

# Immunologic and Virologic Analyses of an Acutely HIV Type 1-Infected Patient with Extremely Rapid Disease Progression

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## ABSTRACT

The immunologic and virologic factors that impact on the rate of disease progression after acute infection with human immunodeficiency virus (HIV) type 1 are poorly understood. A patient with an extraordinarily rapid disease course leading to AIDS-associated death within 6 months of infection was studied intensively for the presence of anti-HIV immune reactivities as well as changes in the genetic and biologic properties of virus isolates. Although altered humoral responses were evident, the most distinctive immunologic feature was a nearly complete absence of detectable HIV-specific CTL responses. In addition to a rapid decline in CD3<sup>+</sup>CD4<sup>+</sup> cells, elevated percentages of CD8<sup>+</sup>CD45RA<sup>+</sup> and CD8<sup>+</sup>CD57<sup>+</sup> cells and diminished CD8<sup>+</sup>CD45R0<sup>+</sup> and CD8<sup>+</sup>CD28<sup>+</sup> cells were evident. Primary viral isolates recovered throughout the course of infection exhibited limited sequence diversity. Cloned viral envelopes were found to have unusually broad patterns of coreceptor usage for cell–cell fusion, although infectivity studies yielded no evidence of infection via these alternative receptors. The infectivity studies demonstrated that these isolates and their envelopes maintained an R5 phenotype throughout the course of disease. The absence of demonstrable anti-HIV CTL reactivities, coupled with a protracted course of seroconversion, highlights the importance of robust HIV-specific immune responses in the control of disease progression.

## INTRODUCTION

**I**NFECTION WITH human immunodeficiency virus type 1 (HIV-1) leads to a highly variable rate of disease progression (i.e., progressive loss of CD4<sup>+</sup> T cells), typically ranging from less than 3 years to well over 15 years.<sup>1–4</sup> In the United States, before the introduction of highly active antiretroviral therapy (HAART), the median interval between infection and the development of acquired immunodeficiency syndrome (AIDS) defining illnesses was estimated to be 10–11 years.<sup>2,4,5</sup> In other parts of the world this interval may be shorter and confounded by disparate levels of clinical care and prophylaxis against common opportunistic infections. Among female sex workers

in Kenya, one study estimated median time from seroconversion to an AIDS-defining illness was 3.4 years.<sup>6</sup>

Although the characteristics of individuals with typical disease progression and the so-called long-term nonprogressors are reasonably well defined,<sup>7–15</sup> the virologic and immunologic characteristics of patients with rapid HIV disease progression are poorly understood. The primary immune response to HIV infection in individuals with typical or nonprogressing HIV disease is not well characterized, nor is its relationship to disease progression known. One possibility to better define the relationship between the virologic and immunologic events occurring during primary infection and disease pathogenesis is through the study of rapid progressors. Rapid progressors, de-

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fined as those patients developing clinical AIDS in less than 5 years of infection,<sup>3,6,16</sup> tend to exhibit a highly biased T cell repertoire at some point during early infection<sup>7,18</sup> and an incomplete resolution of their initial peak viremia resulting in a high viral "set point."<sup>19-23</sup> In addition, their viral quasiespecies exhibit limited genetic diversity.

In this report, we describe an individual with an extremely rapid course of HIV disease involving AIDS-defining illness and subsequent death within 6 months of infection. HIV-1 primary isolates from this patient maintained an R5 non-syncytium-inducing (NSI) phenotype throughout the course of infection. Comprehensive virologic and immunologic analyses suggest that a functional immune impairment, rather than a cytopathic viral phenotype, was responsible for this greatly accelerated disease course.

## MATERIALS AND METHODS

### *Sample collection and confirmation of acute HIV-1 infection*

After obtaining informed consent, sequential samples of whole blood were collected by venipuncture. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll sedimentation and cryopreserved in liquid nitrogen. Serology for HIV-1 was assessed by conventional immunoassay (Cambridge Biotech, Rockville, MD) and Western blot (Cambridge Biotech). HIV-1 p24 positivity was determined by an antigen capture assay with a neutralization confirmation step (DuPont de Nemours, Boston, MA).

### *Quantitation of HIV-1 plasma RNA*

Plasma was prepared from acid-citrate-dextrose (ACD) anticoagulated whole blood within 2 hr of sample collection and stored at  $-70^{\circ}\text{C}$  until analyzed. HIV-1 RNA copies were determined by the Roche Amplicor HIV-1 Monitor assay (Roche Molecular Systems, Branchburg, NJ).

### *Genotypic and phenotypic characterization of HIV-1*

Low-passage primary isolates were prepared from cocultures of patient SC50 PBMCs (days 57, 64, and 71) with activated normal donor PBMCs, and from heparinized plasma (days 90 and 106) from patient SC50. Virus isolates were titrated on activated normal PBMCs and tested for syncytium-inducing (SI) phenotype with MT-2 cells as described.<sup>24</sup> Total DNA was isolated from PBMC cocultures with day 57 PBMCs and from normal PBMCs infected with plasma-derived virus isolates recovered from the day 90 and day 106 samples. Full-length proviral envelope DNAs were amplified by nested polymerase chain reaction (PCR) using the 5'-E0 primer (5'-TAG AGC CCT AGC ATC CAG GAA ATC AGC CTA-3') and 3'-Nef 9023 primer (5'-CAT TGG TCT TAA AGG TAC CTG AGG T-3') for the first round of amplification, and the 5'-E00 primer (5'-AGA AAG AGC AGA AGA CAG TGG CAA TGA-3') and the 3'-MG20 primer (5'-TGC TGT ATT GCT ACT TGT GAG-3') for the second round, each at 0.1  $\mu\text{M}$  final concentration. PCR was initiated with a hot start, and the first round consisted of 35 cycles performed as follows: denaturation for 1 min at  $94^{\circ}\text{C}$ ,

annealing at  $57^{\circ}\text{C}$  for 1 min, and extension for 5 min at  $70^{\circ}\text{C}$ . These cycles were followed by a final 10-min extension at  $70^{\circ}\text{C}$ . One microliters of the first-round reaction was amplified for either 25 cycles (for the QH1116-derived *env*) or 35 cycles (for the QH1193-derived *env*) in the second-round reaction, employing conditions identical to those described above. Amplified envelope DNAs were gel purified and recovered with a QIAEX II gel extraction kit (Qiagen, Valencia, CA) and T/A cloned into either the pCR3.1 expression vector (Invitrogen, San Diego, CA) (for all QH1116-derived *env*) or the pTarget expression vector (Promega, Madison, WI) (for all QH1193-derived *env*) and sequenced in both directions on an ABI377 automated sequencer using dye-terminator chemistries (PE Biosystems, Foster City, CA).

### *Cell-cell fusion and luciferase reporter virus assays*

The fusion and reporter virus infectivity assays were performed essentially as described in detail elsewhere.<sup>25,26</sup> Briefly, for cell-cell fusion assays, effector quail QT6 cells were infected with recombinant vaccinia virus expressing T7 polymerase (vTF1.1) and transfected with envelope plasmids driven by the T7 promoter. These cells were mixed the following day with quail QT6 cells transiently expressing CD4 and various HIV-1 coreceptors (under the control of the cytomegalovirus [CMV] promoter) and the luciferase gene under the control of the T7 promoter. Fusion was quantified 7 hr after cell mixing by measurement of luciferase activity in cell lysates. For virus infection assays, envelope and NL-luc-E<sup>-</sup>R<sup>-</sup> constructs were transfected into 293T cells. Pseudotyped virions were harvested from the medium 2 days post-transfection and used to infect feline CCC or human U87 transiently expressing CD4 and various coreceptors. Infection by reporter viruses was quantified by measurement of luciferase in cell lysates 2 days postinfection.

### *Measurement of HIV-1-neutralizing antibodies*

Neutralizing antibodies were assessed by testing serial serum dilutions for their ability to inhibit 90% of input virus infectivity as described.<sup>27</sup>

### *Measurement of antibody-dependent cell-mediated cytotoxicity*

HIV ENV-specific serum antibody-dependent cell-mediated cytotoxicity (ADCC) reactivities were measured in a standard assay using normal donor effectors, dilutions of patient serum, and gp120-coated CEM.NKR target cells.<sup>28</sup> A percent specific lysis value greater than 10% above the background was considered positive.

### *Assessment of HIV-specific cytotoxic T lymphocyte activity*

Cryopreserved PBMC samples were carefully thawed and stimulated *in vitro* with OKT3 (50 ng/ml; Ortho Biotech, Raritan, NJ) and recombinant interleukin 2 (rIL-2, 100 U/ml; Hoffman-LaRoche, Nutley, NJ) in the presence of irradiated (5000 rads) allogeneic feeder cells. Cultures were maintained for 15-27 days before assessment of HIV-specific cytotoxic T lymphocyte (CTL) activity in a standard chromium release assay as described elsewhere.<sup>29</sup> An autologous B lymphocyte cell line

(BLCL) was used as target cells. The parental vaccinia virus vector and recombinants expressing HIV proteins were provided by J. Tartaglia (Vac, Env, Pol, and Nef; Virogenetics, Troy, NY) and D. Kuritzkes (Gag; University of Colorado Health Sciences Center, Denver, CO). Results were expressed as the percentage chromium release for the HIV target minus that observed for the control vaccinia vector.

#### Analysis of lymphocyte subsets

Quantitative and qualitative analysis of peripheral blood lymphocyte subsets was performed by flow cytometry as described elsewhere.<sup>30</sup> Samples were analyzed with an EPICS XL flow cytometer (Coulter, Hialeah, FL). Total white blood cell count (WBC) and percent lymphocytes were used to calculate the absolute number of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes per microliter of blood.

#### Immunogenetic profile

HLA class I and II (A, B, C, DR, and DQ) typing was performed by routine PCR-SSOP (sequence-specific oligonucleotide probe)<sup>31-33</sup> and TAP alleles were identified by ARMS (amplification refractory mutation system)-PCR technology.<sup>34</sup>

## RESULTS

#### Clinical presentation

A 35-year-old black seaman was evaluated on July 18, 1995 (day 0) at the centralized sexually transmitted disease (STD)

clinic in Port of Spain, Trinidad, for a painful genital ulcer of 5 weeks' duration. He reported no other symptoms at the time. Routine HIV-1 testing revealed p24 antigenemia and negative HIV-1 serology. He was heterosexual and reported a total of seven sexual partners in his lifetime, two of whom were in the preceding year. On physical examination, a 3-cm ulcer with an indurated edge located on the midshaft of the penis, painless inguinal lymphadenopathy of 1-2 cm, and a macular hyperpigmented rash on the palms and soles were noted. He was treated for syphilis with two weekly doses of intramuscular benzathine penicillin G, 2.4 million units per dose, counseled about prevention of HIV-1 transmission, and scheduled for immediate follow-up. The standard of care present at the time did not include the institution of antiretroviral therapy in this setting. After informed consent was obtained, the patient was enrolled in a study of acute HIV-1 seroconversion and is hereinafter referred to as patient SC50. The salient clinical features of his disease course are summarized in Table 1.

The patient did well clinically until days 42-50, when he began to develop symptoms associated with primary HIV-1 infection,<sup>19</sup> including fever, night sweats, and diarrhea. There was never complete resolution of this symptom complex, but he was well enough to be monitored as an outpatient. On day 150, he was admitted to the hospital with fever, diarrhea, and shortness of breath. The chest X-ray showed ground glass infiltrates in the left lung and an infiltrate in the right middle lung fields. Despite treatment with trimethoprim/sulfamethoxazole for presumed *Pneumocystis carinii* pneumonia, he died 3 weeks later in the hospital, 25 weeks (day 172) after he initially presented to the STD clinic.

TABLE 1. CLINICAL EVENTS DURING ACUTE HIV-1 INFECTION

Clinical symptoms	Days postpresentation												
	0	8	42	50	64	71	83	128	130	136	150	169	172
Genital ulcer	+	+	+	+	+								
Lymphadenopathy	+		+	+	+	+							
Rash (feet/palms)	+												
Fever			+									+	
Night sweats				+									
Diarrhea				+			+					+	
Arthralgias					+	+							
Headaches					+	+	+						
Rash (face)					+	+							
Fatigue							+						
Myalgia							+						
Vomiting							+	+	+				
Weight loss <sup>a</sup>							+	+	+				
Bloody diarrhea								+	+				
Oral candidiasis								+	+				
Productive cough								+	+				
Shortness of breath												+	
Death													+
Laboratory findings													
Chest radiograph					—	—	—					+	<sup>b</sup>
Stool culture							—	<sup>b</sup>	+				
Total WBC ( $\times 10^9$ /liter)		3.3		3.5	4.2	3.8	4.0	5.7		4.7			12.8

<sup>a</sup>>10% of baseline body weight.

<sup>b</sup>See text for description.

### Viral serology

Patient SC50 presented with p24 antigenemia in the absence of HIV antibodies on day 0 (Fig. 1), having only preexisting genital ulcers for 5 weeks. The patient remained seronegative until day 50 postpresentation, when reactivity with two viral proteins (gp160 and p24) first became apparent (Fig. 1). Antibodies specific for p66 did not appear until day 106 whereas those for p51 and p17 were absent until day 136. No band for p55 was detected throughout the course of study. Interestingly, the intensity of the p24 band was much weaker near the time of death (day 169), consistent with earlier reports describing greatly diminished p24-directed antibodies in individuals with progressive HIV disease.<sup>9,35,36</sup> Serum immunoglobulin levels measured at weeks 2, 6, and 19 were within normal range and the patient was seropositive for cytomegalovirus, herpes simplex virus, and *Treponema pallidum* on days 8 and 42 (data not shown), suggesting the absence of a generalized immunologic defect.

### CD4<sup>+</sup> lymphocytes and viral load

As shown in Fig. 2, plasma viremia at study entry (day 0) was  $2.6 \times 10^5$  copies of HIV-1 RNA per milliliter of plasma and peaked at  $9.4 \times 10^6$  by day 8. Viral RNA levels failed to resolve below  $10^5$  copies/ml plasma throughout the observation period (days 40–138) and sera from SC50 remained p24 antigen positive throughout the course of disease (data not shown). The absolute number of CD4<sup>+</sup> lymphocytes per microliter of blood increased from 302 (day 50) to a peak of 517 (day 64) followed by a rapid decline to 118 CD4<sup>+</sup> cells by day 90 (Fig.

2). Thus, the interval from peak to nadir CD4<sup>+</sup> cell levels was an extremely short 26 days. The sharp decline in CD4<sup>+</sup> cells occurred despite a stable yet relatively high viral load. It is unclear whether this was due solely to direct or indirect HIV-1 effects or whether intercurrent infection with other agents could also have been contributing factors.

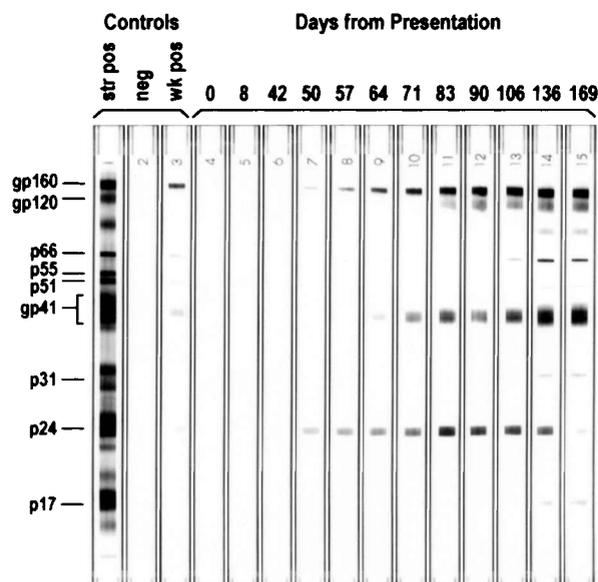
### Lymphocyte subset profiles

Flow cytometric analysis of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocyte subsets was performed with fresh SC50 samples received on days 7, 14, 21, 42, and 56 postseroconversion. The results of the day 56 analysis are presented in Table 2. Also included are the mean values for the same phenotypically defined subsets measured at a comparable postseroconversion interval for other members of the Trinidad acute seroconverter cohort. Other than a decrease in the overall percentage of CD3<sup>+</sup>CD4<sup>+</sup> cells, all CD4<sup>+</sup> lymphocyte subsets from patient SC50 appeared similar to the mean values for the other members of the cohort. Several differences were observed, however, among CD8<sup>+</sup> lymphocyte subsets. Compared with the cohort mean values, SC50 PBMCs contained decreased percentages of CD8<sup>+</sup> cells expressing CD45R0 or CD28 and increased percentages of CD45RA- and CD57-expressing lymphocytes. HLA-DR expression on SC50 lymphocytes increased from 12 to 22% among CD4<sup>+</sup> cells and from 38 to 71% among CD8<sup>+</sup> cells during the 8-week testing interval (data not shown), indicating their relative capacities for activation.

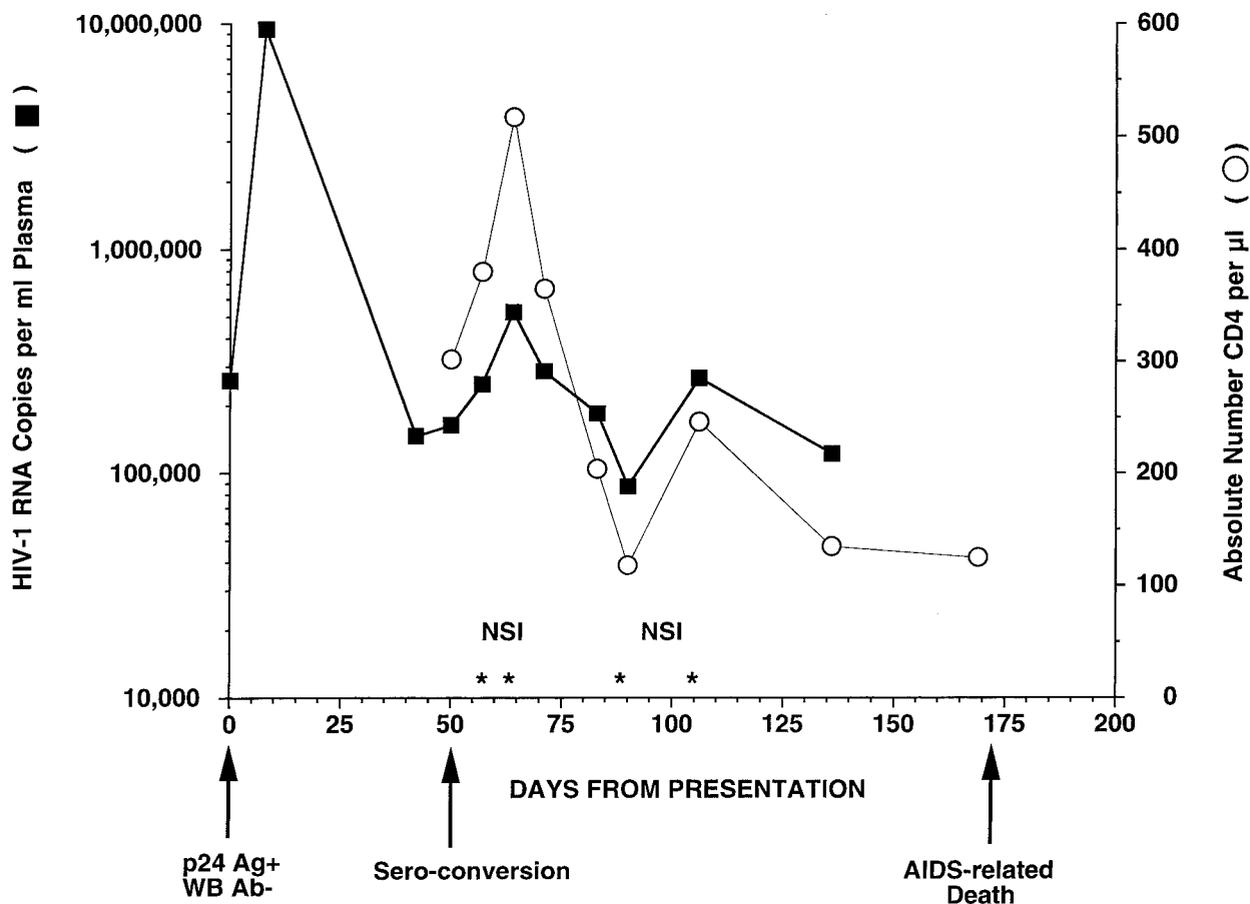
### Functional immune reactivities

Longitudinal assessments of the cellular and humoral reactivities in patient SC50 revealed an impaired immune response to HIV infection. As shown in Fig. 3A, no significant CTL activity ( $\geq 10\%$  lysis above background) specific for either HIV gp160, Gag, Pol, or Nef antigens could be detected at any time, with the exception of a 14% lysis value for HIV gp160-expressing target cells on day 83. These data contrast with those of the 12 other seroconvertors studied thus far from the Trinidad acute seroconverter cohort, where HIV-specific CTL activity was detected at all points tested (J.F. Demarest, unpublished observation). Consistent with the absence of detectable reactivities and delayed CD8<sup>+</sup> T cell activation during acute/early HIV infection, analysis of the T cell receptor (TCR) V $\beta$  repertoire on days 57, 71, and 90, using a semiquantitative RT-PCR technique,<sup>17</sup> did not reveal any significant perturbations in T cell subsets (data not shown).

Significant ADCC reactivities (i.e., lysis  $>10\%$  above background) were also absent in serum samples collected over time from patient SC50 (Fig. 3B). In a majority of other seroconvertors from Trinidad studied thus far, significant ADCC reactivities were present at the time of seroconversion or emerged within 1 to 2 months thereafter (J.F. Demarest, unpublished observation). In comparison with other seroconvertors from Trinidad whom we have studied, SC50 developed low but increasing titers of antibodies capable of neutralizing the laboratory strains HIV-1<sub>SF2</sub> (Fig. 3B) and HIV-1<sub>MN</sub> (data not shown). In contrast, neutralizing antibodies specific to autologous primary isolates recovered from



**FIG. 1.** Serial Western immunoblots. Lanes 1 to 3 represent strong positive, negative, and weak positive Western blot controls, respectively. Lanes 4–15 represent activity found in serial serum samples from days 0, 8, 42, 50, 57, 64, 71, 83, 90, 106, 136, and 169 relative to the day of presentation.



**FIG. 2.** Longitudinal measurement of plasma viremia and CD4<sup>+</sup> cell counts. Plasma viremia (HIV RNA copies per milliliter of plasma; ■) and the absolute number of CD4<sup>+</sup> T cells per microliter of blood (○) were measured over time. Bottom arrows indicate serostatus at time of presentation, time of seroconversion, and time of AIDS-related death. Asterisks indicate time of virus isolation with determination of viral phenotype. For purposes of comparison, the mean viral load for all 22 members of this acute infection cohort at the time of ascertainment was 1,500,361 copies/ml (range, 14,756,436 to 3000 copies/ml), while the average viral load during the interval of 5–7 months postascertainment was 39,793 copies/ml (range, 174,252 to 264 copies/ml).

TABLE 2. COMPARISON OF LYMPHOCYTE SUBSETS

Lymphocyte subset	Cohort mean (± SD)	SC50 (day 56)
CD3 <sup>+</sup> CD4 <sup>+</sup>	32 (8)	18
CD4		
HLA-DR <sup>+</sup>	25 (13)	22
CD45R0 <sup>+</sup>	60 (17)	67
CD45RA <sup>+</sup>	45 (10)	39
CD28 <sup>+</sup>	96 (6)	100
CD3 <sup>+</sup> CD8 <sup>+</sup>	44 (9)	48
HLA-DR <sup>+</sup>	71 (18)	71
CD38 <sup>+</sup>	78 (18)	88
CD45R0 <sup>+</sup>	51 (15)	19
CD45RA <sup>+</sup>	59 (17)	92
CD28 <sup>+</sup>	48 (11)	25
CD57 <sup>+</sup>	28 (12)	52

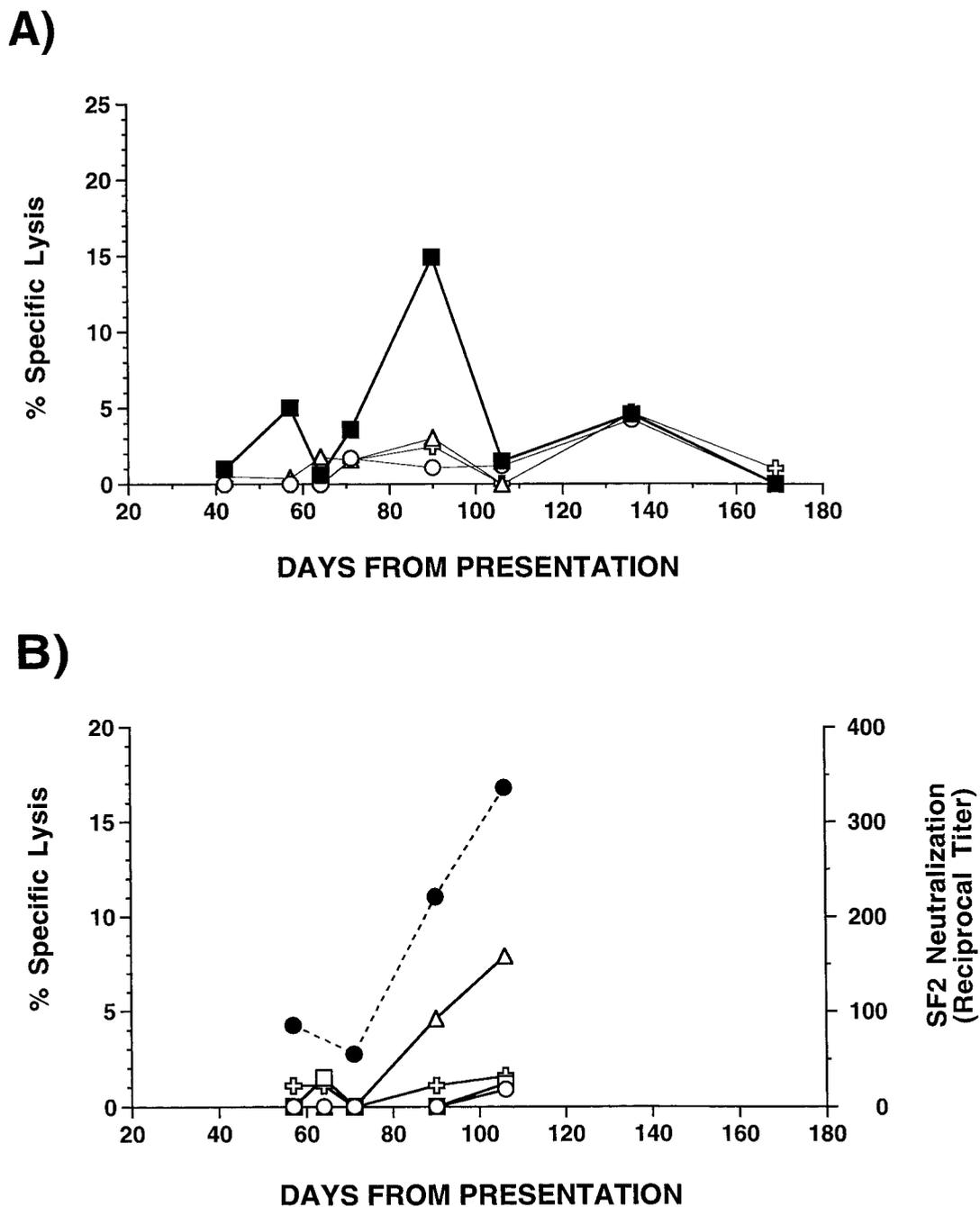
the subject's PBMCs or the HIV-1<sub>IIIB</sub> prototypic laboratory strain were absent.

#### Immunogenetic markers

Host immunogenetic factors have been suggested to impact disease progression.<sup>37</sup> The HLA phenotype of SC50 was determined as HLA-A\*2301, 3601; HLA-B\*1801, 1503; HLA-Cw\*02022, 0704; HLA-DRB1\*1101, \*1503; HLA-DRB3\*0101; HLA-DQB1\*0302, \*0602. Patient SC50 was positive for the TAP 1.2, 1.4, 2.2, and 2.4. alleles and homozygous for wild-type CCR5 and CCR2.

#### Virologic analyses and coreceptor use

All virus isolates that were recovered from subject SC50 failed to induce syncytia or replicate in MT-2 cells and are therefore designated NSI variants. The isolates were also unable to



**FIG. 3.** Longitudinal analysis of cellular and humoral immune responses. Sequential samples of cryopreserved PBMCs and serum from patient SC50 were used to assess HIV-specific cellular (i.e., CTL) and humoral (i.e., ADCC and neutralizing antibody titer) responses throughout the course of disease. **(A)** Longitudinal assessment of HIV-specific CTL activity was performed with mitogen-stimulated PBMCs. Results shown represent percentage specific lysis above the background control for gp160-MN (■), Gag (○), Pol (△), and Nef (+) at an effector-to-target cell ratio of 80:1 (days 42, 57, 64, 71, and 106) or 100:1 (days 90, 136, and 169). Lysis of the parental vaccinia vector was less than 1% at all points tested. CTL precursor (CTLp) frequencies reflected the activity observed in the bulk assays; HIV-specific CTLp were low and comparable to the vaccinia control (data not shown). **(B)** HIV-specific humoral reactivities over time were assessed with cryopreserved specimens of patient serum. For ADCC, serum samples from days 57, 64, 71, 90, and 106 were tested for reactivities specific for gp120-SF2 and -MN (not shown). Results shown represent data at 1:20 (○), 1:200 (△), 1:2000 (□), and 1:20,000 (+) serum dilutions. HIV-1<sub>SF2</sub> neutralization titers (●) were determined with patient serum from days 42, 64, 71, and 106.

<b>Clade B</b>	CTRPNNNTRK	SIHIGPGRAFYT	TGEIIGDIRQAH	35 AA
<b>DAY 57</b>	.....	... <b>M</b> ... <b>L</b> ..-	.....	34 AA
<b>DAY 90</b>	.....	... <b>M</b> ... <b>L</b> ..-	.....	34 AA
<b>DAY 106</b>	.....	..... <b>L</b> ..-	.....	34 AA
<b>TRINIDAD</b>	.....	.....	.....	34 AA

**FIG. 4.** Alignment of V3 consensus sequences for clade B, subject SC50, and Trinidad isolates. Subject SC50 primary viral isolate V3 loop sequences were derived on days 57, 90, and 106. Two substitutions were noted versus the consensus clade B and Trinidad sequences that are not predicted to affect viral phenotype.

replicate in the CEM or AA5 transformed lymphoblastoid cell lines. We examined the genetic complexity of the viral isolates recovered from this subject. Comparison of proviral HIV *env* sequences encoding the V3 to V5 region from four clones derived from the day 57 sample indicated that the sequences were nearly identical, differing by less than 1%. Similar findings were obtained when we compared cloned, full-length proviral *env* sequences from the day 90 and day 106 isolates with each other and with the day 57 clones, implying limited viral diversity. The consensus V3 loop amino acid sequences derived from these samples were similar to the consensus for clade B viruses<sup>38</sup> and for a Trinidad consensus obtained from 19 other infected individuals (Fig. 4). The V3 loop sequences depicted in Fig. 4 are consistent with the NSI phenotype that we observed for these isolates.<sup>39,40</sup> These findings suggest that the viruses recovered from SC50 infect cells via the CCR5 coreceptor and are unable to utilize CXCR4 for infection.

To examine further whether isolates recovered from this patient could infect cells via coreceptors other than CCR5 (specifically through CXCR4 or other coreceptors expressed on activated primary CD4<sup>+</sup> T lymphocytes), we titrated the virus isolates on activated PBMCs obtained from both normal individuals and a CCR5  $\Delta$ 32 homozygous individual. Isolates derived throughout the disease course of the patient readily replicated in the PBMCs from normal donors with titers ranging from  $6 \times 10^3$  to  $2 \times 10^6$  (50% tissue culture infective dose (TCID<sub>50</sub>)/ml. Despite these levels of infectious virus in the isolates prepared from this subject, we did not observe reproducible or significant levels of replication in the CCR5  $\Delta$ 32 cells. In contrast, the X4 IIB laboratory strain replicated well in these CCR5  $\Delta$ 32 target cells. These results strongly suggest that the viruses recovered from this subject primarily used CCR5 for virus entry.

To determine the coreceptor use by virus isolates from SC50, Env clones were tested in cell-cell fusion and luciferase reporter virus assays. Cloned Envs from both early and late virus isolates primarily used CCR5 for cell-cell fusion and virus infection (Fig. 5<sup>25,26,40a-d</sup>). Several Envs demonstrated unusually broad coreceptor use in cell-cell fusion (Fig. 5A), but the ability to mediate efficient entry into target cells expressing these receptors was not observed in virus infectivity assays (Fig. 5B). Coupled with the failure of uncloned virus from SC50 to replicate in PMBCs lacking CCR5, the patterns of coreceptor use by cloned Envs strongly suggest that CCR5 is the primary coreceptor for viruses from SC50.

## DISCUSSION

Among HIV-infected individuals, progression to AIDS and death within 1 year of seroconversion is exceedingly rare. In analysis of the Multicenter AIDS Cohort Study (MACS) cohort, the probability of developing an AIDS-defining illness within 6 months of seroconversion was 0% and within 12 months was less than 1%.<sup>2,3</sup> Isolated cases of such rapid progression have been reported, but the virological and immunological analyses around the time of seroconversion were either limited or not performed.<sup>16,41-44</sup> Although this patient died before the widespread availability of HAART in the United States, the standard of care for HIV-1 in Trinidad and Tobago, as in most developing countries, still does not include antiretroviral therapy. The authors are actively engaged in efforts to make such treatment available to current and future study participants.

Reports describing the appearance and predominance of SI isolates in infected patients just before the development of AIDS-defining illness<sup>8,45</sup> strongly suggest that viral phenotype is a determinant of rapid disease progression. Studies have found that the SI/CXCR4 specificity of HIV-1 is a causal factor in CD4<sup>+</sup> cell depletion *ex vivo* and postulated that the same specificity *in vivo* accelerates the loss of CD4<sup>+</sup> cells and leads to immunodeficiency.<sup>46</sup> In contrast, Michael and co-workers described a case of rapid disease progression with a predominant NSI strain of HIV<sup>44</sup>; however, the immunologic analysis of this patient was limited and the time of seroconversion was inferred from clinical history.

We have described comprehensive longitudinal analyses of virologic and immunologic parameters in an individual who experienced rapid HIV disease progression after heterosexual infection with a clade B HIV-1 genetically similar to that found in 19 other HIV-infected Trinidadians. Longitudinal analyses of the immune response to acute HIV infection in this patient suggested an impairment early after infection. The incomplete antibody profiles and absent ADCC reactivities (i.e., <10% specific lysis) during seroconversion were unique to this individual compared with all other cohort members and were consistent with altered humoral responses (Figs. 1 and 3B). However, the neutralizing antibody response to prototypic laboratory strains was generally similar to that of other Trinidad seroconvertors not undergoing rapid disease progression (data not shown). Dampened cellular immune responses were evidenced by the absence of detectable CTL activity, a feature also unique to this patient. In the limited number of individuals described in the literature to date, the absence of detectable CTL activity

throughout primary and early HIV-1 infection is a relatively uncommon finding.<sup>47-50</sup> Consistent with this observation was the absence of T cell receptor repertoire perturbations and the low percentage of CD8<sup>+</sup>CD45RO<sup>+</sup> lymphocytes, a subset that should include functional CTLs. There were no apparent defects in cellular activation since expression of HLA-DR by both CD4<sup>+</sup> and CD8<sup>+</sup> cells as well as CD38 by CD8<sup>+</sup> cells was comparable to the mean levels seen in the other members of the cohort at the same postseroconversion interval. The increased percentage of CD8<sup>+</sup> cells expressing CD57 in this rapid progressor is of interest since this subset has previously been reported to define putative suppressor T cells (Ts) capable of inhibiting HIV-1 specific CTL.<sup>51</sup> An increase in these Ts cells among alveolar lymphocytes harvested from HIV-1-infected patients carried with it a poor clinical prognosis.<sup>52</sup> The inability to detect TCR V $\beta$  repertoire perturbations might have been due to the sampling interval, in that the day 57 sample was the first available specimen, yet the data were consistent with the undetectable cellular immune response to HIV infection.

The observed failure to mount a robust immune response to HIV infection, particularly within the cellular arm, provided compelling indirect evidence to explain the rapid disease course in patient SC50. Hay *et al.* reported that the inability to maintain a robust CTL response correlated with AIDS-related death within 45 months of presentation.<sup>53</sup> Furthermore, in the SIV model, Evans *et al.* demonstrated that the absence of CTL responses was linked to rapid disease progression whereas a robust CTL response was associated with a more typical disease course and selection of CTL escape variants.<sup>54</sup> The viral and/or host factors that led to the dampened HIV-specific immune responses in SC50 remain unclear. The stable genotype (Fig. 4) of primary isolates from SC50 was consistent with earlier reports of limited HIV-1 heterogeneity during progressive disease<sup>55,56</sup> and with the absence of both immune pressure (Fig. 3) and therapeutic intervention. The genotypic and phenotypic analyses of HIV-1 isolates from SC50 suggested that he was not infected with a uniquely cytopathic strain of HIV-1 although the predominant NSI species was highly virulent in this individual.

Host genetic factors or preexisting immune status might also have contributed to the observed disease progression. SC50 did have an immunogenetic profile associated with rapid HIV disease progression (HLA-A23<sup>+</sup>/TAP2.3<sup>-</sup>),<sup>37</sup> although the functional significance of this profile remains unclear. Interestingly, SC50 BLCLs were capable of presenting antigen to an HLA-A23-restricted CTL population derived from another Trinidad seroconverter (J.F. Demarest, unpublished observation). One cannot exclude the possibility that the patient's active syphilis at the time of HIV-1 infection impacted on the immune response to HIV. Arguing against such interference for patient SC50 is data from other cohort patients among whom, despite having active STDs (including syphilis), detectable HIV-specific immune responses and resolution of peak plasma viremia were observed (J.F. Demarest, unpublished observations).

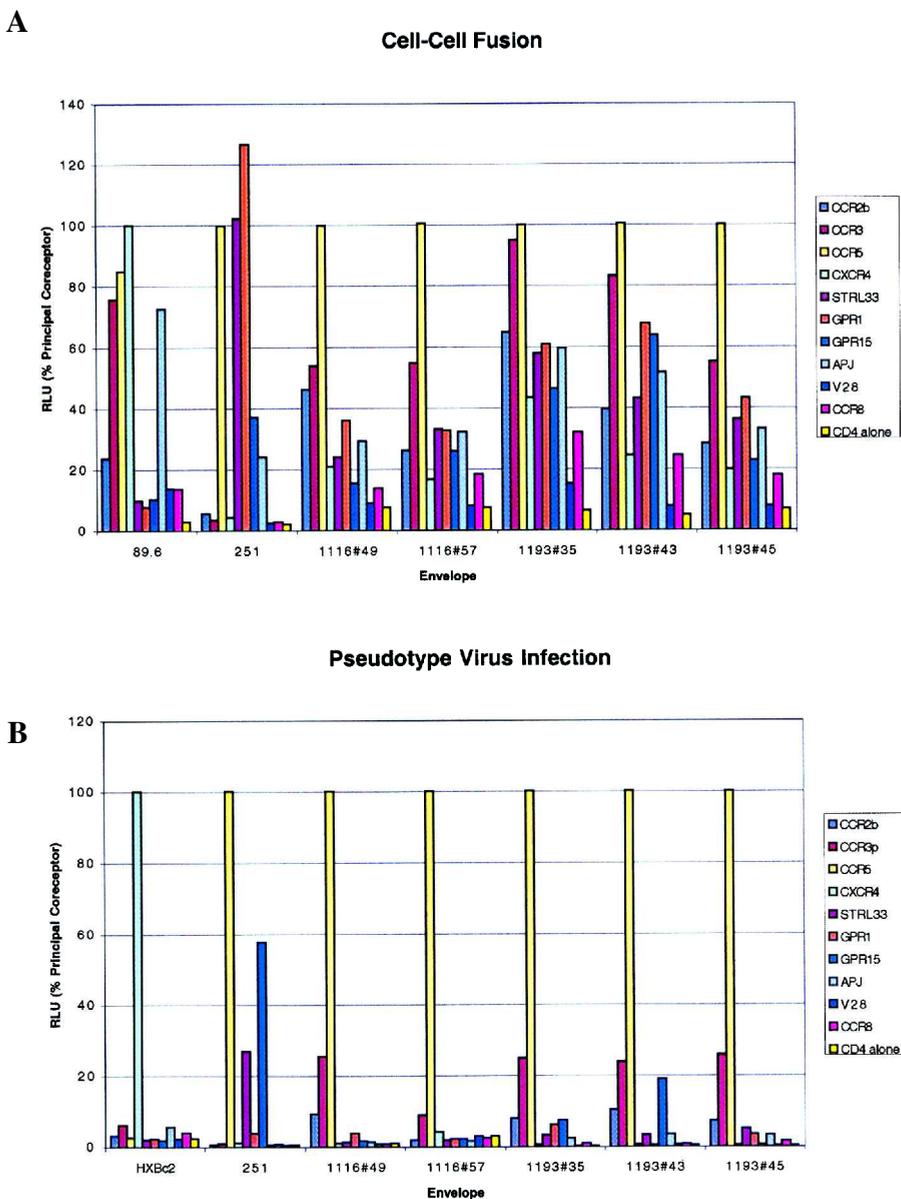
Long-term clinical and laboratory follow-up of most patients studied during acute HIV infection is either incomplete or unavailable.<sup>47-50</sup> Nevertheless, the available data suggest that the early development of CTLs during primary HIV infection to-

gether with the phenotype of the infecting strain are important determinants of subsequent HIV-1 disease progression. In the present report, peak plasma viremia is partially resolved by two orders of magnitude in the absence of detectable CTL activities, suggesting possible contribution by the developing antibody response,<sup>57</sup> CD8<sup>+</sup> cell noncytolytic cellular viral suppression,<sup>58</sup> and tissue redistribution.<sup>59</sup>

Although incapable of eradicating the pool of infected cells, the HIV-specific cellular immune response during acute HIV infection with an NSI virus could contribute to the significant reduction from the initial peak in plasma viremia and a subsequent slow or typical rate of disease progression.<sup>48,50,60</sup> Similarly, on infection with an SI strain of HIV-1, the presence of a broad and persistent HIV-specific immune response may contribute to either downregulation of plasma viremia<sup>17,18,47,61</sup> or SI clearance with replacement by an NSI quasispecies.<sup>62</sup> However, in the presence of a restricted cellular immune response, infection with an SI virus may lead to a rapid rate of progression to AIDS (e.g., WEAU/patient 1 in Refs. 17, 18, 47, and 61), suggesting a stronger contribution by this viral phenotype to disease progression.

In a review article, Klein *et al.* noted that the viral load in rapid progressors generally increases in the face of a strong CTL response, suggesting viral escape.<sup>63</sup> The studies in this report as well as those of others<sup>16,41-44</sup> suggest that a quantitative defect in the HIV-specific cellular immune response to acute HIV infection may also predispose an individual to rapid disease progression. The studies of patient SC50 represent a comprehensive virologic and immunologic analysis of an individual who experienced a rapid rate of progression to AIDS and AIDS-related death. The data from this patient indicate that, in the absence of a cellular immune response, even an R5 NSI species of HIV-1 can contribute to a rapid course of HIV-1 disease progression. Several viral Env clones from the day 57 and day 106 samples exhibited broad coreceptor usage in the cell-cell fusion assays. The ability of primary envelopes recovered from these early time points to display activity with such an array of coreceptors is highly unusual.<sup>64</sup> How, and indeed if, these properties of the viral envelope may have impacted the striking disease course of this individual is not apparent, particularly when the absence of evidence for infection via these alternative coreceptors is considered.

The patient samples available for analyses were severely limited by a rapidly deteriorating clinical status. We, therefore, were unable to assess whether a compromised HIV-specific helper T component of the cellular immune response could also be a determinant of disease progression,<sup>12</sup> a finding that would be consistent with the severely diminished to undetectable CTL and humoral responses observed in this patient. Likewise, samples are no longer available for further in-depth analyses using the more recently developed ELISpot and intracellular cytokine assays and an expanded set of potential targets, including Rev and Tat determinants. The need for further analysis of virus and host factors that influence HIV-1 disease progression remains. Much emphasis has been placed on the virus and its coreceptors as determinants of disease stability/progression. The immunologic and virologic analyses described in this report, together with those of others,<sup>47-50,65</sup> serve as an important reminder that the relative capacity to mount a robust anti-HIV-



**FIG. 5.** Coreceptor use by SC50 Envs. Several viral Env clones from day 57 and day 106 samples were tested for coreceptor use in cell-cell fusion (**A**) and luciferase reporter virus infection (**B**) assays.<sup>25,26</sup> Both techniques measure the ability of Env to mediate membrane fusion against a target cell containing CD4 and a given coreceptor through production of luciferase, which is quantified in target cell lysates. Results (in relative light units) are normalized to the principal coreceptor for each Env and represent the average of at least two independent experiments. SIVmac251 (clone BK28), HIV-1 HXBc2, and HIV-1 89.6 were used as positive controls.<sup>40a</sup> Discordance between the pattern of coreceptor use by a given Env in these assays has been noted previously, and may relate to differences in the physical requirements of fusing cells and viruses or in levels of expression between the two assays.<sup>40a-d</sup>

1 immune response during the early phase of infection may be an equally important determinant.

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