Syngeneic Adoptive Transfer of Anti-Human Immunodeficiency Virus-1 (HIV-1)-Primed Lymphocytes From a Vaccinated HIV-Seronegative Individual to His HIV-1-Infected Identical Twin


Immunotherapy by adoptive transfer of lymphocytes was attempted in identical twins, one who was virus-free and the other who was infected with human immunodeficiency virus-1 (HIV-1), at the stage of acquired immunodeficiency syndrome. The uninfected twin was vaccinated by priming with a recombinant vaccinia virus expressing the envelope glycoprotein of one of his brother's viruses and boosting with the same purified gp160 adsorbed on alum. Vaccination elicited major histocompatibility complex class I-restricted CD8+ cytolytic T lymphocytes specific for HIV-1, but no antibody response. The diseased brother, a 38-year-old homosexual who had developed repeated opportunistic infections since 1990 and had a CD4+ count reduced to practically zero, was treated by infusions of lymphocytes collected from the vaccinated brother by lymphopheresis. After a first transfer of the whole lymphocyte population, no changes were observed in the clinical status and biologic or virologic parameters. A second transfer was then applied with activation of the cells with purified envelope glycoprotein before infusion. The outcome of the treatment was an increase in total lymphocytes, in CD4+ and activated CD8+ DR+ cell counts, and in proliferative responses to HIV antigens. A marked but transient 3-log increase in cellular and plasmatic virus loads was also observed after the second adoptive transfer. These observations will be considered with attention to improve the future adoptive transfer protocols, especially in patients with severe CD4- depletion.

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MATERIALS AND METHODS

Subjects

The identical twins enrolled in this study are 38-year-old adults of HLA genotype A2 B44 CW5 DRW6/A24 B41 DR-.

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SIMI, the HIV-1–infected twin was diagnosed as seropositive in 1986. Since then, he developed severe and disseminated mollusc contagiosum in 1987, pneumocystis carinii pneumonia (PCP) in 1990, and mycobacterium avium (MAI) infection in 1992. He was under zidovudine treatment from 1988 to 1991 and was moved to ddI in March 1991. He was maintained under ddI treatment (134 mg/day) during the whole study period. The low ddI dosage was justified by peripheral neuropathy. Other concurrent therapies included dapsone (50 mg/d) for PCP prophylaxis and clotiazemine (100 mg/d), myambutol (1,200 mg/d), clarithromycin (2,000 mg/d), and rifabutin (450 mg/d) as maintenance treatment for MAI. His performance status based on the Karnofsky’s score was 30. His CD4+ cell count was less than 5 CD4+ cells/μL and CD8+ cells ranged from 121 to 225 cells/μL during the 6 months preceding initiation of this immunotherapy.

SH, the healthy HIV-seronegative twin, had been vaccinated against smallpox in the first year of life.

The study was approved by the local ethics committee and both the patient and his brother gave their informed consent.

Recombinant Vaccinia Virus Constructs

WR87, the vaccinia recombinant used to prime SH, carried the HIV-1 envelope gene from one of SIMI’s viruses. The vaccinia recombinant was constructed in the following way.

In March 1991, SIMI’s peripheral blood mononuclear cells (PBMCs) were purified by ficoll gradient centrifugation and cocultured with activated donor PBMCs. Eleven days after initiation of cell culture, virus proliferation was evidenced by p24 enzyme-linked immunosorbsent assay (ELISA) tests and genomic DNA was purified from the cultured PBMCs. Polymerase chain reaction (PCR) amplification of the HIV-1 envelope gene was applied using the following degenerated primers: 5’ primer: 5’ GAGTCTAGAGCGGCCGCA-CTGACGCAGCTTTCG-GAGGAGGAGCTCAG-AGCTGCGGCA-AG(C/G)ATGG(A/G)AGT(C/G)GA 3’; 3’ primer: 5’ GAGGCGGCCGCCTCAGAT(T/C)TGGACCA(C/T)C(T)TG-TCG(T/C)TACCAT(T/C)TTA 3’.

The Xba I and Not I sites designed at the 5’ end of the primers and the initiation and stop codons of the env coding sequence are underlined.

Conditions for amplification were 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 2 mmol/L MgCl2, primers at 1 mmol/L each, 3% dimethyl sulfoxide (DMSO), 1 μg genomic DNA purified from the cocultured PBMCs, and 2.5 U of Taq polymerase (Cetus, Sunnyvale, CA) in 100 μL. Thirty cycles of amplification were as follows: 1 minute of denaturation at 94°C, 2 minutes of annealing at 55°C, and 3 minutes of elongation at 72°C. The 2.6-kb fragment containing the env gene was then cloned using the Xba I restriction sites in vector pBS (Stratagene, La Jolla, CA). The complete nucleotide sequence of the clone (pUL5384) used for construction of the recombinant vaccinia virus WR87 and WR89 was determined (GenBank accession number L07421). The env gene in pUL5384 was subcloned in the transfer vector for vaccinia virus pSC11. Homologous recombination with wild-type vaccinia virus WR (ATCC VR1354; American Type Culture Collection [ATCC], Rockville, MD) in CV1 cells (ATCC CCL-70) and for Lac+ vaccinia plaques was applied as previously described and led to production of vaccinia virus recombinant WR87.

The vaccine virus stock was prepared in Vero cells (ATCC CCL81). The cell monolayer infected for 48 hours with WR87 was washed twice with phosphate-buffered saline (PBS). Cells were scraped and pelleted by centrifugation at 1,500g, resuspended in PBS, and then ruptured by three cycles of freeze-thaw and stored at −70°C. The resulting suspension had a titer of 2 × 10^6 pfu/mL.

The same env gene was also subcloned in transfer vector pMJ601. Recombination with wild-type vaccinia virus WR generated recombinant vaccinia virus WR89, which was used for envelope glycoprotein production and purification.

Purification of the gp160 Glycoprotein

Infection of Vero cells with vaccinia recombinant WR89 produces the uncleaved gp160 glycoprotein, as evidenced by Western blot analysis (data not shown).

Twenty 125-cm2 Petri dishes containing monolayers of Vero cells were infected at a multiplicity of infection of 10 with WR89. After 20 hours, the monolayers were washed with TBS (25 mmol/L Tris-HCl, pH 7.4, 137 mmol/L NaCl, 2.7 mmol/L KCl) supplemented with protease inhibitors 0.01 mmol/L phenylmethylsulfonyl fluoride (PMSF) and 100 U/mL aprotinin (Sigma, St Louis, MO); the cells were then scraped and collected.

Purification of gp160 involved sodium deoxycholate (DOC; Boehringer, Mannheim, Germany) solubilization of the cell pellet and lentil-lectin affinity chromatography as described by Barrett et al, with minor modifications.

The cell pellet for extraction of gp160 was resuspended in 10 vol of TBS, pH 7.4, with protease inhibitors and 1 mmol/L CuSO4 and 0.5 mmol/L ZnCl2. The suspension was homogenized and the cell membrane suspension was pelleted at 9,000g for 30 minutes. The membrane pellet was resuspended in 15 vol of 50 mmol/L Tris-HCl, pH 8.3, 1% DOC, 1 mmol/L CuSO4, and 0.5 mmol/L ZnCl2, with protease inhibitors and left at 25°C for 30 minutes. The suspension was submitted to ultrasonication for 30 seconds and then clarified by centrifugation at 100,000g for 1 hour. The supernatant was incubated for 2 hours with lentil-lectin sepharose 4B (Pharmacia, Uppsala, Sweden), which was then washed with 50 mmol/L Tris-HCl, pH 8.3, 0.25% DOC, and protease inhibitors. Bound proteins were eluted with 5% a-methyl mannoside in the same buffer.

The procedure yielded 200 μg of 15% pure gp160 glycoprotein per gram of recombinant vaccinia virus-infected cells, as measured by an ELISA test using purified gp160 (strain W61D; Smith Kline Beecham Biologicals, Rixensart, Belgium) as a standard. One hundred and fifty micrograms of the purified material was adsorbed on 0.5 mg of alum (Superfos, Vedbaek, Denmark) by overnight incubation at 4°C, centrifugation for 10 minutes at 1,500g, and resuspension in TBS. 95% of the purified gp160 was adsorbed on alum in these conditions. Such a preparation of partly purified recombinant gp160 adsorbed on alum was used to boost the healthy twin (SH) after priming by scarification with recombinant vaccinia virus WR87 and for ex vivo activation of SH’s lymphocytes before transfer to SIMI.

Biologic Studies

The assays consist of a complete blood cell count and differential and T-cell phenotyping assayed by flow cytometry through an EPICS system by using commercial monoclonal antibodies for CD3+, CD4+, CD8+ DR+, CD8+ DR- , NK+, and NK- DR+ (flow Becton Dickinson, Mountain View, CA). The routine monitoring of the serum β2-microglobulin level was performed by a commercial ELISA test (Pharmacia). α-Interferon (α-IFN) was measured by means of a cytopathic effect (CPE) reduction assay with vesicular stomatitis virus (VSV) as the challenger virus on Madin-Darby bovine kidney (MDBK) cells. Briefly, serial twofold dilutions of the sample were made in Dulbecco’s modified Eagle’s medium (DMEM) and indicator cells were added. After 24 hours, the appropriate dilution of VSV was added. CPE was read under the microscope after 48 hours. All data are expressed in laboratory units per milliliter. Interleukin-10 (IL-10) was assayed by two ELISA tests recognizing human IL-10 and viral IL-10 (BCRF-1), respectively.

Transfers

The following protocol was performed: (1) lymphopheresis of the vaccinated brother; (2) ex vivo activation by incubation of the collected cells for 1 hour at 37°C with 220 μg of gp160 purified from recombinant vaccinia virus WR89-infected cells and adsorbed on...
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Alum (not performed during the first cell transfer); (3) plasmapheresis of the recipient (the rationale of this step was to reduce the circulating viral load as well as to clear blood from potentially immune suppressive circulating factors such as α-IFN); and (4) transfusion of the cells from the donor to the infected patient.

Immunologic Studies

T-cell proliferation assay. PBMCs were suspended in culture medium (RPMI 1640 supplemented with 10% heat-inactivated normal human serum AB) at 2.5 × 10^6 cells/mL. One hundred microliters of the cell suspension was added to 100 μL of culture medium containing heat-inactivated HIV IIIB or HIV MN viruses (1 μg/mL), gp160 IIIB antigen (100 μg/mL, 2.5 μg/mL; Transgene, Strasbourg, France), SEB superantigen (0.6 μg/mL; Sigma, St Louis, MO), purified protein derivative PPD as recall antigen (gift from Institut Merieux, Lyon, France; 1,000 U/mL), or heat-inactivated vaccinia virus (2.5 × 10^7 pfu/mL). Plates were incubated for 6 days and 0.5 μCi 3H-thymidine (Amersham, Amersham, UK) was added during the last 18 hours of incubation. Cells were harvested and 3H-thymidine incorporation into DNA was measured. All determinations were performed in quadruplicates. The data are presented as the stimulation index (SI) calculated as follows: SI = Experimental Mean/Control Mean.

Cytotoxic T-cell analysis. PBMCs isolated from peripheral blood were stored in liquid nitrogen after freezing in fetal calf serum containing 10% DMSO. In vitro stimulations of CTLs were performed using the 15 amino acid peptide P18 MN (RIHGPGRAPFYTPYKK; amino acid coordinates 315-329 from the HIV-1 MN strain) that carries an immunodominant CTL epitope located in the hypervariable V3 loop of the HIV-1 envelope glycoprotein. A peptide homologous to P18 MN was synthesized according to the cloned pULB5384 sequence, P18 SIMI (amino acid sequence: TLHMGPKRAFYATGD), and used in parallel to P18 MN for in vitro stimulation of CTLs. Cells (4 × 10^6) were pulsed for 1 hour at 37°C with 5 μg of the peptide. The cells were cultured and resuspended at 10^6 cells/mL. Cultures were established in 24-well plates (Falcon; Becton Dickinson, Lincoln Park, NJ) in RPMI1640 medium supplemented with 1 mmol/L sodium pyruvate, 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 10% fetal calf serum (PAA, Linz, Austria) and 100 U/mL recombinant IL-2 (Roussel UCLAF, Romainville, France) for 6 days.

B-lymphoblastoid cell lines (B-LCL) were used as target cells. They were generated from SH’s PBMCs by transformation with recombinant vaccinia virus (WR) supplemented with 100 μCi of sodium chromate 51Cr (Amersham) for 60 minutes at 37°C, washed three times, and then resuspended to the appropriate concentration. They were subjected to lysis by effector cells in a 6-hour chromium release assay performed in duplicate. Percent specific cytotoxicity was determined from the formula: 100 × (release in assay – spontaneous release)/maximal release – spontaneous release). Maximum release was determined by 1 N HCl lysis. Spontaneous chromium release for all cultures was 25% of the counts released by HCl.

Inhibition of specific cytotoxicity using monoclonal antibodies directed to the CD3, CD4, or CD8 antigens was performed by incubation of effector cells for 30 minutes at 37°C with the monoclonal antibodies (Coulter clone; Coulter, Hialeah, FL) at a concentration of 1:50, before the initiation of the chromium release assay.

Virologic Studies

Cellular and plasmatic virus loads. The quantification of HIV-1 in the plasma and PBMCs of the patient was performed according to the endpoint dilution culture method. Briefly, decreasing numbers of PBMCs (2 × 10^9, 2 × 10^8, 2 × 10^7, 2 × 10^6, 2 × 10^5, and 1 × 10^5) isolated by Ficoll Hypaque gradient centrifugation were cocultured with 2 × 10^6 phytohemagglutinin (PHA)-activated PBMCs from healthy blood bank donors in 1.5 mL of RPMI 1640 medium supplemented with 10% fetal calf serum, human serum diluted 1:20 as a source of complement, 5 U anti-α-IFN (Boehringer), and 14 U of human recombinant IL-2 per milliliter (Boehringer).

Twenty-four hours later, all cultures were washed four times with medium. The cocultures were subsequently monitored weekly up to 28 days for the presence of p24 antigen in the supernatant. During this period, 5 × 10^7 PBMCs were added weekly and 50% medium changes were performed twice a week. The lowest number of PBMCs required to produce a positive culture was taken as endpoint and the titers of infectious HIV-1 were then expressed as 0.5, 5, 50, 500, or 10,000 tissue culture infectious doses (TCID) per 10^6 PBMCs.

The plasma was obtained by centrifugation of blood at 3,000 rpm for 15 minutes and filtered through a 0.22-μm millipore filter to remove all cells. Decreasing volumes of plasma (1,000, 200, 40, 8, 1.6, and 0.32 μL) were cultured with 2 × 10^6 PHA-activated PBMCs from healthy blood bank donors as described above. The cultures and monitoring of p24 antigen expression were as described above. The smallest volume of plasma required to produce a positive culture was taken as the endpoint and HIV-1 titers were then expressed as 1, 5, 25, 100, 500, or 2,500 TCID per milliliter of plasma.

PCR proviral load. Semiquantitative PCR using nested gag primers was applied on twofold dilutions of purified PBMCs DNA as described by Simmons et al.

p24 antigenemia. To disrupt immunocomplexes, 90 μL of clarified plasma was treated with 90 μL of glycine buffer (pH 2.2) and 30 μL of 5% Triton X-100 and incubated for 1 hour at 37°C. The samples were neutralized with 90 μL of 1.5 mol/L Tris-HCl, pH 8.6, and left for 5 minutes at room temperature. The p24 antigen was then quantified by capture ELISA as described by the manufacturer (Kit DuPont De Nemours, Bad Homburg, Germany).

Sensitivity to didanosine (ddI). In vitro sensitivity to ddI was evaluated by infection of PHA-stimulated donor PBMCs with titrated patient’s isolates in the presence of different concentrations of ddI. Virus growth was assessed by evaluation of reverse transcriptase activity.

Ex vivo sensitivity to ddI was evaluated by cocultivation of patient’s lymphocytes with PHA-activated donor PBMCs in the presence of ddI at concentrations of 6 and 24 μmol/L.

RESULTS

Characterization of the env Genes of SIMI’s Viruses

The envelope glycoprotein gene used in the vaccination protocol of the healthy twin was cloned after PCR amplification of genomic DNA from SIMI’s short-term cocultured PBMCs. Clone pULB5384 was obtained and its nucleotide sequence was determined. The predicted envelope amino acid sequence of clone pULB5384 was 85% identical to that of the HIV-1 MN, LAI, or RF strains. Envelope V3 loop domains (amino acid coordinates 296-330 on the HIV-1 MN envelope map) are hypervariable among HIV-1 strains; they are strongly immunogenic and carry neutralizing as well as helper and cytotoxic T-cell epitopes. This domain is consequently of major importance for vaccination. Amino acid sequences of V3 loop domains from envelope genes cloned at three different time points during the study period of 1 year and 2 months were obtained. Alignment of these sequences (Fig 1) shows the remarkable conservation of
SIMI's viruses V3 loop sequences during the study period. When compared with V3 loop sequences from reference strains HIV-MN, LAI, and RF, divergence was much broader. Because of (1) the divergence of SIMI's viruses V3 loop sequences with available reference env genes, (2) their conservation in time, and (3) the fact that an homologous antigen has a better chance to induce in the vaccinee an immune response targeted to the infecting virus, we decided to construct a recombinant vaccinia virus expressing the cloned pULB5384 envelope gene. Construction of the recombinant virus is detailed in the Materials and Methods.

**Vaccination of SH**

SH was vaccinated by standard intradermal scarification with a stock of recombinant vaccinia virus WR87 at 2 × 10⁶ pfu/mL (December 27, 1991). A typical lesion was observed at the site of vaccination. Eleven weeks later (March 16, 1991), SH was boosted by intramuscular injection of 150 µg of purified gp160 adsorbed on alum prepared as described in Materials and Methods and 2 mg of P40 immunostimulant (Pasteur Institute, Paris, France).

HIV-specific humoral and cellular responses were repeatedly tested before and after boosting. HIV-specific antibody response to vaccination was evaluated by ELISA and Western blot assays. Antibodies to HIV envelope were undetectable during the whole study period (data not shown).

Proliferative responses specific for HIV-1 virus and HIV gp160 antigen developed 17 days after the boost (Fig 2). Two months after the boost (May 21, 1992), the cytotoxic activity of SH's PBMCs was determined before and after stimulation with peptide P18, a V3 loop peptide carrying an immunodominant CTL epitope and synthesized according to the sequence of the vaccine envelope glycoprotein (P18 SIMI), and interleukin-2 (IL-2). Fresh PBMCs did not show any detectable HIV-specific CTL activity (data not shown). However, after stimulation with P18 SIMI and IL-2, a strong cytotoxic activity developed. Indeed, as shown in Fig 3B, effector cells stimulated with P18 SIMI were able to efficiently lyse (65%) autologous lymphoblastoid target cells (HLA A2 B44 CW5 DRW6/A24 B41 DR-) infected with the recombinant vaccinia virus WR89 at a ratio of effector to target cells of 10:1.

Most of the cytotoxic activity was specifically targeted to gp160 because control target cells infected with wild-type vaccinia showed only 25% of lysis in the same condition (Fig 3B). When SH's PBMCs collected before immunization (June 1991, 7 months before initiation of the vaccination protocol) were stimulated in vitro under the same experimental conditions, no CTLs could be generated (Fig 3A). It is interesting to note that we were also able to generate a CTL activity (60% of specific lysis at a ratio of effector to target of 10:1; data not shown) from SH's PBMCs collected 17 days after the boost by in vitro stimulation with the P18 MN peptide despite the divergence in amino acid sequence between this peptide and the corresponding region in the vaccine envelope glycoprotein.²⁸

Heterologous B-LCL (HLA A1 B14 C7 BW4 DRW-/A B8 C BW6 DR+) infected with WR89 were not significantly lysed by the effector cells (Fig 3C). Further experiments were performed to characterize the phenotype and the specificity of these effector cells. Envelope-induced lysis of autologous EBV-transformed B-cell blasts was blocked by preincubation of the effector cells with anti-CD3 or anti-CD8 but not with anti-CD4 antibodies (Fig 3D). This result shows that the lytic activity towards the envelope is mediated by CD8+, CD3+ T cells.

We conclude that the vaccination protocol led to priming of HIV-specific HLA class I-restricted CD8+ CTLs.

**Transfers**

Two adoptive transfers of lymphocytes from SH to SIMI were performed 2 months after the boost (May 6, 1992) and 15 days later (May 21, 1992).

The adoptive transfer protocol involved (1) plasmapheresis of SIMI to reduce the plasma virus load and the presence of factors susceptible to suppress immune reactivity (α-IFN), (2) lymphopheresis to collect 6 × 10⁶ leukocytes (including

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**Fig 1. Alignment of deduced amino acid sequences from SIMI's HIV virus V3 loops. V3 loop amino acid sequences of independent clones obtained by PCR amplification at different time points before and during treatment are aligned with the consensus of American and European isolates and V3 loop sequences of reference strains MN, RF, and LAI. Clone 1 represents the V3 loop of the vaccine pULB5384 env gene. The sequences are compared with the consensus of SIMI's V3 loop. A dash (-) indicates concurrence with the top sequence in the alignment. A period indicates a deletion.**
Fig 2. Lymphoproliferation of SH's PBMCs during the vaccination protocol. Stimulation index (SI) of $^3$H thymidine incorporation by SH's PBMCs incubated with gp160 antigen from the IIIB isolate or with heat-inactivated HIV MN virus or vaccinia virus or PPD recall antigen, 7 months before initiation of the vaccination protocol, 4 weeks postprimovaccination with recombinant vaccinia virus WR87, and 17 days after boosting with purified gp160 adsorbed on alum. For each of these parameters, no changes were noted during the 15-day period after the first transfer. However, after the second transfer, the proportion of activated peripheral CD8+ DR+ cells increased from 20% to 80%, in the absence of a significant increase of the absolute number of circulating CD8+ lymphocytes. A moderate increase in CD4+ cells (from less than 5 CD4+/μL for an extended period of time before the second transfer up to 10 to 18 CD4+/μL) also appeared between 5 and 15 days after the second transfer. It was followed by an increase in total lymphocytes between 30 and 60 days (from 100 to 200 up to 400 to 500 lymphocytes). No clinical adverse reaction was reported during or after the transfers.

Evolution of Biologic Parameters After Adoptive Transfer

Evolution of total lymphocytes, CD4+, CD8+, and both CD4+ DR+ and CD8+ DR+ cell counts from SIMI were evaluated at least once weekly after the first and the second transfer.

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Evolution of Immunologic Parameters After Adoptive Transfer

Fresh PBMCs from SIMI were repeatedly tested after transfers for cell-mediated immunity to HIV-1 IIIB gp160 antigen and HIV-1 IIIB or MN viruses, to PPD recall antigen and to SEB superantigen. Figure 6 summarizes the proliferative responses of mononuclear cells from SIMI. Lymphoproliferations towards HIV antigens and SEB were at basal line before and during the first posttransfer period. An impact of the treatment appeared at 20 days post-second transfer (June 11, 1992) and was maintained for at least 1 month. Indeed, significant lymphoproliferative responses were detected towards HIV antigens and SEB superantigen. However, the proliferative response to recall antigen PPD detected on the vaccinee’s PBMCs (Fig 2, proliferation index of 27) was not recovered in the

day2. This led us to test the sensitivity of SIMI’s isolates to ddI. Virus from three time points (June 1991, 3 months after initiation of ddI treatment; April 1992, 1 week before the first transfer; and June 1992, during the post-second transfer period) were tested for ddI sensitivity as detailed in Materials and Methods. The IC50 inhibitory concentrations determined on a dose-response curve were 1.4 and 1 μmol/L for the two first isolates. Ex vivo transmission assay performed on PBMCs collected in June 1992 showed a complete inhibition at 6 μmol/L ddI. These inhibition values fall into the range generally observed for virus isolates from ddI-naive individuals. Genetic analysis, performed by PCR search for the Leu 74 to Val mutation most commonly linked to resistance to ddI, showed the presence of the Leu codon at this position, supporting the sensitive phenotype of SIMI’s isolates.

Evolution of Virologic Parameters

Cellular and plasmatic virus loads and p24 antigenemia were evaluated during the study period (Fig 5A, B, and C). Cellular virus load was evaluated at 0.5 to 50 TCID/10^6 cells during the whole study period, with a transient peak at more than 5,000 15 days after the second transfer that rapidly ceased 1 week later. PCR determination of the proviral load faithfully reflected the trend in cellular virus load. The increase in cellular virus load was followed 1 week later by a peak in plasmatic virus load, with a value exceeding 3,000 TCID/mL of plasma that also ceased 2 weeks later, coming back to a mean value of 25 TCID/mL. Plasma p24 antigenemia measured after acid dissociation of the Ag/Ab complexes was 10 to 15 pg/mL and slightly raised to 40 to 60 pg/mL at the end of the study period (1 month after second transfer). Plasmapheresis did not appear to have an impact on the circulating virus load, as shown by determinations of the plasmatic virus load and of the HIV p24 antigenemia (Fig 5B and C).

The increase in plasmatic and cellular virus loads was obviously not controled despite treatment of the patient with ddI. This led us to test the sensitivity of SIMI’s isolates to ddI. Virus from three time points (June 1991, 3 months after initiation of ddI treatment; April 1992, 1 week before the first transfer; and June 1992, during the post-second transfer period) were tested for ddI sensitivity as detailed in Materials and Methods. The IC50 inhibitory concentrations determined on a dose-response curve were 1.4 and 1 μmol/L for the two first isolates. Ex vivo transmission assay performed on PBMCs collected in June 1992 showed a complete inhibition at 6 μmol/L ddI. These inhibition values fall into the range generally observed for virus isolates from ddI-naive individuals. Genetic analysis, performed by PCR search for the Leu 74 to Val mutation most commonly linked to resistance to ddI, showed the presence of the Leu codon at this position, supporting the sensitive phenotype of SIMI’s isolates.

Evolution of Simi’s Blood Biologic Parameters After the First and Second Adoptive Transfers.

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recipient (Fig 6). This finding suggests that the HIV-specific proliferative responses observed after transfer were induced by priming of naive T cells exposed to HIV antigens in the recipient rather than already primed cells originating from the donor.

When PBMCs were in vitro stimulated with P18 V3 loop peptides, no CTL activity was detected (data not shown).

**Clinical Outcome**

During and after the adoptive transfers of his brother's cells, SIMI did not experience any adverse effect. His clinical evaluation showed no significant changes. SIMI unfortunately died accidentally on July 27, 1992; the cause of his death was unrelated to HIV infection.

**DISCUSSION**

In this report, we describe the outcome of adoptive transfers of lymphocytes from an HIV-free individual vaccinated against HIV-1 envelope glycoprotein to his HIV-1-infected identical twin.

The AIDS patient was in the terminal phase of the disease, with opportunistic infections and CD4⁺ counts reduced to less than 5 CD4⁺ cells per microliter for months. During the study period of 1 year and 2 months, we observed that variation among envelope hypervariable V3 loop sequences was very restricted in this terminal stage patient and that these sequences were highly divergent from known V3 loops. Based on the observed ability to induce protective immunity by using HIV-1 envelope glycoprotein homologous to the challenge virus in the HIV-1 chimpanzee¹⁶,¹⁷ and SIV-macaque¹⁸ models, we decided to vaccinate the healthy twin with the envelope glycoprotein of one of his brother's viruses.

The vaccination protocol that was applied to SH, a smallpox vaccinated individual, was shown to be safe and to induce MHC class I-restricted CD8⁺ precursor CTLs that could be activated in vitro by V3 peptides. Our work thus confirms that HIV-specific CD8⁺ CTL can be induced in HIV-seronegative individuals through a vaccination protocol using recombinant vaccinia viruses for priming and purified envelope glycoprotein for boosting.²⁰,²¹ No antibody response was detected in the vaccinee. This latter point is in accordance with the observations by Cooney et al.¹⁴ and Graham et al.²⁰ showing that smallpox-vaccinated vaccinia-seropositive subjects developed weaker and less frequently HIV-
specific antibody responses to env recombinant vaccinia priming and recombinant envelope glycoprotein boosting than did vaccinia-naive subjects.

The evidence of a cellular immune response in the vaccinated healthy twin was the basis of the treatment; leukocytes were harvested from the vaccinated brother by lymphopheresis and transferred to the infected twin. The impact of the treatment was evidenced by changes in surrogate markers, ie, a slight increase in CD4+ cell counts and a decrease in β2-microglobulin. However, these effects were only transient and did not lead to clinical change.

Other important changes were the appearance in the recipient of activated CD8+ DR+ cells and significant proliferative responses to HIV antigens when compared with the baseline values. This finding suggests that the cellular immune response developed in the vaccinee could at least be partly transferred from the donor to the recipient. Because of the chronology of the response and the low value for proliferation to recall antigen, it is unlikely that the immune response developed in the recipient could be a direct effect of primed CD4+ cells. Furthermore, it is not clear whether the increase in CD8+ DR+ cells and proliferative responses observed in the patient after transfer was the result of in vivo priming of naive T cells from the donor or of activation of the recipient's own T cells. Labeling of donor cells could give an answer to the origin of the responding cells. Finally, the failure to detect HIV-specific CTLs after transfer could reflect the fact that the absolute number of CTLs transferred in the peripheral blood infusion was quite low.

The treatment also lead to a short-lived but strong increase in virus load. This observation was also made by others after immune stimulation of HIV-infected chimpanzees. The cellular virus load peaked in 1 week and then decreased rapidly during the following week at a time point when lymphoproliferative responses were developing. This finding suggests that the transferred or in vivo-stimulated lymphoproliferation activity had some protective value.

The patient was treated with a low dose of ddI during the whole study period to take into account peripheral neuropathy. This dose was clearly inefficient in controlling the increase in cellular and plasmatic virus loads despite the sensitivity of the patient isolate to this drug.

Two adoptive transfers were applied at a 15-day interval; they only differed by a step of incubation of the leukocytes harvested from the vaccinee with envelope glycoprotein (second transfer). The outcome was clearly different because the biologic, immunologic, and virologic parameters were invariant 15 days after the first transfer and changes only appeared after the second transfer. These changes could thus be the consequence of the activation of the cells before infusion in the patient and/or the cumulative effect of the two transfers.

Plasmapheresis was applied to the AIDS patient before transfer. This treatment had a clear impact on the level of circulating α-IFN that was very high in this patient. However, the effect was only transient and relapse occurred 24 hours later. Plasmapheresis appeared to be inadequate to sustainably eliminate this cytostatic agent.

T4 cells were included in the transfer to reconstitute CD4+ cell population and to provide IL-2. It should be mentioned that the number of CD4+ cells infused is about 100 times the number of circulating CD4+ cells in the patient (2 × 10^9 CD4 cells infused v 2 × 10^7 circulating CD4 cells). Because the number of CD4+ cells only slightly increased after transfer, it is supposed that the infused CD4+ cells were rapidly trapped in lymphoid organs or possibly infected and killed by the virus. It is noteworthy that the slight elevation of CD4+ cells was only observed after the second transfer. The impossibility to recover the infused CD4+ cells and the rapid increase in viremia after transfer points to and gives support to the use of CD4+ cells genetically protected against HIV infection.

In conclusion, the vaccination appeared to be safe for the healthy twin. The vaccination protocol led to priming of cellular immune responses, as shown in vitro by lymphoproliferation and cytotoxicity assays. These primed cells may be of some help in treating HIV-infected patients in whom cellular functions are deficient or absent. Prior activation of transferred cells obtained by lymphopheresis could enhance the immune response. Passive transfers of high numbers of...
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unprotected CD4+ cells should be avoided to decrease the risk of viral replication even for a short period of time and even under an efficient antiretroviral therapy. The dramatic increase of virus load could be explained by the rapid infection of these CD4+ cells and focuses on the idea that such a treatment should involve either other ways to provide IL-2 or gene therapy to protect CD4+ cells against HIV-1 infection. Before we gain insight into such solutions, we are focussing on clinical studies with substitutive immunotherapy by using selected syngeneic CD8+ cells.

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Syngeneic adoptive transfer of anti-human immunodeficiency virus (HIV-1)-primed lymphocytes from a vaccinated HIV-seronegative individual to his HIV-1-infected identical twin

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