American Journal of Hematology 59:199–207 (1998)

Viral Burden and Disease Progression in HIV-1–Infected Patients With Sickle Cell Anemia

Omar Bagasra,[1] Robert M. Steiner,[1,2]* Samir K. Ballas,[1] Oswaldo Castro,[3] Geethanjali Dornadula,[1] Stephen Embury,[4] Donald Jungkind,[5] Lisa Bobroski,[1] Abdullah Kutlar,[6] and Sandra Burchott[7]

[1]Division of Infectious Disease/Center for Human Virology and The Cardeza Foundation of the Department of Medicine, Jefferson Medical College of Thomas Jefferson University, Philadelphia, Pennsylvania
[2]Department of Radiology, Jefferson Medical College of Thomas Jefferson University, Philadelphia, Pennsylvania
[3]Sickle Cell Center of Howard University Hospital, Washington, D.C.
[4]Division of Hematology of the San Francisco General Hospital of the University of California, San Francisco, California
[5]Department of Pathology, Jefferson Medical College of Thomas Jefferson University, Philadelphia, Pennsylvania
[6]Sickle Cell Center of the Medical College of Georgia, Augusta, Georgia
[7]Children's Hospital of Boston, Harvard Medical School, Boston, Massachusetts

The spleen and lymph nodes are major sites of human immunodeficiency virus type 1 (HIV-1) replication, mutation, and genetic variation in vivo. If a major portion of the lymphatic tissue, such as the spleen, is removed or otherwise is unavailable for invasion by the HIV-1 virus, will the course of the infection be altered, resulting in a prolonged symptom-free interval or even increased survival? The spleen of most adults with sickle cell anemia (SS) is nonfunctional due to recurrent episodes of microinfarction. If autosplenectomized SS patients are exposed to HIV-1, they may be ideal candidates to examine the question of whether absence of splenic function at the time of infection will positively alter the course of HIV-1-related disease. All SS patients with a diagnosis of HIV-1 infection at five university sickle cell centers were included in the patient cohort. Patients in active treatment or in follow-up (group A, n = 11) underwent a series of quantitative viral studies to determine their HIV-1 viral burden. The studies included the branched-DNA signal amplification assay, quantitative DNA-polymerase chain reaction (PCR), quantitative reverse transcription (RT)-initiated–PCR, and in situ PCR. All patients who died of the complications of the acquired immunodeficiency syndrome (AIDS) or of SS, lost to follow-up, or were otherwise unavailable for study (Group B: n = 7) were included in the total patient group. None of the patients in group B underwent quantitative viral studies. In addition, a control population (group C, n = 36) of HIV-1–infected African Americans without SS, of similar age and gender to the SS patients, were compared with the study population for outcomes. In eight of 11 active patients (group A), the CD4+ T-lymphocyte counts were normal and viral burdens were low for an average of 10.25 years following diagnosis. These eight patients all from group A were the only long-term nonprogressors (44%) among a total of 18 SS patients (groups A and B). In group C (control), only five patients of 36 were long-term nonprogressors (13.9%). Five patients (28%) of the total SS group (groups A and B) succumbed to AIDS. One of the five was from Group A. The evaluation of a limited number of adult individuals suggests that a significant proportion of HIV-1–seropositive SS patients (44%) may be asymptomatic long-term nonprogressors. In these patients, the CD4+ T-lymphocyte counts remained high and their viral burdens were remarkably lower than in non-SS HIV-1–seropositive individuals. Whereas this study does not prove an ''autosplenectomy'' hypothesis, it suggests that in patients with both SS and HIV-1 infection, the retroviral disease may be ameliorated by host factors of which absence of splenic function prior to HIV-1 infection may be one. Am. J. Hematol. 59:199–207, 1998. **© 1998 Wiley-Liss, Inc.**

Key words: HIV-1 sickle cell; RNA; DNA; anemia

*Correspondence to: Robert M. Steiner, M.D., New York Methodist Hospital/Cornell University Medical Center, 506 6th Street, New York, NY 11215-9008.

Received for publication 8 December 1997; Accepted 8 July 1998

INTRODUCTION

The rapidity of viral replication, mutation, and genetic variation are important factors in the progression of human retroviral disease concentrated in plasma, white blood cells and particularly in the lymph nodes and spleen [1–8]. The size of the viral burden can be closely correlated with clinical stage, CD4+ T-lymphocyte count, and the level of viral nucleic acids as measured by highly sensitive and reproducible assays in blood [9–16].

Whereas most individuals infected with the human immunodeficiency virus type 1 (HIV-1) progress to acquired immunodeficiency syndrome (AIDS) [4], 5% of all HIV-1-seropositive individuals fail to develop the clinical manifestations of HIV-1 infection and remain ''long-term nonprogressors'' [3,17–21]. There are several hypotheses given for this delay or absence of the clinical manifestations of HIV-1 disease. Multiple HIV-1 variants with different degrees of virulence, replication capabilities, and multiple host factors may play important roles in the ultimate outcome of the long-term nonprogressors [22–27]. One possible cause for reduced virulence may be absence of a major site for viral invasion and replication, such as the lymph nodes or the spleen [28].

Sickle cell anemia (SS) may well be the ideal model to test this hypothesis since 95% of individuals with SS have functional asplenia after infancy due to microinfarction [29]. If patients with SS and splenic atrophy are infected with HIV-1, it is possible that they may exhibit lower viral burdens than non-SS HIV-1–infected individuals with normal splenic function leading to a prolonged symptom-free period and possibly improved survival.

The possibility that SS disease has an ameliorating influence on the natural course of HIV-1 disease was first suggested by Waweru and his associates at the Kenya National Hospital [30]. They studied 198 SS children, all of whom had multiple blood transfusions during the years 1987–1988. None were HIV-1–seropositive. In a second study in 1990–1991, one of 44 (2.3%) transfused patients with SS demonstrated seroconversion to HIV-1 [31]. Transfused children from the same hospital without SS, had an HIV-1–seropositive prevalence of 7.4% in 1987–1988. Three of four transfused non-SS children were HIV-1–seropositive in 1990–1991 [30,31].

In another study of 256 SS children aged 12 years or less, 14 or 5.5% were found to be seropositive for HIV-1 compared with the general population of 20% [32]. In yet another communication, Mbewe [33] found the prevalence of HIV-1 infection in children and adults with SS disease to be significantly lower than in the general population (Zambia). More recently, Castro et al. [34] suggested that the risk of HIV-1 infection in transfused SS patients is low, based on the study of 116 SS adults. None were seropositive for HIV-1 antibodies [34]. Finally, Godeau [35] screened 283 adult SS patients for HIV-1 infection. Eight HIV-1 seropositive patients were found but none progressed to AIDS over a 9.6 year follow-up.

The effect of surgical splenectomy on the progression of HIV-1 disease has also been considered. McKernan and Hay [36] described a dramatic sustained increase of CD4 T-lymphocyte levels in their HIV-1–seropositive patients following splenectomy. The increase in the CD4+ T-lymphocyte count was profound, measuring up to seven times the presplenectomy values. In their patients, a three-year follow-up showed either minimal or no further increase in CD4+ T-lymphocytes after the initial postsplenectomy rise. Kemeny et al. and others [37,38] found that splenectomy reversed the patients' thrombocytopenia and there was no accelerated progression to AIDS. Tunkel et al. [39], in a retrospective study, reviewed the records of five HIV-1–infected patients who underwent splenectomy for HIV-1–associated thrombocytopenia and splenomegaly. Four of the five had marked sustained increases in their absolute CD4+ T-lymphocyte counts after surgery. In the same patients, CD8+ T-lymphocytes counts increased even further and the CD4/CD8 ratio was reduced.

To study a possible effect of SS on the course of HIV-1–seropositive patients, available patients with both conditions were identified. These patients underwent a series of quantitative studies to provide objective evidence of the state of their viral burden, and attempt to elucidate the role of splenic atrophy in SS patients with HIV-1 infection.

METHODS AND MATERIALS

Patient Selection

All active and available HIV-1–seropositive patients with SS followed at five national sickle cell disease centers[a] were included in this study (group A). All pertinent and available data were reviewed, including a history of blood transfusions, relevant social factors, previous CD4+ T-lymphocyte counts and percentages, the source and approximate date of infection, the course of disease, and outcome. Those patients with SS who were HIV-1–seropositive but who died or were lost to follow-up were included in the series based on a retrospective review (group B).

All peripheral venous blood specimens were obtained by phlebotomy, after informed consent, at the patients' sickle cell centers. The serologic status of each blood specimen was reconfirmed at the Molecular Retrovirology Laboratories of the Thomas Jefferson University

[a]The sickle cell centers of Thomas Jefferson University Hospital (TJUH), Howard University Hospital, San Francisco General Hospital, University of Georgia, and Boston Children's Hospital.

Hospital (TJUH) using HIV-1 enzyme-linked immunosorbant assay (ELISA) and Western Blotting tests. Quantitation of viral burden was performed utilizing plasma HIV-1 RNA quantitation by branch DNA signal amplification assay, quantitative DNA-polymerase chain reaction (PCR), HIV-1 RNA by reverse transcriptase (RT)-initiated PCR, and in situ DNA-PCR [11–14,40–47].

The medical records of a control group (group C) consisting of African-American HIV-1–seropositive patients without SS matched for age and gender were studied retrospectively for risk factors, onset of disease, time interval to development of AIDS, treatment, and outcome when known. The control patient records were derived from those of TJUH in random sequence, within comparable age and gender groups.

Quantitative Assays

HIV-1 RNA quantitation by branched DNA signal amplification assay. All plasma specimens were coded and blinded to the investigators performing the assay. The assay was performed, according to the manufacturer's instructions, using a Quantiplex HIV-1 RNA kit. The branched DNA (bDNA) assay is a sandwich nucleic acid procedure for the quantitative of HIV-1 RNA in human plasma [46,47]. To determine the copy numbers of HIV-1 RNA per ml of plasma, the virions are concentrated by centrifugation. After HIV-1 genomic RNA is liberated from virions, it is captured on a microwell surface, utilizing an oligonucleotide probe. Another set of probes (nonoverlapping to the capture probe) are hybridized to the viral RNA and the bDNA amplifiers.

Specifically, the target probes bind to the *pol* gene of the viral RNA. Multiple copies of an alkaline phosphatase-labeled probe are hybridized to the immobilized complex, to amplify the signal. Detection is achieved by incubating the complex with a chemiluminescence substrate, and measuring the light emission generated by the bound alkaline-phosphatase. Light emission is identified and measured by specially designed photosensors and the degree of light emitted is directly proportional to the amount of unspliced HIV-1 RNA present in each sample. A standard curve is defined by light emission from standards, with known concentrations of recombinant bacteriophage. Concentrations of HIV-1 RNA in unknowns are then determined from the standard curve and expressed as equivalents per milliliter (Eq/ml).

Peripheral blood mononuclear cells (PBMCs) were first isolated by density gradient centrifugation, utilizing Ficoll-Hypaque media. Centrifugation at 800xg was used to pellet 5×10^6 cells per aliquot. These cells were then cryopreserved at −70°C for further analysis. HIV-1 RNA from PBMC samples was exracted with guanidine-HCl and then quantitated using the bDNA assay [46,47]. All PBMCs were analyzed simultaneously for the determination of HIV-1 RNA copies to avoid assay-to-assay variations. Results were expressed as HIV-1 RNA Eq/10^7 PBMC.

Quantitative DNA-PCR

The quantitative DNA-PCR study was performed as described by Zhang et al. [43]. Briefly, PBMC samples (50 ml of blood) were subjected to quick lysis in 50 ml of lysis buffer (10 mM Tris HCl, 10 mM KCl, 1% Tween 20, 1% Non-Idet-40 [NP40]), and then boiled. After centrifugation, 50 μl of supernatant was added to 50 μl of PCR cocktail (5 mM $MgCl_2$, 360 μM dNTPs, 50 pmol of HIV-1 *gag*-specific sense and antisense primers [SK38/SK39], two μl of *Taq* DNA polymerase, 10 μl of *Taq* buffer). Each sample was subjected to 30 cycles of amplification (M.J. Research, Watertown, MA), consisting of two long denaturation cycles holding at 94°C for two min decreasing to 53°C over a three-min period and holding at 53°C for 30 sec, and then finally increasing over one sec to 68°C and holding there for 30 sec. Following the first two cycles, the following seven cycles were set at 94°C, 55°C, and 72°C, 10 sec each. The remaining 23 cycles consisted of 90°C, 60°C, and 72°C, 10 sec each. The amplified PCR product of primer pair SK38/SK39 was hybridized with the probe ^{32}P-labeled SK19. The hybridization was completed in an oligomer hybridization diluent (66 mM NaCl and 44 mM ethylenediaminetetraacetic acid [EDTA]) at 55°C for 15 min. The mixture was then analyzed by electrophoresis through an 8% native polyacrylamide gel and quantitated by utilizing a phosphorimager (Molecular Dynamics, Sunnyvale, CA). The primer pair/probe system PC03, PC04, and RS06 was used to detect human β-globin DNA as a positive control. The DNA standard curves utilized for quantitation were derived from lysed ACH-2 cells, which contain one integrated copy of double-stranded proviral HIV-1 DNA. The number of HIV-1 proviruses, within the ACH-2 cells, was verified by performing Southern blot hybridization with a ^{32}P-labeled nick-translated, *Fsp*I to *Nae*I, full-length HIV-1 fragment from HIV-1_{NL4-3}. The mean HIV-1 *gag* copy numbers per ACH-2 cell were also verified by serially diluting the cells in a constant number of HIV-1–negative PBMCs in duplicate and performing PCR with SK38/SK39, and then calculating the Poisson distribution that best fit the data. DNA standard curves were utilized to quantitate the number of copies of HIV-1 proviral DNA per 10^6 PBMC. The results are expressed as the number of proviral copies per million cells.

HIV-1 RNA by RT-Initiated PCR

To further measure the copy numbers of HIV-1 RNA, first virions were captured from the patients' blood plasma by utilizing HIV-1 gp120- and gp41-coated immunobeads, as described previously [43]. The viral genomic RNA was extracted from all the samples and was

TABLE I. Clinical Characteristics of HIV-1–Seropositive SS Individuals (Group A)*

Subject	Age	Sex	Risk factors	Year of Dx (years since Dx)		Last CDC classification[a]	Presentation	Treatment	Outcomes
A	35	M	Sexual, transfusion, cocaine Abuse	1989	(8)	III	Generalized lymphadenopathy	No antiviral therapy	Long-term nonprogressor
B	37	M	Sexual, transfusion	1987	(9)	II	Asymptomatic	No antiviral therapy	AIDS-related lymphoma, died in 1996
C	30	F	Transfusion	1984	(13)	II	Asymptomatic	No antiviral therapy	Long-term nonprogressor
D	37	F	Sexual, transfusion	1992	(5)	IVB	Mucocutaneous candidiasis	AZT (initiated 1995)	Leukopenia under treatment
E	29	M	Transfusion	1985	(12)	II	Asymptomatic	No antiviral therapy	Long-term nonprogressor
F	32	F	Transfusion	1985	(12)	II	Asymptomatic	No antiviral therapy	Long-term nonprogressor
G	35	F	Sexual, transfusion	1985	(12)	II	Asymptomatic	No antiviral therapy	Long-term nonprogressor
H	17	F	Transfusion	1984	(13)	II	Asymptomatic	AZT (short course)	Long-term nonprogressor
I	35	F	Transfusion	1991	(6)	II	Asymptomatic	No antiviral therapy	Long-term nonprogressor
J	25	M	Transfusion	1995	(2)	II	Asymptomatic	No antiviral therapy	Presently asymptomatic
K	36	M	Transfusion	1985	(12)	II	Asymptomatic	No antiviral therapy	Long-term nonprogressor

*HIV, human immunodeficiency virus; SS, sickle cell anemia; Dx, diagnosis; CDC, Centers for Disease Control and Prevention; AZT, azidothymidine.
[a]The CDC HIV clinical classification system of 1987, rather than 1993, was utilized for stratification, as the total CD4+ lymphocyte count is a critical part of the 1993 system and may not be as useful in sickle cell disease.

then resuspended in DEPC-treated, doubly-deionized distilled water (ddH_2O). Twelve microliters of each sample were reverse transcribed by Moloney murine leukemia virus reverse transcriptase (MMLVRT) (GIBCO BRL, Grand Island, NY), using the HIV-1 *gag* antisense primer SK39. For quantitation, RNA standard copy templates were reverse transcribed at the same time. The in vitro RNA standards were prepared as described previously [43]. The cDNAs from all the samples and the standards were amplified simultaneously by PCR, and the products were analyzed by Southern blotting, utilizing the P^{32}-labeled HIV-1 *gag* probe SK19. Quantitation was performed using a phosphorimager (Molecular Dynamics, Sunnyvale, CA). All quantitation analyses were repeated at least three times.

In Situ PCR

The freshly isolated PBMCs (1×10^6 cells) were transferred to specially-designed, salinated slides, containing a 20 mm single well. The cells were air-dried overnight, heat-fixed at 105°C for 10 sec and then incubated in 4% paraformaldehyde for two hr. Paraformaldehyde was inactivated by incubating slides in 3 × phosphate-buffered saline (PBS) for 10 min, and then the slides were washed twice in 1 × PBS. The endogeous peroxidase was quenched by incubating slides in 0.3% hydrogen peroxide in PBS overnight. These slides were treated with proteinase K (six μg/ml) at room temperature for 12 min. Proteinase K was inactivated by incubating slides on a heat block at 95°C for five min. The in situ amplification of HIV-1 *gag* DNA was performed as described previously [9,42]. Subsequently, amplified DNA signals were detected by the biotinylated probe-SK19. The percentage of HIV-1–positive cells were enumerated by counting at least 10,000 cells/slide, by two independent observers. All the microscopic analyses were performed on coded slides [14].

Statistical Analysis

Analysis of variance was performed as previously described by Todd et al. [45]. Additional analyses for statistical significance were carried out utilizing the unpaired Student's t-test. The control group and the study groups were compared for statistical significance, for the rate of long-term nonprogression, using the ''binomial approximation to the normal distribution'' method.

RESULTS

Clinical Characteristics of ''Active'' SS-Positive, HIV-1 Seropositive Patients (Group A)

As demonstrated in Table I, ten of the 11 SS-positive, HIV-1–infected patients in group A were potentially infected with HIV-1 by blood transfusions before 1985.

One patient (J) with a history of blood transfusions had documented HIV-1 seroconversion in 1995 and denied other risk factors. This patient had repeatedly tested negative for HIV-1 in previous evaluations since 1985, including a study as recently as March 1994. It would be highly unusual for the HIV-1 infection to have arisen from blood transfusions received after 1985. If he was infected through blood transfusions after the implementation of serological testing of donors' blood for HIV-1, then he may be one of those extremely rare and unfortunate cases [48].

Four individuals (A, B, D, and G) had other confounding risk factors, including a sexual route of transmission. One of 11 patients had a history of cocaine abuse (patient A). Patient A also had generalized lymphadenopathy, but did not receive antiviral therapy. Another patient (D) exhibited evidence of AIDS—mucocutaneous candidiasis and cachexia—and recently was begun on antiviral therapy [Azidothymidine (AZT)]. A third patient (H) received a short course of AZT. One subject (patient B) underwent HIV-1 seroconversion in 1987 and at the time of the initial quantitative viral studies was considered to be a long-term nonprogressor. However, in 1996 he developed AIDS-related lymphoma and died in late 1996. All other patients continue to be long-term nonprogressors and have not been treated with antiviral therapy.

If one does not include patient J in the patient tabulation, since the tentative date of infection remains in question, the average interval since identification of HIV-1—seropositive in ten patients is 10.25 years. If one excludes two of the 10 individuals who developed AIDS-related mucocutaneous candidiasis (D), and AIDS-related lymphoma (B) and the one patient who has exhibited generalized lymphadenopathy (A), then the remaining seven patients have remained free of disease for over 11.4 years. Seven SS HIV-1–seropositive patients from two of the five SS centers died prior to the beginning of this study. Four died of AIDS and three of the complications of SS. Importantly, no other SS HIV-1–seropositive patients were thought to have been followed at these sickle cell centers.

Among the 18 SS HIV-1—seropositive patients (groups A and B), there were eight (44%) long-term nonprogressors, all from group A (those patients who underwent quantitative studies for viral burden). Thus, of the 11 patients in group A, eight of 73% remained long-term nonprogressors. Five patients or 28% of the total of 18 patients succumbed to AIDS. Of these, one was group A. In addition, three patients, or 17% of the total patient cohort, all from group B, succumbed to the complications of SS.

The control group consisted of 36 patients who were age- and gender-matched non-SS African-American HIV-1–seropositive patients (group C). Of these patients, 13 were intravenous drug abusers, six had heterosexual exposure, six were homosexual, three had multiple risk factors, two were hemophiliacs, and one other had transfusion-related HIV-1 seroconversion. Five had unknown risk factors. Twenty-two progressed from HIV-1 infection to AIDS in an average of 1.9 years. In eight patients, there was documented HIV-1 seroconversion but not documented progression to AIDS. In one patient, conversion to AIDS was documented but the date of initial HIV-1 seroconversion was not identified. The remaining five patients were long-term nonprogressors for an average of 10.2 years (13.9%). Of the long-term nonprogressor patients in group C, two had a heterosexual route of transmission, two were hemophiliacs, and one patient had a history of drug abuse.

The rate of long-term nonprogression among the SS HIV-1–seropositive groups (A and B) and the control group (C) were compared for statistical significance using the binomial approximation to the normal distribution method. The Z-statistic was 2.343. The corresponding P value was $P = 0.0193$. Therefore, there was a highly significant difference in the percentage of long-term, nonprogressors between these groups.

Levels of HIV-1 Virions in Plasma

The plasma levels of HIV-1 virus were determined by particle-associated HIV-1 RNA bDNA-signal amplification assays and by the quantitative reverse transcriptase-initiated PCR (Q-RT-PCR) method (Table II). By using bDNA-signal amplification assays, HIV-1 RNA in the plasma was detectable in only two of 11 SS, HIV-1–infected individuals (18%) (patients A and D). In recent studies, bDNA analyses detected HIV-1–specific RNA in the plasma of 38% to 71% of HIV-1–infected individuals with CD4+ T-lymphocyte counts greater than 500/mm^3 [46,47].

Relatively high CD4+ T-lymphocyte counts were demonstrated in the SS-positive patients (Table I). However, it is not unusual for splenectomized (in this case auto-splenectomized) individuals to exhibit relatively high levels of CD4+ T-lymphocytes, as compared with progressors with intact spleens [47]. The CD4 percentage, another immune-function marker measured as part of the immune profile of HIV-1–infected patients, may be a better indicator than the total CD+ T-lymphocyte count for predicting the development of AIDS in splenectomized individuals, although this is controversial [39]. As shown in Table I, the CD4 percentages varied extensively. In the SS-positive HIV-1–positive group, CD4 percentages ranged from 1% to 30%.

Levels of HIV-1 Proviruses in PBMC

The number of HIV-1 proviral DNA copies were also measured in the PBMCs of SS-positive HIV-1–infected individuals. The HIV-1 DNA was quantitated by solu-

TABLE II. Quantitative Virological and Serological Studies of Eleven HIV-1–Seropositive SS Individuals (Group A)*

Subject	Most recent CD4 (%)	Most recent CD4 count per mm^3	bDNA analysis of HIV-1 RNA copies per ml in plasma or in (10^7 PBMCs) × 10^{3a}	Q-RT-PCR: copies of HIV-1 RNA in plasma per ml × 10^3	Q-DNA-PCR: copies of HIV-1 DNA per 10^6 PBMCs	In situ DNA-PCR: % HIV-1 DNA-positive PBMCs
A	7	1,107	70 (20)	420	200	4.1%
B	6	933	<10 (<10)	ND	ND	< 0.1%
C	4	1,062	<10 (<10)	ND	ND	< 0.1%
D	1	100	70 (73)	800	150	8.0%
E	15	1,566	<10 (<10)	ND	ND	< 0.1%
F	20	1,185	<10 (<10)	12	ND	< 0.1%
G	15	960	<10 (<10)	8	ND	< 0.1%
H	30	1,025	<10 (<10)	10	50	< 0.1%
I	23	915	<10 (<10)	3	10	< 0.1%
J	11	300	<10 (<10)	15	70	1.0%
K	12	1,157	<10 (<10)	20	80	0.9%

*All patients were HIV-1 ELISA and HIV-1 Western blot positive, with p24, gp 41 and gp 120/160 bands present on all patients' Western blots. HIV, human immunodeficiency virus; SS, sickle cell anemia; bDNA, branched DNA; PBMCs, peripheral blood mononuclear cells; Q-RT-PCR, quantitative reverse transcriptase-initiated polymerase chain reaction.
[a]RNA copies in PBMCs are noted in parentheses.

TABLE III. All Additional SS patients With HIV-1 Infection From Five University Sickle Cell Disease Centers (Group B)*

Subject	Age	Gender	Risk factors	Year of Dx	Years since Dx	Latest CD4 count	Clinical presentation	Treatment	Outcome
AA	45	F	IVDA Transfusion	1986	6	NA	Asymptomatic	NA	Lost to follow-up 1988–1992. Died of complications of SS in 1992
BB	37	M	Transfusion	1986	8	1883	Asymptomatic	No antiviral Rx	Died of complications of SS in 1994
CC	38	M	Transfusion	1985	9	NA	Disseminated varicella	NA	Died of AIDS in 1991
DD	35	F	Transfusion, sex partner	1989	5	310	Lymphaden., CMV, C diff., pneumonia	Acyclovir	Died of AIDS in 1994
EE	39	M	Homosexual, transfusion	1984	8	1870	Lymphaden., pneumonia	No antiviral Rx	Died of complications of SS in 1992
FF	32	M	Homosexual, transfusion	1985	4	430	C diff., pneumonia, encephalitis	AZT, pentamidine	Died of AIDS in 1989
GG	33	M	Transfusion	1986	6	20	Fever, lymphaden	AZT, pentamidine	Died of AIDS in 1992

*SS, sickle cell anemia; HIV, human immunodeficiency virus; Dx, diagnosis; IVDA, intravenous drug abuse, NA, not applicable; CMV, cytomegalovirus; Lymphaden., lymphadenopathy; C. diff., clostridium difficile-induced diarrhea.

tion-based quantitative DNA-PCR, as well as by in situ PCR methods.

As shown in Table II, the number of copies of HIV-1 proviral DNA in the PBMCs of the asymptomatic SS-positive individuals, by quantitative DNA-PCR were 52.5 ± 31 copies per 10^6 cells. Thus, levels of HIV-1 DNA were generally low, less than 100 copies per million PBMCs by the quantitative DNA-PCR technique. Only two individuals (subjects A and D), both of whom were symptomatic, exhibited the presence of >100 copies per 10^6 PBMCs (200 and 150 per 10^6 PBMCs, respectively). These two subjects also exhibited very high copy numbers of HIV-1 RNA in the plasma, as measured by the bDNA signal amplification assay and by quantitative HIV-1 RT-PCR methods. These two SS-positive, HIV-1–positive subjects also had the highest percentages of HIV-1–infected PBMCs, as measured by the in situ PCR method [8,30], 4.1% and 8.0%, respectively.

Analysis of HIV-1 provirus in the PBMCs isolated from these patients, and measured by the highly sensitive in situ PCR method, were also performed. Of importance, utilizing large numbers of historical controls (n = 98) from our laboratories [11,16], the levels of proviral DNA by in situ PCR were quite low in nine of 11 of the SS-positive patients, and the entire SS-positive cohort had significantly lower proviral loads as compared with

this non-SS, HIV-1–seropositive cohort ($P < 0.04$). Therefore, it appeared that in SS-positive HIV-1–infected patients, HIV-1 expression, as well as HIV-1 proviral load, was significantly lower than in several large cohorts of non-SS, HIV-1–seropositive individuals.

Serological Studies

Each patient's serum was evaluated independently for the presence of anti–HIV-1 antibodies by standard ELISA, as well as by Western blotting. Since splenectomy is known to alter the normal antibody response to certain antigens, especially T-lymphocyte–independent antigens [38], we performed Western blot analyses on all the sera to determine if slow progression in these long-term survivors may be related to the absence of certain anti–HIV-1 antibodies. However, the Western blot analysis revealed no unusual anti–HIV-1 antibody patterns (data not illustrated).

DISCUSSION

Delayed development or even the absence of manifestations of AIDS in HIV-1–infected patients has been studied extensively to characterize both the natural history of disease and in the quest for clues to ways of retarding or even preventing disease progression [17–28,49–55]. Among the factors that appear to temporarily alter disease progression is splenectomy, performed in many patients with AIDS who develop hypersplenism. Reports based on a number of groups of HIV-1–infected individuals who have undergone splenectomy suggest a protracted course of disease with at least a transient increase in CD4+ and CD8+ T-lymphocyte counts and percentages [36–39,56–58]. Oksenhendler et al. [57] studied the course of 68 HIV-1–infected patients prospectively with severe immune thrombocytopenia (ITP) who underwent splenectomy 2–41 months (mean of 10 months) after ITP was diagnosed. In this cohort, the platelet count increased from as low as 18×10^9/l to as high as 223×10^9/l, a percentage increase of 82% in 56 patients. The mean CD4+ T-lymphocyte count increased from 47×10^6/l to 725×10^6/l in the same group. However, the study also showed there was no statistically significant long-term improvement as a result of splenectomy when compared with nonsplenectomized patients. Leissinger et al. [59] also studied viral burden in four HIV-1–infected patients with ITP finding no consistent changes in HIV-1 viral burden, suggesting that splenectomy after HIV-1 infection does not influence the rate of progression of HIV-1 disease.

Joag et al. [60] studied the effect of splenectomy in a simian immune deficiency virus (SIV) model. In this study, unsplenectomized and splenectomized Indian and Chinese rhesus macaques were infected with SIV_{239}. Splenectomized macaques had lower viral burden, longer survival time, and more effective viral control. In this study, one of seven splenectomized animals died of sepsis—a common sequelae of splenectomy in SS-positive patients. One of two deceased nonsplenectomized macaques died of SIV infection and immune suppression. Survival in the splenectomized animals was significantly longer than in those with intact spleens [60]. It is interesting that none of the reports in humans has addressed the issue of splenectomy before HIV-1 infection and its possible influence on survival.

Several reasons for the low viral burden in our patients in addition to autosplenectomy prior to HIV-1 seroconversion may be considered. Pandit [27] suggested that the β-globins in the red blood cells of SS patients may be the source of HIV-1 antibodies. These same antibodies which may yield selective resistance to malaria infection might also serve the same role in other infections, such as HIV-1, or in malignancies [27].

The possibility that free hemoglobin in the SS individual's blood might alter measurement of HIV-1 virions was also considered. Todd et al. [45] tested the potential interference of hemoglobin levels in the quantitation of HIV-1 RNA using the bDNA assay and found that hemoglobin up to one mg/ml did not interfere with HIV-1 RNA quantitation by bDNA. Thus, extracellular hemoglobin is unlikely to contribute to the bDNA data presented in the present study, as the plasma hemoglobin levels are significantly less than one mg/ml in SS-positive individuals. In addition, virions were captured by immunobeads prior to RT-PCR and, thus, hemoglobin was washed away prior to reverse transcription and cDNA amplification.

Although an explanation for the longer survival of SS-positive, HIV-1–infected individuals may be the functional absence of the spleen as manifested by low viral burden in the plasma, as well as in the PBMCs, another mechanism(s) may also be active. For example: 1. the longer survival may be the result of higher proportions of CD 4+ T-lymphocytes in PBMCs, which has been considered a marker for staging HIV-1 infection [16–18]; and 2. Changes in the trafficking of lymphocytes resulting in more efficient elimination of viral particles or lower production of HIV-1.

Our control group shows striking differences in the prevalence of AIDS compared with our study group. However, there are built-in confounding factors making a direct comparison between the two groups difficult. The patient cohort was carefully monitored annually for HIV-1 seroconversion, which was not performed in the control group. The study group had, for the most part, a single risk factor—contaminated blood—whereas the risk factors in the control group were varied.

Our observations in HIV-1–infected SS patients suggest that the natural history of these patients may result in long-term nonprogression related to autosplenectomy.

This possibility deserves further investigation. Since the numbers of such patients are relatively small in the United States, studies of a larger population of SS-positive HIV-1–infected individuals in Sub-Sahara Africa might be instructive. These studies may lead to a better understanding of in vivo HIV-1 pathogenesis and allow the development of novel therapeutic approaches to combat this human retroviral disease.

ACKNOWLEDGMENTS

The authors thank Kelley Burke, Betsy Clair, Dr. Jeffrey Mondschein, and Dr. Chen Huang for their assistance; Dr. Peter Dailey for advice, critical discussions, and for providing the Quantiplex HIV-1 RNA kits; Dr. Karen Gilbert for statistical support; and Ms. Rita M. Victor, Mr. Todd Conway, and Ms. Brenda O. Gordon for their excellent secretarial assistance.

REFERENCES

1. Wei X, Ghosh SK, Taylor ME: Viral dynamics in human immunodeficiency virus type 1 infection. Nature 373:117, 1995.
2. Ho DD, Neumann AU, Perelson AS: Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 373:123, 1995.
3. Coffin JM: HIV population dynamics in vivo: Implications for genetic variation, pathogenesis and therapy. Science 267:483, 1995.
4. Levy JA: Pathogenesis of human immunodeficiency virus infection. Microbiol Rev 57:183, 1993.
5. Dianzani F, Antonelli G, Riva E, Uccini S, Visco G: Plasma HIV viremia and viral load in lymph nodes. Nature Med 2:832, 1996.
6. Piatak M, Jr, Saag MS, Yang LC: High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science 259:1749, 1993.
7. Graziosi C, Pantaleo G, Burtini L: Kinetics of human immunodeficiency virus type 1 (HIV-1) DNA and RNA synthesis during primary HIV-1 infection. Proc Natl Acad Sci USA 90:6405, 1993.
8. Pantaleo G, Graziosi C, Demarest JF: HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature 362:355, 1993.
9. Ho DD, Moudgil T, Alam M: Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N Engl J Med 321:1621, 1989.
10. Connor RI, Mohri H, Cao Y, Ho DD: Increased viral burden and cytopathology correlate temporally with CD4+ T-lymphocyte decline and clinical progress in human immunodeficiency virus type 1-infected individuals. J Viral 67:1772, 1993.
11. Bagasra O, Hauptman SP, Lischner HW, Sachs M, Pomerantz RJ: Detection of human immunodeficiency virus type 1 provirus in mononuclear cells by in situ polymerase chain reactions. N Engl J Med 326:1385, 1992.
12. Bagasra O, Pomerantz RJ: In situ polymerase chain reaction and HIV-1. Clin Lab Med 14:351, 1994.
13. Ma TS: Applications and limitations of polymerase chain reaction amplification. Chest 108:1393, 1995.
14. Patterson BK, Till M, Otto P: Detection of HIV-1 DNA and messenger RNA in individual cells by PCR-driven in situ hybridization and flow cytometry. Science 260:976, 1993.
15. Embretson J, Zupancic M, Ribas JL: Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature 362:359, 1993.
16. Bagasra O, Seshamma T, Oakes JW, Pomerantz RJ: High percentages of CD4 positive lymphocytes harbor the HIV-1 provirus in the blood of certain infected individuals. AIDS 7:1419, 1993.
17. Hu DJ, Dondero TJ, Rayfield MA: The emerging genetic diversity of HIV. J Am Med Assoc 275:210, 1996.
18. Sheppard HW, Lang W, Ascher MS, Vittinghoff E, Winkelstein W: The characterization of non-progressors: Long-term HIV-1 infection with stable CD4+ T-cell levels. AIDS 7:1159, 1993.
19. Buchbinder SP, Katz MH, Hessol NA, O'Malley PM, Holmberg SD: Long term HIV-1 infection without immunologic progression. AIDS 8:1123, 1994.
20. Lifson AR, Buchbinder SP, Sheppard H: Long term human immunodeficiency virus infection in asymptomatic homosexuals and virological characteristics. J Infect Dis 163:959, 1991.
21. Deacon NJ, Tsykin A, Solomon A: Genomic structure of an attenuated quasispecies of HIV-1 from a blood transfusion donor and recipients. Science 270:988, 1995.
22. Kestler HW, Ringler DJ, Mori K: Importance of the nef gene for maintenance of high virus loads and/or development of AIDS. Cell 65:651, 1991.
23. Huang Y, Zhang L, Ho DD: Characterization of nef sequences in long term survivors of human immunodeficiency virus type 1 infection. J Virol 69:93, 1995.
24. Kirchhoff F, Grenough TC, Brettler DB, Sullivan JL, Desrosiers RC: Brief report: Absence of intact nef sequences in a long term survivor with nonprogressive HIV-1 infection. N Engl J Med 332:228, 1995.
25. Huang Y, Paxton WA, Wolinsky SM: The role of the mutant CCR5 allele in HIV-1 transmission and disease progression. Nature Med 2:1240, 1996.
26. D'Souza MP, Harden VA: Chemokines and HIV-1 second receptors. Nature Med 2:1299, 1996.
27. Pandit HM: Potential use of globins and their derivatives of abnormal blood cells in the treatment of cancer and related immune disorders. Med Hypotheses 40:332, 1993.
28. Steiner RM, Ballas S, Castro O: The role of sickle cell hemoglobinopathy in AIDS. 14th National Sickle Cell Conference, Mobile, Alabama, 1996.
29. Pearson HA, Gallagher D, Chilcote R: Developmental pattern of splenic dysfunction in sickle cell disorders. Pediatrics 76:392, 1985.
30. Waweru SEN, Meme JS, Kinuthia DM, Kitoyi GW: Absence of HIV seropositivity in children with sickle cell anemia at Kenyatta National Hospital, Nairobi, Kenya (abstract MBP160). Int Conf AIDS 5:248, 1989.
31. Waweru SEN, Oogo S, Muniu E, Libondo J: Persistent low HIV infection in transfused sicklers as compared to high HIV infection in children presenting with other types of anaemia requiring blood transfusion. Nairobi J Med 8:26, 1992.
32. Lukin ML, Binda P, Mbensa I: Seroprevalence of HIV infected children with sickle cell anemia (abstract 0441). Int Conf AIDS 10:253, 1994.
33. Mbewe AL: HIV-1 in patients with sickle cell anemia in Zambia (abstract PC 0350). Int Conf AIDS 10(2):247, 1994.
34. Castro O, Saxinger C, Barnes S: Prevalence of antibodies to human immunodeficiency virus and to human T cell leukemia virus type 1 in transfused sickle cell disease patients. J Infect Dis 162:743, 1990.
35. Godeau B: Severe pneumococcal sepsis and meningitis in human immunodeficiency virus. Infected adults with sickle cell anemia. Clin Infect Dis 15:327, 1992.
36. McKernan, Hay CR: Early rapid decline in CD4 count released by splenectomy in HIV infection. Haemophilia 1:67, 1995.
37. Kemeny MM, Cooke V, Melester TS, Halperin IC, Burchell AR, Yee JP, Mills CB: Splenectomy in patients with AIDS and AIDS-related complex. AIDS 7:1063, 1993.
38. Zurlo JJ, Wood L, Gaglione MM, Polis MA: Effect of splenectomy on T lymphocyte subsets in patients infected with the human immunodeficiency virus. Clin Infect Dis 20:768, 1995.

39. Tunkel AR, Kelsall B, Rein MF, Innes DJ, Saulsbury FT, Vollmer K, Wispelung B: Case report: Increase in CD4 lymphocyte counts after splenectomy in HIV-infected patients. Am J Med Sci 306:105, 1993.
40. Todd J, Pachl C, White R: Performance characteristics for the quantitation of plasma HIV-1 RNA using branched DNA signal amplification technology. J Acquir Immune Defic Syndr Hum Retrovirol 10:S35, 1995.
41. Pachl C, Todd JA, Kern DG: Rapid and precise quantitation of HIV-1 RNA in plasma using a branched DNA signal amplification assay. J Acquir Immune Defic Syndr Hum Retrovirol 8:446, 1995.
42. Dailey PJ, Wilber JC, Collins MC: Correlation of HIV RNA viral load in PBMCs and plasma with clinical status using branched DNA (bDNA) signal amplification (abstract). Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando, FL, 1994.
43. Zhang H, Bagasra O, Niikura M, Poiesz BJ, Pomerantz RJ: Intravirion reverse transcripts in the peripheral blood plasma of human immunodeficiency virus type 1-infected individuals. J Virol 68:7591, 1994.
44. Bagasra O, Seshamma T, Hansen J, Pomerantz RJ: In situ polymerase chain reaction and hybridization to detect low abundance nucleic acid targets. In Ausubel FM, ed. Current Protocols in Molecular Biology, New York: John Wiley & Sons, 1995, Sec 14.8.1.
45. Todd J, Yeghiazarian T, Hoo B: Quantitation of human immunodeficiency virus plasma RNA by branched DNA and reverse transcription coupled polymerase chain reactions assay methods: A critical evaluation of accuracy and reproducibility. Serodiagn Immunother Infect Dis 6:1, 1994.
46. Cao Y, Ho DD, Todd J: Clinical evaluation of branched DNA signal amplification for quantifying HIV type 1 in human plasma. AIDS Res Hum Retroviruses 11:353, 1995.
47. Dewar RL, Highbarger HC, Sarmiento MD: Application of branched DNA signal amplification to monitor human immunodeficiency virus type 1 burden in human plasma. J Infect Dis 170:1172, 1994.
48. Lackritz EM, Satten GA, Aberle-Grasse J: Estimated risk of transmission of the human immunodeficiency virus by screened blood in the United States. N Engl J Med 333:172, 1995.
49. Ferbas J, Kaplan AH, Hausner MA, et al.: Virus burden in long-term survivors of human immunodeficiency virus (HIV) infection is a determinant of anti-HIV CD8+ lymphocytic activity. J Infect Dis 172:329, 1995.
50. Cocchi F, DeVico AL, Garzino-Demo A: Identification of RANTES, MIP-1α and MIP-1β as the major HIV suppressive factors produced by CD8+ T cells. Science 270:1811, 1995.
51. Bagnarelli P, Menzo S, Valenza A: Molecular profile of human immunodeficiency virus type 1 infection in symptomless patients and in patients with AIDS. J Virol 66:7328, 1992.
52. Cocchi F, DeVieo AL, Garzino-Demo A, Cara A, Gallo RC, Lusso P: The V3 domain of the HIV-1 gp120 envelope glycoprotein is critical for chemokine mediated blockade of infection. Nature Med 2:1244, 1996.
53. Michael NL, Chang G, D'Arcy LA: Defective accessory genes in a human immunodeficiency virus type 1-infected long-term survivors lacking recoverable virus. J Virol 69:4228, 1995.
54. Clerici M, Stocks NI, Zajac RA: Detection of three distinct patterns of T-helper cell dysfunction in asymptomatic human immunodeficiency virus-seropositive patients. J Clin Invest 84:1892, 1989.
55. Jurriaans S, Gemen B, Weverling GJ: The natural history of HIV-1 infection: Are virus load and virus phenotype independent determinants of clinical course? Virology 204:223, 1994.
56. Henry K, Erice A, Dailey P, Posalak Z, Melroe H: Acute effect of splenectomy on the levels of HIV RNA in plasma (abstract) 3rd Conference on Retroviruses and Opportunistic Infections, Washington, DC, #245, January 28–February 1, 1996.
57. Oksenhendler E, Bierling P, Chevret S: Splenectomy is safe and effective in human immunodeficiency virus-related immune thrombocytopenia. Blood 82(1):29, 1993.
58. Gobarre J, Azar N, Othman T: Long-term effects of splenectomy for immune thrombopenic purpera related to human immunodeficiency virus: A retrospective study from two groups with and without splenectomy. Presse Med 20(44):2239, 1991.
59. Leissinger C, Haislip A, Tenenbaum S, Sander D, Garry R: Changes in HIV-1 proviral DNA levels and lymphocyte population in HIV infected patients after splenectomy. Blood 88(10):931A, 1995.
60. Joag SV, Stephens EB, Adams RJ, Foresman L, Narayan O: Pathogenesis of SIV_{mac} infection in Chinese and Indian rhesus macaques: Effects of splenectomy on virus burden. Virology 200:436, 1994.