Absence of Recoverable Infectious Virus and Unique Immune Responses in an Asymptomatic HIV+ Long-Term Survivor

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ABSTRACT

We have studied a woman with transfusion-acquired HIV who appears to have contained infectious virus to consistently undetectable levels over a 13-year period without antiviral treatment. She received the infected transfusion for intra- and postpartum blood loss immediately after delivery of her second child in 1981. She had no acute febrile syndrome and has never had HIV-associated clinical signs or symptoms in the 13 years since infection. She was first tested and found positive for HIV antibodies in 1985, and the infected blood donor was diagnosed with AIDS in 1986 and died of AIDS-related complications in 1989. Two other recipients of packed erythrocytes from this donor (in 1980 and 1982) also became infected and were subsequently diagnosed with AIDS. Between January 1986 and April 1994, in the setting of continuous and unambiguous Western blot HIV-specific antibodies and intermittently positive low-level HIV DNA signal after polymerase chain reaction (PCR) amplification, more than 30 separate cell cocultures performed in several independent laboratories failed to yield evidence of infectious virus, despite special efforts to induce and detect HIV replication. Immunologically, a strong in vitro proliferative response to HIV envelope proteins also distinguished this subject from other asymptomatic HIV+ individuals.

INTRODUCTION

The natural history and pathophysiology of HIV disease are complex and variable, dependent on a multitude of viral and host factors and their interactions. The recognition of viral quasispecies mutation and selection of escape variants, in the presence of antiviral drugs or neutralizing antibodies, has had a sobering effect on drug and vaccine development. On the other hand, cohorts of (1) long-term survivors¹² and (2) uninfected, persistently high-risk individuals³⁴ suggest that the balance between disease vs. effective immunity or infection vs. resistance, respectively, may not be far from the equilibrium point in these groups of HIV-infected or -exposed individuals. Provocative evidence from studies by Clerici, Shearer, and colleagues demonstrating “memory” T cell responses to HIV antigens in vitro among uninfected but repeatedly exposed individuals⁵⁶ has been interpreted by some as indicative of protective immunity.⁷⁸ In addition, certain primates harbor SIV or HIV at low or undetectable levels of replication without evidence of disease.⁹¹⁰
These observations in humans and primates offer hope that the balance between host immunity and viral replication and mutation can be tipped in favor of disease suppression by use of immune-based and drug therapies. We have studied an individual with transfusion-acquired HIV who appears to have contained infectious virus to consistently undetectable levels over a 13-year period without antiviral treatment.

**SUBJECT AND METHODS**

**Case report**

A 30-year-old Caucasian female, grávida IV, para II, married and monogamous since 1975 and living in a region of high HIV prevalence, was transfused in October 1981 with packed erythrocytes (PRBCs) subsequently determined to have been from an HIV-infected donor. The patient, referred to as patient 3799, received the transfusion for intra- and postpartum blood loss immediately after delivery of her second child. She received HIV-negative PRBC transfusions for intrapartum blood loss in 1984 and 1985. Careful and repeated histories taken from patient and husband by multiple interviewers over several years revealed no other risk factors for HIV. Approximately 1 month following the HIV+ transfusion she developed an urticarial rash on her palms, soles, and buttocks that resolved spontaneously. At no time did she develop fever, sweats, weight loss, diarrhea, malaise, fatigue, or a macular papular rash. Currently 43 years old, grávida 6, para 4, she has never had lymphadenopathy and has been without any HIV-associated clinical signs or symptoms in the 13 years since infection.

Because she recognized herself to be at risk from transfusion, the patient requested HIV testing in August 1985 and was found at that time to be seropositive by enzyme immunoassay (EIA) and Western blot. On the basis of these results, former blood donors of the transfusions she received for intrapartum bleeding in 1981 and 1984 (following the birth of her third child) were identified and tested. Only the donor of the 1981 transfusion was found to be HIV+. He was diagnosed with AIDS in 1986 and died of AIDS-related complications in 1989. Two other recipients of PRBCs from this donor in 1980 and 1982 also became infected and were subsequently diagnosed with AIDS. Lymphocyte analysis of patient 3799 in September 1985 showed 450 CD4/mm³ with a CD4:CD8 ratio of 0.9. Her fourth child, born in November 1985, had seropositive cord blood and subsequently seroreverted. All four children (each breast-fed for 1 year) and patient 3799's husband remain seronegative.

Between January 1986 and April 1994, enzyme-linked immunoadsorbent assays have been unequivocally positive. Similarly, antibodies binding all Western blot bands have been detected consistently since initial testing in August 1985 (see Fig. 1). During this same period, HIV DNA signal after polymerase chain reaction (PCR) amplification has been intermittently positive, and only at the limits of detection, using multiple aliquots of cells.

**Cell separations**

Peripheral blood mononuclear cells (PBMCs) were isolated from heparin- or citrate-anticoagulated blood by Ficoll-

**FIG. 1.** Typical Western blot reactivity pattern of patient 3799. Positive (lanes 1 and 2), and negative (lane 3) WB reactivities are shown with the positive reactivity of serum from patient 3799 obtained on August 22, 1991 (lane 4).

Hypaque density gradient centrifugation. Bone marrow mononuclear cells (BMMCs) were similarly obtained from heparinized aspirates of posterior superior iliac crest.

**Leukocyte phenotyping and selection**

HLA haplotyping was determined by serological panel in the tissue-typing laboratory of D. Kostyn (Duke University, Durham, NC). Two-color flow cytometric analysis of PBMCs was performed in multiple laboratories using fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies (MAbs) directed to surface leukocyte antigens CD3, CD4, CD8, and CD20 (Becton-Dickinson, Mountain View, CA), PE-conjugated MAbs specific for CD29 and CD45RA (Coulter Immunology), and activation markers HLA-DR, CD38, CD25, and CD57 (Becton-Dickinson). Natural killer cells were identified with a mixture of CD3-FITC, CD16-PE, and CD56-PE (Becton-Dickinson). Similar results were obtained at all sites. For some cultures, CD8+ T cell and CD20+ B cell subsets were depleted by immunomagnetic bead separation (Dynal, Great Neck, NY) or anti-CD8 coated MicroCelllector flasks (Applied Immune Sciences, Inc., Menlo Park, CA).

**Lymphocyte proliferation and cytotoxicity assays**

Proliferation assays using 1 × 10⁶ PBMCs in 200 μl/well and various HIV and recall antigens were performed in quadruplicate as described previously. Recombinant CHO cell-produced HIV-1MB gp120 and HIV-1MN gp120 antigens (Genentech, South San Francisco, CA) were used, with 5 and 50 μg/ml giving equivalent results. Recombinant gp160 (MicroGeneSys, Meriden, CT) produced in insect cells was used at a range of 0.125–12.5 μg/ml, with 1.5 μg/ml generally giving optimal responses. Tetanus toxoid, diphtheria toxoid, and Candida antigens were used in a standard range of concentrations. Cells were cultured for 7 days in the presence of antigen or control media, pulsed with [³H]thymidine for 12 hr, and harvested for scintillation counting. Lymphoproliferative stimulation indices (LSIs) were calculated by dividing the mean thymidine incorporation for each antigen by the mean thymidine incorporation of cells in control medium for each lymphoproliferation assay. For each antigen, maximum LSIs are presented.

Cytotoxicity assays were performed as described previously, using as effector cells either fresh PBMCs or CD8-
enriched PBMCs activated with phytohemagglutinin (PHA) and expanded in interleukin 2 (IL-2). Target cells were Epstein–Barr virus (EBV)-transformed autologous B cell lines (BLCLs) infected with vaccinia vectors containing HIV-1 env (vPE16), gag (vDK1), or pol (vCP21) genes or, as a control, the bacterial lac gene (vSC8). In vitro-activated CD8+ T cells were also tested for lysis of Daudi cells as a measure of lymphokine-activated killer activity. Cytolysis was assessed in a 6-hr 51Cr-release assay at the indicated effector:target ratios.

Serology assays

Sera and plasma were tested on licensed commercial HIV-1 enzyme-linked immunosorbent (EIA) kits (Abbott, Abbott Park, IL) and Western blot strips (Cambridge Biotech, Worcester, MA) according to manufacturer instructions. Enzyme-linked immunospot assays (ELISPOTs) were performed as described previously,14,15 using HIV-1 gp120-based recombinant gp160 envelope protein (MicroGenSys). Fusion inhibition assays were performed using 5000–10,000 CEM cells chronically infected with various HIV strains, and mixed with 70,000 uninfected Molt-4 cells as described previously.16 Titters were determined 24 hr later as the reciprocal of serum dilutions yielding 90% reduction in visually scored syncytia compared to cells maintained in control sera. Envelope peptide-binding EIs were performed as described previously,17 using the V3 loop regions of HIV-1MN (CYNKRKRIHIGPGRAFYTVKNIIG) and HIV-1Gp1 (CNYKRKRIHIGPGRAFYTVKNIIG), and the conserved immunodominant gp41 “Avery peptide” (ERYLKDQQLLIWGCSGKLICG).

Cell infection and viral culture studies

Fresh PBMCs, or PBMCs depleted of B and/or CD8+ T cells, were exposed to various laboratory strains of HIV-1 at 105 TCID50 (50% tissue culture infective dose). Viral replication was assayed by sequential p24 production (Abbott). Tittered stocks of HIV-1MN, HIV-1MN, and HIV-1Bal, were purchased from ABI (Columbia, MD). HIV-1p24 and HIV-1p24 were generous gifts from L. Ratner, Washington University, St. Louis.

Efforts to recover autologous virus by coculture studies generally employed 5 × 106 fresh PBMCs from patient 3799 cultured with an equal number of uninfected donor PBMCs that had been stimulated with PHA and IL-2 for 48–72 hr. These standard cultures were established and maintained in accordance with the AIDS Clinical Trials Group consensus protocol18 in the Johns Hopkins AIDS Clinical Trials Unit Virology Laboratory (Baltimore, MD), which has one of the highest rates of viral recovery among participating institutions. Standard cultures were maintained by twice weekly feeding with fresh uninfected PBMCs for an extended period of at least 8 weeks. Similar standard coculture experiments were performed at the Irwin Memorial Blood Center (San Francisco, CA), University of Maryland (College Park, MD), Centers for Disease Control (Atlanta, GA), and UCSF Medical Center (San Francisco, CA).

Alternatively, PBMCs from patient 3799 and uninfected cocultured cells were depleted of CD8+ T and CD20+ B cell populations prior to mixing and culture as described above. In some cases soluble synthetic HIV-1 MN Tat peptide was added at a concentration of 5 μg/ml, and in some cultures anti-CD3 MAb (OKT3; Ortho, Ranitan, NJ) was used in lieu of PHA to stimulate T cells.

Cocultures were routinely assayed at 3-day intervals for viral replication by measurement of supernatant p24. In some cases cocultures were also tested for the presence of cell-associated HIV DNA by PCR amplification, using published primers and reaction conditions.19,20

HIV DNA polymerase chain reaction amplification

Bone marrow aspirates were processed and amplified by DNA PCR as previously detailed.21 The PBMCs were processed and amplified in the gag and env regions, using published techniques,19,20 or using a highly sensitive nested PCR employing primers spanning the env V3 or long terminal repeat (LTR) region. For V3, the outer primer set was 5′ CAGCAGTACAATGACATCTGAAT 3′ and 5′ ATACAGTAGAAATTCCTCACC 3′; the inner set was 5′ TGGCAAGTCGACAGAAAG 3′ and 5′ ACAATTCTGTGGTCCTCCTC 3′. For the LTR region, the outer primer pairs were 5′ GATCTGGATCATTACACACACAGGC 3′ and 5′ GACACTAAGGCAAGCTTTATGAGGC 3′; the inner set was 5′ CCGTGAATGGACAGACTACACACCG 3′ and 5′ GTGGGCTCTTAGGTAGGAGCCG 3′.

Unfractionated PBMCs or enriched PBMC subsets were used for nested PCR analysis. Adherent macrophages were collected on plastic petri dishes by incubation of PBMCs at 37°C in 5% CO2, removal of nonadherent cells by washing, and recovery of adherent cells by scraping in the presence of ice-cold Ca2+-free phosphate-buffered saline (PBS). CD4-enriched populations were obtained from nonadherent populations by positive selection with Dynal immunomagnetic anti-CD4 beads as described above. Cells were resuspended (8 × 106 cells/ml) in PCR lysis buffer (2.5 mM MgCl2, 50 mM KCl, Tris- HCl [pH 8.4], and gelatin [200 mg/ml]) containing 0.1% Tween 20 and 0.1% Nonidet P-40 (NP-40), and stored at −20°C. Before PCR, the lysed cells were treated with proteinase K (100 μg/ml; Boehringer Mannheim, Indianapolis, IN) overnight at 57°C and boiled for 5 min to inactivate the enzyme.

An aliquot of cellular material equivalent to 1–2 × 109 cells was first denatured at 95°C for 2 min, and then amplified for 30 cycles using outer primers (for either V3 or LTR, as shown above) in 100 μl of PCR buffer containing dNTPs (0.2 mM each), 25 pmol of each primer, and 2 U of Taq polymerase (Boehringer Mannheim). The outer PCR product was diluted 1:100 in deionized H2O and 10 μl of the diluted PCR product was reamplified using the appropriate V3 or LTR nested inner primers detailed above. Cycle conditions were set at 94°C for 15 sec to denature the DNA, 45°C for 1 sec to anneal primers, and 72°C for 30 sec for DNA synthesis. After 30 cycles the products were extended at 72°C for 10 min. Conditions for the outer and inner nested PCR amplifications were identical, and a Perkin-Elmer Cetus (Norwalk, CT) 9600 thermal cycler was used throughout. The nested PCR products were separated by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining.

In situ PCR was performed on PBMCs as previously described.22–25 In brief, cells were plated into 3-well heavy Teflon-coated (HTC) slides (Cel-Line Associates, Inc., Northfield, NJ) at 1 × 105 cells/well, and allowed to settle and
air dry. Slides were then placed on a heat block at 105°C for 90 sec and then placed in 2% paraformaldehyde–PBS for 1 hr. The paraformaldehyde was then removed by washing six times with PBS. Endogenous peroxidase activity was quenched by overnight incubation with 3% hydrogen peroxide at 37°C. The slides were then treated with proteinase K (5 µg/ml in PBS for 15 min at 55°C), which was then inactivated by placing the slides on a heat block at 96°C for 2 min, washing them with distilled water, and air drying. The previously published gag region PCR primers SK38 and SK39 were used at 20 pM each, along with dNTPs (10 µM each), 1.0 U of Taq polymerase, 50 mM KC1, 10 mM Tris (pH 8.3), and 2.5 mM MgCl2 in a total volume of 8 µl. Two wells per slide were used for sample amplification, with the third well serving as a negative control (no primers added). Slides were sealed with coverslips and clear nail polish, placed on a thermocycler modified to hold slides (M. J. Research Watertown, MA), and subjected sequentially to 30 cycles each at 92, 45, and 72°C for 1 min each. Slides containing U1 (HIV infected) and U937 (uninfected) cells in various ratios were used as positive and negative controls.

Following amplification, slides were dipped in 100% ethanol for 35 min, coverslips removed, and slides placed on a 92°C heating block for 30 sec. After washing in 2× SSC buffer (0.3 M NaCl and 0.03 M sodium citrate), PCR amplification products were probed with the previously described SK19 biotinylated gag oligonucleotide. After a 4-hr incubation at 48°C, slides were washed in 2× SSC buffer, incubated with streptavidin–peroxidase, and developed with aminoethylcarbazol and hydrogen peroxide. Slides were analyzed for in situ PCR-amplified gag by direct microscopic examination, with positive cells appearing brownish red against a background of colorless or pink negative cells. Samples were evaluated in two independent experimental preparations (total of 1.2 × 106 cells examined) performed in triplicate.

**RESULTS**

**HLA and lymphocyte subset phenotyping**

The patient's haplotype was determined to be A1 A2, Bw57 Bw4, Cw6, DR7, DQw2. No lymphocyte enumeration data are available prior to 1985, at which time absolute CD4+ lymphocytes were calculated to be 450/mm³, with a CD4:CD8 ratio of 0.9. Subsequent CD4+ lymphocyte counts have ranged between 385 and 600/mm³ over a period of 8 years, with a CD4:CD8 ratio ranging between 0.7 and 0.9. A large number of cell surface activation markers were analyzed in conjunction with CD4 and CD8 by two-color fluorescence. There was little change in percentages of subsets over the most intensively studied 3-year period (1990–1993). The proportion of CD4+ and CD8+ lymphocytes expressing activation markers CD25 (IL-2 receptor), CD38, and/or HLA-DR were slightly elevated compared to cells from HIV-uninfected individuals, and within the range seen in our laboratory among individuals with early asymptomatic HIV infection.

**Antibody and B cell studies**

No unique humoral anti-HIV responses were noted. In fact, numbers of B cells spontaneously secreting anti-gp160 (80/10⁶ PBMCs) were in the low end of the range routinely seen for asymptomatic HIV+ individuals (mean of 86 HIV+ individuals = 737/10⁶ PBMCs; see Ref. 14). Similarly, total binding to rgp120 from MN and SF-2 strains was at the lower end of the range typically seen for sera from asymptomatic HIV+ individuals. The ability of sera to block gp120–CD4 binding–mediated cell–cell fusion was assessed using CEM cells infected with LAI (IIIB), MN, and SF-2 strains of HIV-1 and added to Molt-4 cells. Binding to the V3 region peptides of these three strains, as well as the conserved “Avery peptide” region of gp41, was also assessed by EIA. LAI-specific syncytium blocking and V3-binding titers were undetectable in patient 3799, but this is common seen for HIV+ sera. MN- and SF-2-specific syncytium blocking and V3-binding titers were somewhat lower in patient 3799 than the mean of 11 sera from asymptomatic HIV+ individuals (Table 1). In contrast, Western blot band reactivity was typically very strong over the time period studied (Fig. 1). Serum from patient 3799 did not compete for neutralizing epitopes with F105 MAb (data of M. Posner, not shown).

**Circulating HIV-1 envelope-specific cytotoxic T lymphocyte and cytotoxic T lymphocyte precursors**

Using fresh PBMCs as effectors and autologous BLCL targets, direct cytotoxic T lymphocyte (CTL) activity was detected only against env-expressing targets (Fig. 2A). However, fol-

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**Table 1. Patient 3799 Syncytium-Blocking and V3-Binding Titers Compared to Those of HIV-Infected Asymptomatic Individuals**

<table>
<thead>
<tr>
<th>Serum source</th>
<th>Syncytium inhibition†</th>
<th>EIA titre‡</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MN</td>
<td>SF-2</td>
<td>V3 MN</td>
</tr>
<tr>
<td>Patient 3799</td>
<td>85</td>
<td>100</td>
<td>145</td>
</tr>
<tr>
<td>11 HIV+</td>
<td>120</td>
<td>340</td>
<td>465</td>
</tr>
</tbody>
</table>

†Results expressed as reciprocal of serum dilution giving 90% inhibition of syncytium formation between CD4+ Molt-4 cells and CEM cells chronically infected with indicated strain of HIV-1.

‡Results expressed as 50% maximal binding titers on V3 and immunodominant gp41 (Avery) peptides. V3 MN, CYNKKKRIHIGPRAFYTTKINIG; V3 SF-2, CNNKSIY1PGRFHTGTRIG; gp41, ERYLKDQQLLGIWGCGKIG.

Geometric mean titers of sera from 11 asymptomatic HIV-infected individuals.
In contrast to essentially all HIV+ asymptomatic subjects studied in our laboratories or reported in the literature,14 PBMCs from patient 3799 proliferated strongly (LSIs > 100) in vitro PHA stimulation and IL-2 expansion of CD8+ enriched cells, significant specific killing was seen against both env- and gag-expressing targets (Fig. 2B). Similar results were obtained in two independent laboratories at separate time points.

**A. DIRECT CTL REACTIVITY (PBMC)**

![Diagram](image)

**B. MITOGEN-ACTIVATED CTL (CD8+ CELLS)**

![Diagram](image)

**FIG. 2.** HIV-1-specific cytotoxic T cell activity in patient 3799. CTL effector cells were either fresh PBMCs (A), or CD8+-enriched PBMCs activated for 72 hr with PHA and expanded for 21 days in IL-2 (B). CTL lysis of EBV-transformed autologous B cell lines was measured after infection of targets with recombinant vaccinia expressing either a non-HIV protein (Control), or one of the HIV proteins encoded by env, gag, or pol. PHA-activated, in vitro-expanded CD8+ effectors were also tested for killing of Daudi cells as a measure of lymphokine-activated killer (LAK) cell activity.
response to stimulation with rgp120 from either HIV-1MN or HIV-1IB (Fig. 3). Although less dramatic, an LSI of 10 in response to baculovirus-produced rgp160 was also significantly higher than the mean LSI of 361 asymptomatic HIV* volunteers similarly studied. Responses to non-HIV recall antigens were all within normal limits (LSIs > 60, data not shown).

Infection of patient cells with exogenous HIV-1

Phytohemagglutinin-stimulated PBMCs or PBMCs depleted of CD8+ cells were exposed in individual cultures to five HIV-1 laboratory strains: IIIB, MN, BaL, p120, and p125. Unfractionated PBMCs from patient 3799 appeared resistant to 10^3 TCID_{50} of HIV-1MN or HIV-1Bat; however, both strains produced detectable p24 when cultured in CD8-depleted PBMCs from patient 3799 (Fig. 4). Furthermore, HIV-1MN, and the IIIB-related p120 and p125 HIV strains, grew well in whole or CD8-depleted PBMCs from patient 3799 (not shown). Thus, at the single CD4+ cell level, there was no intrinsic genetic resistance to HIV infection by laboratory strains.

Coculture of patient cells

Peripheral blood mononuclear cells from patient 3799 were first cocultured in January 1986 and again in November of the same year. These cultures were negative by the criterion of detectable supernatant p24. Between August 1991 and April 1994, many additional unsuccessful attempts were made to recover infectious HIV by coculture. Techniques used (sometimes in combination) to increase sensitivity included (1) depletion (>90% reduction) of CD8+ T cells and B cells from patient 3799 and normal donor cells, (2) use of anti-CD3 MAAb-stimulated cocultures as well as PHA-stimulated cocultures, (3) addition of soluble synthetic Tat (5 μg/ml), (4) detection by HIV DNA PCR amplification, (5) coculture of PBMCs or purified monocytes on primary macrophage lines, (6) cocultures maintained for >8 weeks with weekly addition of new, uninfected mitogen-stimulated cells, and (7) use of bone marrow aspirate mononuclear cells as a source of patient HIV.

None of these methods resulted in detectable in vitro propagation of patient virus. To date, more than 30 separate cultures performed in multiple independent qualified laboratories from 1986 to 1994, using the various culture enhancement techniques noted above, have been uniformly negative.

Proviral DNA polymerase chain reaction amplification

From 1988 to 1994, more than a dozen attempts at PCR amplification of HIV DNA were performed on PBMCs or bone marrow aspirates using various primer pairs for conserved regions in the gag, env, and LTR genes. Typically, low or absent signal was obtained when standard procedures employing DNA template from 1–2 × 10^5 cells were used. Signal could often be obtained from samples when 5- or 10-fold more cells were used as template, or when multiple standard aliquots were amplified. For example, using gag-specific primers to amplify signal from PBMCs obtained in August 1992, two of eight samples were positive with SK38/39 and one of eight were positive with SK101/145 when probed with 32P-labeled SK19 specific for HIV-1 gag. These results are consistent with a peripheral proviral burden at least 10-fold lower than typically seen in asymptomatic HIV+ individuals. The in situ PCR amplification technique of Bagasra et al.,22–25 which has detected surprisingly high proportions of HIV DNA in PBMCs of asymptomatic HIV+ individuals, was performed three times with cells from patient 3799 obtained in 1992. No PBMCs containing HIV DNA were detected.

**FIG. 3.** Lymphoproliferative stimulation indices (LSIs) of patient 3799 vs. asymptomatic HIV* volunteers with CD4 cell counts > 300/mm^3. Compared with large numbers of asymptomatic HIV* volunteers tested during the same period, patient 3799 had significantly higher LSIs to recombinant gp120 envelope proteins from two strains of HIV-1 (MN and IIIB). These proteins were produced in Chinese hamster ovary cells. A lesser, but still significant, LSI was observed in response to IIIB strain rgp160 produced in a baculovirus–insect cell system.

**FIG. 4.** Permissiveness of patient 3799 CD8-depleted PBMCs for HIV. PBMCs (5 × 10^6 in 5 ml) or PBMCs depleted of CD8+ cells (5 × 10^6 in 5 ml) from patient 3799 were exposed to 10^3 TCID_{50} of HIV-1MN (circles) or monocyte tropic HIV-1Bat (squares). No p24 was detected in whole PBMC cultures (closed symbols) over 30 days, but both strains of virus showed p24 production by day 10 in cultures depleted of CD8+ cells (open symbols).
Attempts to localize HIV DNA to PBMC subsets from two closely spaced time points indicated a predominance of signal in the CD4+ population, as opposed to the adherent cell population, using either V3 region or LTR region amplification (Fig. 5). Sequence analysis of the V3 regions obtained by PCR amplification revealed genotypes typical of less than 5% of North American isolates (unpublished data). This makes it extremely unlikely that PCR signal was obtained as a result of repeated laboratory strain or patient isolate cross-contamination.

DISCUSSION

Patient 3799 represents a rare documented case of 13-year survival with HIV-seropositive immune status in the absence of detectable circulating replication-competent virus. Polymerase chain reaction amplification of HIV DNA signal in peripheral blood and bone marrow intermittently revealed the presence of provirus, but this does not permit distinction between persistence due to viral replication or mitotic division of progenitor cells with integrated HIV DNA. High levels of humoral and cellular HIV-specific immunity suggest persistence of HIV antigenic stimulation, but this could occur in the absence of fully infectious virions. The presence of multiple quasispecies with nonsilent point mutations in the V3 region (unpublished data) suggests selection of mutants by immune system pressure, but does not indicate when in the course of infection that selection may have occurred. We plan to retrieve stored cell samples from pre-1990 time points in order to document the rate of change in the V3 region.

This case differs from the group of healthy asymptomatic transfusion recipients reported by Learmont et al.1 in that all of those individuals and the donor remained healthy, whereas the donor and two other recipients in the present case progressed to AIDS. Also, it was not reported by Learmont et al. that extraordinary efforts were made to cultivate HIV from this cohort over a period of years, and it is not uncommon to recover no HIV in standard cultures of very healthy HIV+ individuals with normal CD4 counts. We are aware of only one similar case of transfusion-associated persistent seroconversion with confirmation by PCR amplification of HIV DNA, but consistently negative cultures using state-of-the-art in vitro manipulations over a period of several years.26 We are currently studying four potentially similar cases. In most of these rare cases, the individuals who seem to have suppressed HIV replication were identified by known transfusion risk factors. It is possible that a much larger number of unidentified individuals with no symptoms and no self-identified risk factors have also encountered and contained HIV at a level that is nonprogressive. Such seropositive PCR-positive individuals represent points on a spectrum that is bounded by the well-described asymptomatic culture-positive long-term survivors on one end, and on the other end by seronegative, PCR-negative, high-risk individuals with in vitro evidence of cellular immune memory for HIV antigen exposure.

As remarkable as the absence of recoverable infectious virus is the presence of a vigorous T cell proliferative response to rgp120 in vitro. The lack of such a response in virtually all asymptomatic HIV+ patients may reflect the migration from the peripheral circulation, and subsequent infection and destruction, of CD4+ T cells specific for HIV envelope antigens.27 This unresponsiveness can be transiently overcome by repeated immunizations with exogenous subunit.13 Could an effective immune response in patient 3799 have suppressed all replication-competent quasispecies originally transmitted or arising by mutation? If so, then replication-inept vent provirus must be maintained in a dividing cell pool to maintain sufficient provirus for PCR detection. Furthermore, at least some of this provirus must be transcriptionally active to account for the persistently strong cellular and humoral immune responses seen 12 years after infection. Likewise, the low but stable CD4 counts might be explained, in the absence of direct infection-mediated destruction, by viral proteins inducing mechanisms of indirect killing of CD4 cells (e.g., apoptosis, syncytium formation, innocent bystander targeting).

Although the absence of recoverable virus makes it difficult to speculate on the role of neutralizing antibodies (Abs) in controlling disease, it is interesting to note that assays such as V3 loop and gp41 binding assays, and syncytium inhibition, dependent largely on conformational and/or strain-specific epitopes of HIV-1 strains LAI, MN, and SF-2, revealed absent or lower than average titers, whereas WB reactivity with denatured, conserved epitopes of HIV-1 was intense. This may reflect limited broadening of the humoral response (normally seen over the course of infection) owing to an immune response that effectively limited HIV quasispecies diversity. Similarly, the ability of whole PBMCs (but not CD8- and B cell-depleted populations) to suppress exogenous infection with MN or BaL strains of HIV, but not IIIB-related strains, could reflect greater diver-
gence of the latter from endogenous sequences with consequent ability to escape CD8+ CTL and neutralizing Abs in vitro. Of course, it is also possible that strain IIIB is an intrinsically more efficient strain with respect to in vitro replication.

Harrer et al.28 have described a long-term survivor with strong CTL activity for HIV pol gene-derived peptides in the context of the HLA A2 allele (present in patient 3799), but there are no conclusive data to support the protective role of particular HLA alleles, and pol-specific CTLs were not detected in our patient. In fact, direct CTL activity was seen exclusively against env determinants, in contrast to the more typical pattern obtained from asymptomatic patients of a dominant response to gag and pol determinants. Patient 3799 may have been alloimmunized owing to multiparity prior to HIV exposure. If so, this may have played a role in the earliest stages of infection, as HIV is known to carry alloantigens on its coat as it buds from the host cell membrane.29

Lymph nodes are reservoirs of noncirculating HIV in asymptomatic humans and primates and may be a site of persistent HIV in patient 3799, even in the absence of lymphadenopathy. Alternatively, long-lived thymic dendritic/mesenchymal cells may transmit relatively defective HIV to resident thymocytes at some low rate, dependent on high thymocyte turnover. Replication-competent provirus within the residual adult thymic milieu might be destroyed along with the majority of thymocytes, which never leave the thymus. Any escaping HIV might find the microenvironment and much lower rate of cell division in nodes and periphery to be suboptimal, especially in the face of an effective immune response. Evidence that some HIV recovered from hosts may be replication defective,30 along with in vitro demonstration of complementation among defective HIV strains, suggests that a critical mass of quasispecies may be required to overwhelm an effective response by the immune system. At present we have no information on the status of this individual’s thymus, and she has declined lymph node biopsy.

Any of these interpretations would have positive implications: prophylactic vaccines might prime for a response resembling that of patient 3799, and early therapeutic vaccination or even passive immunotherapy combined with antivirals might tilt the “balance of power” in favor of the host, resulting in prolonged clinical latency. We have undertaken a detailed genetic analysis of PCR-amplified structural and regulatory gene regions obtained from patient 3799. Additional rare individuals should be studied for clues to effective host responses.

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