundation, Birkenfeld, Germany) and Martin Meixner (Department of Biochemistry, Charité Universitätsmedizin, Berlin) for performing sequencing; Stephan Fuhrmann and Mathias Steitz (Department of Immunology, Charité Universitätsmedizin, Berlin) for providing HIV p24 antigens; Alexander Thielten (Max-Planck-Institut für Informatik, Saarbrücken, Germany) for performing 454 ultradepth-sequence data analysis; Lutz Uharek (Department of Haematology, Charité Universitätsmedizin) for clinical supervision of the allogeneic stem-cell transplantation; and Martin Raftery (Institute of Medical Virology, Charité Universitätsmedizin, Berlin) for reading an earlier version of this article.

REFERENCES