Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation

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Objective: To determine whether sustained HIV remission after stem cell transplantation (SCT) can be achieved by the combination of pretransplantation CCR5Δ32/Δ32 gene correction or haploidentical SCT (H9004/H9004 32/32) in an HIV-infected patient.

Patients and Methods: The patient is a 22-year-old man with relapsed acute myeloid leukemia (AML) and documented CXCR4-tropic (X4 HIV) and CCR5-tropic (R5 HIV) variants (X4 HIV) were present within the patient's pretransplantation HIV reservoir population. It was reasonable to hypothesize that HIV from the viral reservoir could reseed the body, even after the immune system has efficiently been restored with X4 HIV-susceptible target cells.

Results: After SCT, the patient remained viremia-negative during the first 3.5 years. To verify the ability of the recovered CD4 + T cells to improve our knowledge about the curative potential of CCR5-targeted treatment strategies, but uncertainty has remained over whether a cure for HIV infection has been achieved in this patient.

Conclusion: The reasons for nonadherence to ART or treatment discontinuation include the effects of pretransplantation conditioning and the time course of ART discontinuation may allow for incomplete elimination of HIV, as demonstrated by previous studies in which researchers demonstrated that HIV-exposed patients who undergo stem cell transplantation generally experience a viral rebound when ART is discontinued.12-17 For this reason, together with the fact that CXCR4-tropic HIV variants (X4 HIV) were present within the patient's pretransplantation HIV reservoir population, it was reasonable to hypothesize that HIV from the viral reservoir could reseed the body, even after the immune system has efficiently been restored with X4 HIV-susceptible target cells.

Accordingly, key questions that remain to be answered are (1) whether CD4 + T cells have been efficiently restored throughout the body; (2) whether or not the patient's immune system includes HIV-exposed target cells; and (3) how stable the size of the HIV reservoir has been reduced over time. In conclusion, our results strongly suggest that cure of HIV infection has been achieved in this patient (Blood. 2011;117(10):2791-2799).
as HIV target cells, their activation status, CXCR4 expression profile, and susceptibility to producers of HIV infection was analyzed. Moreover, because the absence of the CCR5 wild-type gene variant in donor cells precludes us with the possibility to discriminate between donor- and host-derived immune cells, we were able to examine the persistence of potential viral reservoirs, in addition to the detection of viral sequences, in distinct compartments.

**Methods**

**Subjects**

In February 2007, an HIV-infected patient underwent SCT because of a relapse of AML with a graft consisting of CCR5/H9004 and H32. Thirteen months later the patient received a second transplantation with TBI (day +1), 6 mg/m2 gemtuzumab (day +1), and a 400-cGy total body irradiation (TBI; day +3); 5.5 mg/kg rabbit antithymocyte globuline (in 3 doses between day +10 until and +15). The pretransplantation conditioning regimen included 100 mg/m2 amsacrine, 30 mg/m2 fludarabine, 2 g/m2 cytarabine (day +1), 6 mg/m2 gemtuzumab (day +10 until and +15), and 200 mg of TBI (day +10. Five animal days, and further details, see Hitte et al. At 5.5, 26, and 90 months after the first CCR5/H11001 SCT, the patient underwent colonoscopy, and biopsy specimens were taken as the result of suspected intestinal graft-versus-host disease (GVHD) while tapering immunosuppressive treatment. With the patient's informed consent for this procedure, 10-11 additional colon biopsy specimens were collected at each time point for research purposes of the present study. Examination of histologic sections included the diagnosis of intestinal GVHD. Twelve months after transplantation, the patient underwent a first biopsy, and histologic examination confirmed intestinal GVHD grade 3, which was confirmed in a second biopsy at the end of follow-up 48 months after transplantation. A biopsy was performed for diagnostic purposes. Previous colon biopsies were reserved for diagnostic purposes. Mitogen resonance imaging of the form unidentified signal adenoma malignum with leukoencephalopathy. For further evaluation, colonoscopy had been performed. Pathologic examination revealed multiple lesions in the distal colon, rectum, and sigmoid colon. Enlarged lymph nodes were found in the mesentery and the peripancreatic region. Magnetic resonance imaging of the form unidentified signal adenoma malignum with leukoencephalopathy. For further evaluation, colonoscopy had been performed. Pathologic examination revealed multiple lesions in the distal colon, rectum, and sigmoid colon. Enlarged lymph nodes were found in the mesentery and the peripancreatic region. A biopsy was performed for diagnostic purposes. Previous colon biopsies were reserved for diagnostic purposes.

**Flow cytometric analysis and cell sorting**

Flow cytometric analysis was performed by the use of antibodies against CD4 (clone 2G2; BD Biosciences), CD8 (clone RPA-T4; BD), CD3 (clone UCHT1; BD), CD123 (clone 9F10; BD), CD14 (clone HCD14; BD), CD19 (clone HIB19; BD), CD27 (clone M-T20; BD), CD62L (clone DREG-56; BD), CXCR4 (clone 12G5; BD), HLA-DR (clone IMMU357; Beckman Coulter), and CD45RO (clone UCHL1; BD). CD45RO+ T-cells were classified by coexpression of CD45RO and CD100, and effector memory CD45RO+ T-cells were characterized by lack of CD31. Residual HIV antigen was identified by coexpression of CD100 and CD62L, and CD4+ monocytes were labeled by CD14 and CD68. T-cells were labeled by lack of CD31. CD4+ T-cells was evaluated for their coexpression of CD62L and CD25 in the lymphoid gate, and intracellular cytokines were selected by their expression of CD3 and CCR5 in the lymphoid gate, and intracellular cytokines were selected by their expression of CD3 and CCR5 in the lymphoid gate.

**HIV susceptibility assay**

CCR5-inhibiting HIV-1 strain R5-CXCR4-inhabited human T-cells (H9004) were propagated in PHA. A stock of CCR5-inhibiting HIV-1 strain MA10 was propagated from the HIV (chimeric gene p2HIV45) from the EV A Center for AIDS Reagents (Berlin, Germany) and propagated in PHA. Immunostaining on paraffin sections was performed as described previously.24 Primary antibodies were mouse anti-CD4 (1F6; Novocastra), mouse anti-CD3 (clone UCHT1; BD Biosciences), mouse anti-CD68 (PGM1; DAKO), or goat anti-CCR5 (CKR-5 [C20]; DakoCytomation). Absolute analysis of CD4+ T-cells was determined in each whole blood by the use of T-cell antigen and CD31/CD68+ T-cell (H9004) according to the manufacturer's protocol. Data were acquired by FACSCaliber flow cytometry and analyzed with CELLQuest software and FACSDiva software. Cells were labeled by lack of CD31. CXCR4 expression density on CD4+ T-cells was evaluated as the mean fluorescence intensity (MFI) of CXCR4 expression divided by the MFI value obtained from the corresponding isotype control (BD) and is expressed as the MFI ratio.

**Immunosuppressive therapy**

Immunosuppressive treatment has been stopped 38 months after CCR5/H9004 SCT, the patient underwent colonoscopy and biopsy specimens were taken as the result of suspected intestinal GVHD while tapering immunosuppressive treatment. With the patient's informed consent for this procedure, 10-11 additional colon biopsy specimens were collected at each time point for research purposes of the present study.
3 sections were analyzed. Immunohistochemical evaluations were performed in a blinded manner by the researcher to ensure the patient's clinical characteristics. For CD3+ OR CD8+ T cells, double immuno-  

The horizontal lines in panel B denote the median values of each group. Statistical significances are shown by asterisks (ie, *P < .05, **P < .01, ***P < .001). The horizontal bars in panel A denote the median values of each group. Statistical significances are shown by asterisks (ie, *P < .05, **P < .01, ***P < .001).

Results

Efficient recovery of CD4+ T cells was associated with a characteristic enrichment of activated/effector memory CD4+ T cells.

After CCR5Δ32/Δ32 SCT, dimmension analysis as well as genotyping of CCR5 alleles suggested that host T cells were completely eliminated from the periphery. Numbers of donor-derived peripheral CD4+ T cells increased continuously and, after 2 years, reached levels within the normal range of age-matched healthy patients (Figure 1A). Further phenotypic analysis revealed an increase of memory CD4+ T cells, which, with a parallel, but low, increase of CD69+ memory T cells, was found to be associated with a parallel increase of CD25+ cells. In both the CCR5Δ32/Δ32 SCT patient and the SCT control patients, the proportion of central memory CD4+ T cells was within the normal range, whereas effector memory CD4+ T cells were within the normal range. In accordance, the frequency of cells expressing the activation markers CD69, HLA-DR, and CD40 and the proliferation marker Ki67, at 9 and 36 months after CCR5Δ32/Δ32 SCT, was compared with that of healthy control patients. Data are represented as median 

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recovery of CD4⁺ T cells in the gut mucosal immune system. Interestingly, compared with healthy controls, the peripheral CD4⁺ T cells were exclusively derived from donor hematopoietic cells. Taken together, these results reveal that circulating donor-derived CD4⁺ T cells are efficiently recruited to the GI tract and have repopulated the mucosal CD4⁺ T-cell compartment after CCR5Δ32 SCT.

CXCR4 surface availability is not impaired on recovered CD4⁺ T cells

Reconstitution of the CD4⁺ T-cell compartment after CCR5Δ32/Δ32 SCT was associated with an expansion of activated memory CD4⁺ T cells.25,26 These results demonstrate that circulating CD4⁺ T cells were exclusively derived from donor hematopoietic cells. Taken together, these results reveal that circulating donor-derived CD4⁺ T cells are efficiently recruited to the GI tract and have repopulated the mucosal CD4⁺ T-cell compartment after CCR5Δ32/Δ32 SCT.
such as tissue CD4+ that survive chemotherapeutic and irradiation therapies represent potential productive X4 HIV infection, long-lived HIV-infected host cells impaired CXCR4 expression on recovered CD4+ T cells. In vivo, the availability of CXCR4 may be affected by the chemokine CXCL12, the physiologic ligand of CXCR4.27 During the immune reconstitution period, CXCL12 plasma levels in the CCR5+/H9004 32 SCT patient remained within the normal range of healthy patients (not shown), indicating that the in vivo availability of CXCR4 was not impaired by naturally occurring receptor occupation. Altogether, these results indicate that recovered CD4+ T cells are susceptible to productive infection by X4 HIV.

Because of the fact that recovered CD4+ T cells are susceptible to productive infection, we next analyzed CXCR4 expression on CD4+ T cells upon ex vivo activation and found efficient expression of CXCR4 on CCR5+/H9004 32 SCT T cells (Figure 3B). These data demonstrate that the CCR5+/H9004 32 SCT was not associated with an impaired CXCR4 expression on recovered CD4+ T cells. In vivo, the availability of CXCR4 may be affected by the chemokine CXCL12, the physiologic ligand of CXCR4.27 During the immune reconstitution period, CXCL12 plasma levels in the CCR5+/H9004 32 SCT patient remained within the normal range of healthy patients (not shown), indicating that the in vivo availability of CXCR4 was not impaired by naturally occurring receptor occupation. Altogether, these results indicate that recovered CD4+ T cells are not protected against X4 HIV infection.

Recovered CD4+ T cells are susceptible to productive X4 HIV infection.

Susceptibility of recovered CD4+ T cells in the context as well as the reassembled immune system to productive HIV infection was studied by ex vivo infections of H9004 and MIMCs obtained after CCR5+/H9004 32 SCT. As shown in Figure 4, cells from both compartments were susceptible to productive infection by X4 HIV. Consistent with our previous observation, virus production of the PBMC-propagated X4 HIV strain was greater in peripherally than in mucosal CD4+ T cells. As expected, because of the lack of CCR5 surface expression on allograft-derived cells, both peripheral and mucosal CD4+ T cells were resistant to X4 HIV infection.

Long-lived HIV target cells of host origin were replaced with donor-derived cells during the posttransplantation period. Because of the fact that recovered CD4+ T cells are susceptible to productive X4 HIV infection, long-lived HIV-infected host cells serve chemo- and structurally determinants of potential sources from which HIV to emerge. Noninfecting immune cells such as tissue CD4+ T cells or macrophages are virtually chemo- and structurally resistant and, therefore, represent possible viral reservoirs.

We investigated the presence of residual host immune cells after CCR5+/H9004 32 SCT by in situ immunofluorescence detection of cellular CCR5 expression. Clinical samples from the brain, the brain, and the colon could be used for research purposes in the present study after a diagnosis was given. Brain tissue specimens were available from the white matter and the cortex. From the colon, 1 specimen was obtained in the acute setting. Positive results were obtained with peripheral lymphocytes purified at all time points during the course of immune reconstitution. In the brain, 12 months after CCR5+/H9004 32 SCT, CCR5-expressing CD4+ T cells or macrophages/Kupffer cells were not detectable (Figure 5A). Likewise, 17 months after CCR5+/H9004 32 SCT, no CCR5-expressing macrophages/microglia were found in the brain (Figure 5B).

In the colon, there was no evidence of residual host CD4+ T cells after CCR5+/H9004 32 SCT, as already described previously (Figure 2B).

In situ immunofluorescence staining revealed the presence of CCR5-expressing macrophages of 6.5 months after CCR5+/H9004 32 SCT, which is in agreement with our previous flow cytometric data24 and demonstrates the persistence of host macrophages during the first months after CCR5+/H9004 32 SCT (Figure 5A). Importantly, late in the course of immune reconstitution, CCR5 expression on macrophages became unanalyzable indicating that their replacement by donor-derived cells (Figure 5A).

To further prove the origin of mucosal macrophages, we performed additional genotypic analysis of sorted mucosal macrophages. As shown in Figure 6B, 24 and 20 months after CCR5+/H9004 32 SCT, mucosal macrophages were negative for the CCR5 wild-type gene. The absence of host’s genomic DNA in mucosal macrophages at these time points confirms the phenotypic results and suggests that host macrophages have been replaced with donor-derived cells during the posttransplantation period.

HIV remains undetectable in distinct tissue compartments.

The presence of HIV RNA and HIV DNA was examined in distinct tissue compartments during the course of 45 months after CCR5+/H9004 32 SCT by in situ immunofluorescence detection of CD4+ T-cells compartment. Clinical samples from the liver, the colon, and the brain were available from the white matter and the cortex. From the colon, 1 specimen was obtained in the acute setting. Positive results were obtained with peripheral lymphocytes purified at all time points during the course of immune reconstitution. In the brain, 12 months after CCR5+/H9004 32 SCT, CCR5-expressing CD4+ T cells or macrophages/Kupffer cells were not detectable (Figure 5A). Likewise, 17 months after CCR5+/H9004 32 SCT, no CCR5-expressing macrophages/microglia were found in the brain (Figure 5B).

Antibodies against HIV decrease over time. Previously, we reported the loss of antibodies directed against the HIV envelope as well as a decrease of HIV antibodies and core-specific antibodies during the first 20 months after CCR5+/H9004 32 SCT. Immunogenic analysis revealed a continuously decline of HIV-specific antibodies thereafter demonstrating the process of seroconversion, whereas HIV core-directed antibodies (p24, p17) disappeared completely, the serum level of antibodies against the HIV envelope (gp120, gp160) further decreased. Today, the patient has only HIV envelope-specific antibodies.

Discussion

Immunoreconstitution is critical to the long-term success of the SCT, and in HIV-infected patients, also provides a prerequisite for viral rebound and HIV disease progression. Prognostic infection in turn impairs the reconstitution of CD4+ T cells after SCT. Our results show that systemic recovery of CD4+ T cells after CCR5+/H9004 32 SCT and discontinuation of ART was not impaired compared with that of SCT control patients. In accordance with previous studies,25,26 repopulation of the CD4+ T-cell compartment was associated with peripheral expansion of donor-derived memory
CD4+ T cells, that probably occurs to compensate for the limited thymic capacity in adults.29-31 Generally, this homeostasis-driven expansion of activated memory CD4+ T cells leads to an enrichment of the preferential targets for productive infection with both R5 HIV and X4 HIV32 and likely contributes to the rapid dynamic of HIV rebound after conventional SCT in HIV-infected patients.12,14,15,17 Viral tropism analysis was not in the focus of previous reports of HIV-infected patients with conventional SCT and would be an interesting issue to address in future studies.

In the CCR5Δ32/Δ32 SCT patient, CD4+ T-cell numbers have even returned to the normal range of healthy patients whereas HIV RNA and HIV DNA remain continuously undetectable in plasma and PBMC, respectively. Today, by monitoring the most common prognostic markers, ie plasma viral load and CD4+ T-cell counts in the peripheral blood, HIV disease cannot be assessed in this patient. However, observations from the central immune compartment need not be representative for distinct tissue compartments throughout the body. Only 1%-2% of the body's total CD4+ T cells reside in the peripheral blood, whereas the majority of immune cells are located in the GI tract,33 containing most of the body's activated memory CD4+ T cells with high expression of cellular receptors, the mucosal immune system is highly prone to productive infection with both R5 HIV and X4 HIV.34,35 In fact, profound depletion of CD4+ T cells in the GI mucosa occurs earlier than that in blood or lymph nodes regardless of the infection route, and even with complete suppression of viremia for many years, residual low-level replication in the GI tract prevents full recovery of mucosal CD4+ T cells in ART-treated HIV-infected patients.2,37-39 Poor recovery of CD4+ T cells in the mucosal immune system is therefore an important risk factor for the development of HIV disease progression. After CCR5Δ32/Δ32 SCT, we found that the process of immune reconstitution included a gradual increase of donor-derived CD4+ T cells in the GI mucosa. Compared with HIV-uninfected SCT control patients, mucosal CD4+ T-cell numbers normalized whereas HIV remained undetectable in gut tissue specimens as well as in mucosal HIV target cell populations. These findings argue for the absence of HIV disease progression in the largest component of the lymphoid organ system. Surprisingly, compared with healthy control patients, mucosal CD4+ T-cell numbers in both the CCR5Δ32/Δ32 SCT patient and the SCT control patients were increased. This finding may likely be explained by the high prevalence of activated/effector memory CD4+ T cells in the circulation, for which we have previously found enhanced gut-homing capacity.40 In addition, the normalized frequency of central memory cells within circulating CD4+ T cells suggests that recovered CD4+ T cells have been efficiently directed to peripheral lymph nodes.41,42 Furthermore, the decline of HIV-
In addition to their natural protection from R5 HIV infection, T cells of some persons have been suggested to be less susceptible to X4 HIV entry as a result of down-regulated CXCR4 expression. However, in the patient described here, we found no evidence for an altered CXCR4 expression on mucosal CD4+ T cells. Moreover, the patient’s peripheral and mucosal CD4+ T cells are susceptible to productive infection with X4 HIV, demonstrating that the CCR5Δ32/SCT has not provided protection against X4 HIV infection. Consequently, the patient’s risk of exogenous HIV reinfection is not completely eliminated. Nevertheless, our results demonstrate that the process of immune reconstitution has successfully reversed both the central and the mucosal immune system with CD68+ macrophages expressing CXCR4 surface expression and have susceptibility to productive X4 HIV infection. Consequently, host cells that survived the chemotherapeutic treatment appear to be critical roles in viral reservoir because they are not only for a very limited area of the respective organ, but the influence cannot be definitely excluded the presence of residual, potentially infected, host cells.

However, there is convincing evidence from studies in mice to support that host tissue macrophages were efficiently replaced with donor-derived cells during the course of immune reconstitution. For example, although it is generally accepted that microglia under steady-state conditions are very closely associated by cells of hematopoietic origin, it has been demonstrated that the conditioning procedure efficiently enhances this process after stem cell transplantation. Moreover, the majority of Kupffer cells are replaced already early after SCT and, importantly, increasing conversion rates of tissue macrophages over time after transplantation have been demonstrated in distinct tissue compartments throughout the whole body. Evidence in support of the conclusion that conversion from host to donor tissue macrophages took place in the patient after CCR5Δ32/SCT comes from our serial analysis in the brain. Here, phenotypic results revealed that residual host cells were present within the mucosal macrophage populations during the first months after CCR5Δ32/SCT. Later in the course of immune reconstitution, host-originating macrophages cannot definitely exclude the presence of residual, potentially infected, host cells.
conclude that cure of HIV infection has been achieved in this discontinuation of ART. From these results, it is reasonable to any evidence of HIV infection for more than 3.5 years after support with cell sorting.

In summary, our results demonstrate successful CD4 T-cell reconstitution at the systemic level as well as in the largest immunologic organ after CCGralka23 SCT and in addition provide evidence for the induction in the size of the potential HIV reservoir over time. Although the recovered CD4 T cells are susceptible to infection with HIV, the patient remains without any evidence of HIV viremia for more than 3.5 years after discontinuation of ART. From these results, it is reasonable to conclude that cure of HIV infection has been achieved in this patient.

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Authorship

Contributions K.A. designed experiments; K.A., J.H., and C.L. performed experiments and analyzed data; K.A. and C.L. composed the figures; K.A., G.H., J.H., and T.S. interpreted and discussed the data; G.H., K.R., and E.T. collected data; T.S. critically reviewed the manuscript; K.A. wrote the manuscript; and all authors read and approved the manuscript.

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