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Contribution of immunological and virological factors to extremely severe primary HIV-1 infection

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Abstract

Background—During acute HIV infection, high viral loads and the induction of host immune responses typically coincide with the onset of clinical symptoms. However, clinically severe presentations during acute HIV-1 infection, including AIDS-defining symptoms, are unusual.

Methods—Virus isolates were tested for clade, drug susceptibility, coreceptor usage, and growth rate for two cases of clinically severe sexual transmission. HLA genotype was determined, and HIV-1-specific CTL responses to an overlapping peptide set spanning the entire HIV clade A and clade B proteome were assayed.

Results—The virus isolated from the two unrelated cases of severe primary HIV-1 infection showed R5/X4 dual/mixed tropism, belonged to clade B and CRF02-AG, and were highly replicative in peripheral blood mononuclear cell culture. Impaired humoral responses were paralleled by a profound absence of HIV-1-specific CTL responses to the entire viral proteome in the two study cases. One case for which the virus source was available, showed a remarkable HLA similarity between the transmission pair as all 4 HLA-A and -B alleles were HLA supertype-matched between the subjects involved in the transmission case.

Conclusions—The data suggest that concurrence of viral and host factors contribute to the clinical severity of primary HIV-1 infection and that subjects infected with highly replicative dual tropic viruses are more prone to develop AIDS-defining symptoms during acute infection if they are unable to mount humoral and cellular HIV-1-specific immune responses. Concordant HLA supertypes might facilitate the preferential transmission of HLA-adapted viral variants, further accelerating disease progression.

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Keywords

primary HIV-1 infection; HLA supertypes; CTL responses; R5/X4 dual tropism; rapid disease progression

Introduction

Symptoms of acute retroviral syndrome typically coincide with high-level viremia and the induction of the host's initial adaptive immune response [1,2]. However, clinically severe presentations during acute HIV-1 infection, including AIDS-defining symptoms, are considered to occur infrequently [3]. Furthermore, epidemiological studies have shown that, in the absence of treatment, less than 0.5% of HIV-1-infected individuals progresses to AIDS within a year after primary infection [4]. A complex interplay between multiple viral and host factors is most likely to be involved in accelerating disease progression. Among these myriad factors, CXCR4 tropism has been associated with higher viral virulence [5]. Moreover, HLA class I concordance between individuals and the inability to elicit specific CTL responses have been suggested to increase HIV-1 transmission and disease progression [6,7].

Here, we investigate the immunological and virological factors that contribute to development of AIDS-defining pathogenesis in two independent cases of unusually severe acute sexually transmitted HIV infection. Their clinical and diagnostic outcome is described in Figure 1.

Material and methods

The study subjects gave written informed consent to participate in this study, which was approved by the institutional review boards of the hospitals where they received medical care.

HIV-1 was isolated from the patients' peripheral blood mononuclear cells (PBMC), and the viral stocks were titrated in TZM-bl cells [8]. Coreceptor usage of primary HIV-1 isolates was assessed by infection of U87.CD4 cells expressing either CCR5 or CXCR4 [9]. Syncytia induction was determined in vitro in MT-2 cells. CCR5 was genotyped in genomic DNA extracted from cryopreserved PBMCs to detect the Δ32 deletion. The growth rate of the viral isolates was determined by infecting phytohemagglutinin (PHA)-stimulated donor PBMCs [10]. To assess the presence of drug resistance-associated mutations, we sequenced the HIV protease region (codons 1 to 99) and reverse transcriptase (RT) region (codons 40 to 247) from a plasma sample drawn prior to initiation of antiretroviral therapy.

In order to determine whether case 2 harbored the same virus as the suspected source patient, viral RNA was extracted from plasma. The *pol* (protease and first 235 codons of the RT) and *env* (C2 to V5 regions) genes were sequenced [10,11]. In addition, a total of 46 molecular clones encompassing the *env* gene were used to estimate diversity in the plasma viral RNA for the source and index patients [11]. Sequence alignments were obtained using Sequencher v4.6 (Gene Codes Corporation) and ClustalW, and manually edited in the regions of variable length. Genetic distances and evolutionary rates were computed using a Kimura 2-parameter model. Neighbour-joining phylogenetic trees of each subject's *pol* and *env* sequences were constructed using MEGA3. The reliability of phylogram clustering was assessed by bootstrapping analyses. Co-receptor usage was inferred from *env* clonal sequences using phenotype prediction tools (http://coreceptor.bioinf.mpi-inf.mpg.de/).

HLA class I and class II genotypes were identified by high resolution sequencing in an approved clinical laboratory. HLA class I supertype assignment was based on functional classification

for the many different four-digit high-resolution HLA alleles that overlap in their peptide-binding specificities [12].

Cellular immunity to HIV and EBV was assessed by IFNγ elispot assays. T cell responses were detected to an overlapping peptide (OLP) set spanning the entire HIV clade A and clade B protein sequence [13]. In addition, optimal epitopes known to be presented by the subjects HLA class I alleles were included in either their clade-specific consensus version or based on sequence variants identified in the index or source subject (Table I suppl.). To assess general immune reactivity, three peptide pools containing a previously described set of EBV-derived optimal CTL epitopes were also tested [14]. Specific cut-offs for positive responses were used as previously defined [15].

Results

Case 1

Laboratory assessment of case 1 indicated a change in his HIV-1 antibody reactivity around the time of presentation. Three previous determinations—HIV-1 antibody-, nucleic acid-, and antigen-based assays within nine months before presentation—were all negative. Antibody/antigen and WB tests became partially reactive, and plasma HIV-1 RNA was positive at time of presentation, suggesting HIV-1 primary infection (Figure 1 and Table 1).

The replication-competent virus isolated from PBMCs was able to infect and replicate in both CCR5 and CXCR4-U87.CD4 cells as concluded from the p24 antigen production and the formation of syncytia in the cell cultures (Figures 2A and 2B). The subject did not have a Δ32 genotype in the CCR5 chemokine receptor gene that might have explained an early selection of CXCR4-tropic viruses [16]. The production of p24 antigen in growth kinetics cultures of donor PBMCs was similar to the laboratory-adapted viral strain HIV-1_{NL4-3} (Figure 2C). Phylogenetic analyses with bootscanning methods for the genetic subtyping of *pol* indicated the presence of a subtype B virus. The HIV-1 genotype showed no drug resistance-associated mutations. The results of HLA-typing are shown in Table 1.

Case 2

Clinical symptoms and analytical results in the index patient were consistent with a diagnosis of advanced HIV-1 infection and AIDS. However, the patient denied other HIV risks than sexual contact with her partner for the past two years. Her mother tested negative for HIV-1 infection, thus excluding a potential vertical transmission. Moreover, the viruses isolated from the source and index patients were similar both phenotypically and genotypically (Figure 3 and Table 1).

Laboratory assessment of the index patient's original sample provided clear reactivity data on the presence of HIV-1 antigens, but, despite high levels of immunoglobulins (Igs), antibody-based systems provided partial reactivity, indicating a lack of HIV-1-specific antibodies (Figure 1 and Table 1).

Bootstrap analysis of *pol* and *env* sequences from the index and the source patients revealed values of ≥99% in 1000 replicates (Figure 3A and 3B), indicating that sequence clustering was unlikely to have occurred by chance. The genetic distance of the *pol* sequences between the index patient and the source patient was <0.1%, whereas the mean genetic diversity between randomly selected sequences from local, epidemiologically unrelated HIV-infected individuals with the CRF02-AG subtype was 3.0%. The high degree of similarity between viral sequences indicates a likely viral transmission from one patient to the other. Clonal analysis of *env* sequences indicated that all sequences from the index patient were closely related, with a mean

diversity of 1.8%, while the source patient's sequences had a mean diversity of 2.4% (Figure 3C and 3D).

After five days of culture, the viruses isolated from the source and the index patient were able to infect and replicate in both CCR5- and CXCR4-U87.CD4 cells as indicated by the p24 antigen production and the formation of syncytia in the cell cultures (Figure 3E and 3F). None of the individuals showed a Δ32 genotype in the CCR5 chemokine receptor gene. Phenotypic inference of the V3 amino acid sequence in multiple clones from each individual suggests that all clones from the index patient could use CXCR4 (R5X4 tropism) for viral entry, whereas the source patient contained clones which could only use CCR5 and clones that could use CXCR4 (R5X4 tropism) (Figure 3C). The production of p24 antigen in growth kinetics cultures in PBMCs was equal in the two viral isolates and comparable with the laboratory-adapted viral strain HIV-1_{NL4-3} (Figure 3G).

Genetic subtyping of the *gag*, *pol*, and *env* genes in the patients' virus indicated that both subjects contained the AG circulating recombinant form 02 (CRF02-AG). Drug-resistance genotyping showed no resistance-associated mutations in the RT. Several polymorphisms were detected in the protease gene, which might have been associated with possible tipranavir resistance in non-subtype B viruses (Table 1).

Cellular immune responses

Case 1 showed a single weak response against one OLP, which was not subsequently observed in the reconfirmation test, and only a borderline response to one EBV-peptide pool. This atypical lack of EBV-specific CTL responses suggests a widespread impairment of the ability to mount adequate CTL responses [15].

The index and source patient of case 2 expressed HLA class I alleles that were highly related. In fact, 3 out of 4 HLA-A and -B alleles were HLA supertype-matched between the two subjects (Table 1). As the transmission of escape mutants arising in the source may have prevented the induction of an effective T cell response in the index patient, the cellular immune response to the entire viral proteome was assessed in both subjects at 1 and 9 months after the transmission event. The analyses included two comprehensive sets of OLP spanning HIV clade A and clade B consensus sequences as well as autologous (index and source patient) peptide variants of optimally defined epitopes presented by the subjects' HLA types (Table I suppl.). The source subject showed weak responses to three different regions of the virus (Table 2), representing an overall weak response rate in comparison to more than 300 previously tested chronically HIV-infected subjects with a median of 17 responses [13, unpublished data]. The index case showed an even weaker HIV-specific T cell response to only a single peptide, which was detected before the subject was treated but was subsequently lost 9 months after infection. Importantly, the index subject was able to mount a T cell response to a peptide pool containing EBV-derived CTL epitopes, indicating that the absence of HIV-specific T cells was not due to poor cell viability or a general immune incompetence in this individual.

Although some responses to autologous sequence variants that were not tested may exist, the data are in line with a remarkable absence of HIV-specific T cell immunity in both case 1 and the index patient in case 2, which may be related to the extraordinarily fast disease progression in these individuals.

Discussion

The interplay between the viral and host factors influencing accelerated disease progression is complex and poorly understood. The two temporarily coincident cases reported here suggest immediate progression to AIDS from primary HIV-1 infection after sexual transmission. In

both cases, the diagnosis of primary HIV-1 infection was supported by nucleic acid- and antigen-based screening tests, with an evolving antibody pattern. Case 1 tested negative for HIV several times before presentation and subsequent HIV-1 WB was only partially reactive. The index patient in case 2 lacked previous negative test results, but presented with very high plasma HIV RNA level, which is consistent with acute HIV infection [17]. In addition, HIV-1-specific antibody tests were only partially reactive and did not become positive until nine months after antiretroviral treatment. Although detection of HIV-specific antibodies after the onset of primary infection symptoms can take between 5 and 15 days [18], complete seroconversion may occasionally be delayed until 12 months after identification of infection by antigen, once virological control has been achieved with effective antiretroviral therapy [18,19]. The fact that serum levels of IgG, IgM, and IgA (in the index patient in case 2) were within the reference range or higher suggests polyclonal B cell activation [20]. Moreover, positive IgG responses to cytomegalovirus, *Toxoplasma gondii*, and hepatitis A indicate the ability of antibodies to maintain an appropriate response against microorganisms that cause persistent latent infection. Although plasma viremia had greatly decreased upon treatment, $CD4^+$ T cell recovery increased slowly from total absence at presentation, which could have delayed HIV seroconversion.

The diversity of the viral population in HIV-1 *env* increases in parallel with divergence at a rate of 1% per year for a few years after seroconversion, before reaching a peak and then levelling off or decreasing [21]. Nevertheless, the rates of diversity are higher among patients with a sharp decline in the number of CD4 T cells [22]. In case 2, mean HIV-1 diversity was lower in the index patient than in the source patient, indicating that viral evolution was longer in the latter. Although viral diversity tends to decrease in the later stages of infection, most of the genetic distances would remain above 2% [21], thus supporting the direction of transmission in this pair and the theory of a very early presentation after viral transmission in the index patient.

The development of acute retroviral syndrome typically coincides with high-level viremia and the host's initial immune response. However, these two cases illustrate primary HIV-1 infection with unusually severe clinical symptoms. Other reports have described severe presentations during primary HIV-1 infection, including acute myopericarditis, renal failure, acute liver failure, and opportunistic infections [23-26], but viral and host factors have not been addressed in detail.

In both cases, the virus isolated from the patients' PBMCs was able to use CCR5 and/or CXCR4 as entry co-receptors and replicate very efficiently in PHA-stimulated donor PBMCs. These data indicate that both viral isolates are either dual-tropic viruses or a mixed population of CCR5-tropic and CXCR4-tropic viruses with high replication capacity. This observation would suggest that the transmitted virus had the ability to deplete CCR5+ as well as CXCR4+/CD4 + T lymphocytes, which may help to explain the total loss of the CD4+ T cell population and rapid clinical progression observed in the index patient upon transmission. Infection with dual-tropic HIV-1 variants in injecting drug users has been associated with immediate and rapid total T cell decline and progression to AIDS within four years of the estimated time of infection [27]. Furthermore, CCR5-Δ32/Δ32 seroconverters who showed the uncommon pattern of early syncytia-inducing virus and rapid CD4 decline had a uniformly high viral load and dual-tropic coreceptor usage [28]. A link between the detection of syncytia-inducing variants and a rapid CD4+ T cell decline in vivo has already been established [29].

Despite the fact that the characterized viral subtype CRF02-AG in case 2 is rather unusual in our area (1.1% of *pol* sequences tested for antiretroviral resistance between 1999 and 2007), it is still the second most common non-B subtype. CRF02-AG is the predominant and most rapidly spreading HIV strain in West and West Central Africa [30,31], thus raising concerns

about its superior replication fitness and/or transmission efficiency. In fact, primary HIV-1 CRF02-AG isolates from Cameroon exhibited higher ex vivo replicative fitness than subtype A and G viruses from the same geographic region [32,33]. These observations are consistent with the high replication rate we observed in primary PHA-stimulated PBMCs, although we compared them to the laboratory-adapted B-subtype HIV-1_{NL4-3} strain.

In our cases, concurrent host factors may have also contributed to higher susceptibility to HIV-1 infection or disease progression. For example, specific HLA haplotypes have been proposed as an important risk factor in this context [34]. Among these, HLA-B*35, which is in high linkage disequilibrium with HLA-Cw*04, has been consistently associated with rapid progression to AIDS [35-37]. Specifically, the allele HLA*B3503, present in case 1, has been reported to increase the risk of progression to AIDS 2.7-fold (95% CI: 1.7–4.3, P<.001) [38]. In case 2, the source patient expressed the HLA A*68, B*53, and Cw04 alleles, which have been associated with rapid disease progression [34]. Although, none of the alleles in the index patient have been associated with accelerated disease progression, the donor and recipient expressed 3 out of 4 HLA-A and –B alleles that fell in the same HLA supertypes (i.e. clusters of functionally related four-digit high-resolution HLA class I alleles) [12]. This may have facilitated the transmission of viruses with cytotoxic T lymphocyte escape mutations, thus diminishing the number of epitopes recognized in the newly infected individual [6,7,39,40]. Remarkably, optimal epitope variants representing autologous sequence diversity did not elicit a response, suggesting effective CTL escape (Table I suppl.). This hypothesis would fit with the fact that the index patient in case 2 showed almost complete absence of HIV-1-specific CTL responses, something rather unusual during primary HIV-1 infection. In a previous study, only 1 out of 5 patients presenting with primary HIV-1-infection, showed absence of precursor CTL specific for cells expressing viral proteins [1]. Another study in acute and early infected subjects reported a slightly higher breadth and magnitude of HIV-1-specific CTL responses [41]. However, this study used a less comprehensive pool of overlapping peptides and, contrarily to our case which showed a persistent absence of responses at month 9, CTL responses increased after 6 to 12 months of treatment. Although we could not identify the source patient for case 1, the lack of HIV-1-specific CTL responses also in this case might let us to speculate with a potential HLA class I concordance at transmission. In any case, the inability to elicit HIV-1-specific CTL responses at the time of primary infection was paralleled in these patients with AIDS-defining pathogenesis and severe clinical presentation. Although HIV-1-specific CTL responses have been considered a crucial factor in HIV disease progression, we had limited experimental and clinical evidences of the detrimental effect that the inability to elicit these responses might have in symptomatic primary HIV-1 infection [1, 42]. Moreover, the coincident inability in these 2 cases to mount an effective adaptive immune responses against HIV-1, albeit not to other pathogens, might be a consequence of a potential defect in the innate immunity. Clearly, further studies in these and other subjects with accelerated disease progression will be needed to address these factors.

In conclusion, we describe two cases of sexual transmission of a highly replicative dual-tropic HIV-1 of subtypes B and CRF02-AG that resulted in an aggressive clinical progression to severe symptomatic AIDS in young patients. Adaptive cellular and humoral immune responses in the host might have simultaneously failed to control the virus.

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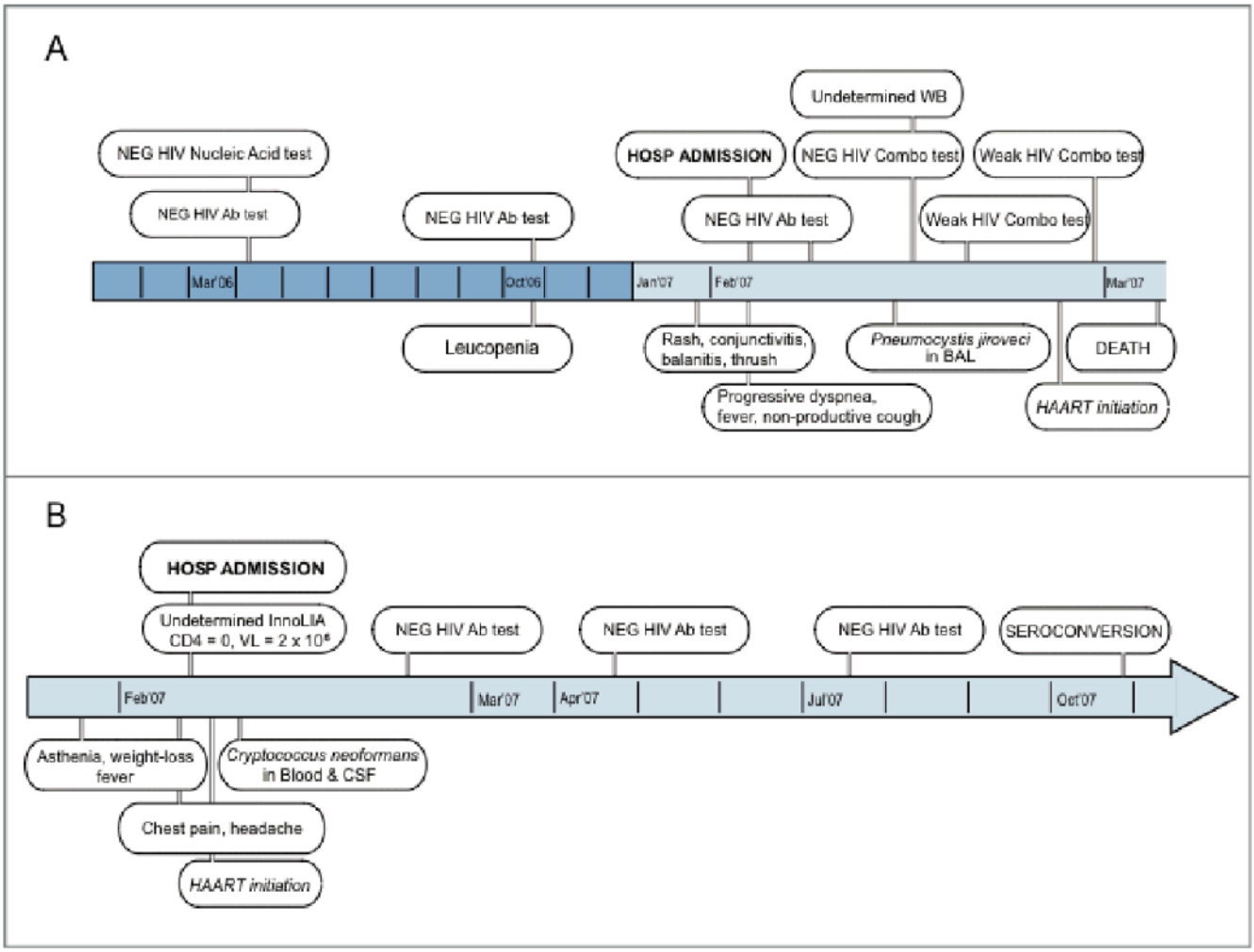

Figure 1.
Description and outline of clinical and diagnostic outcome of the two severe primary HIV-1 infection cases in this study.

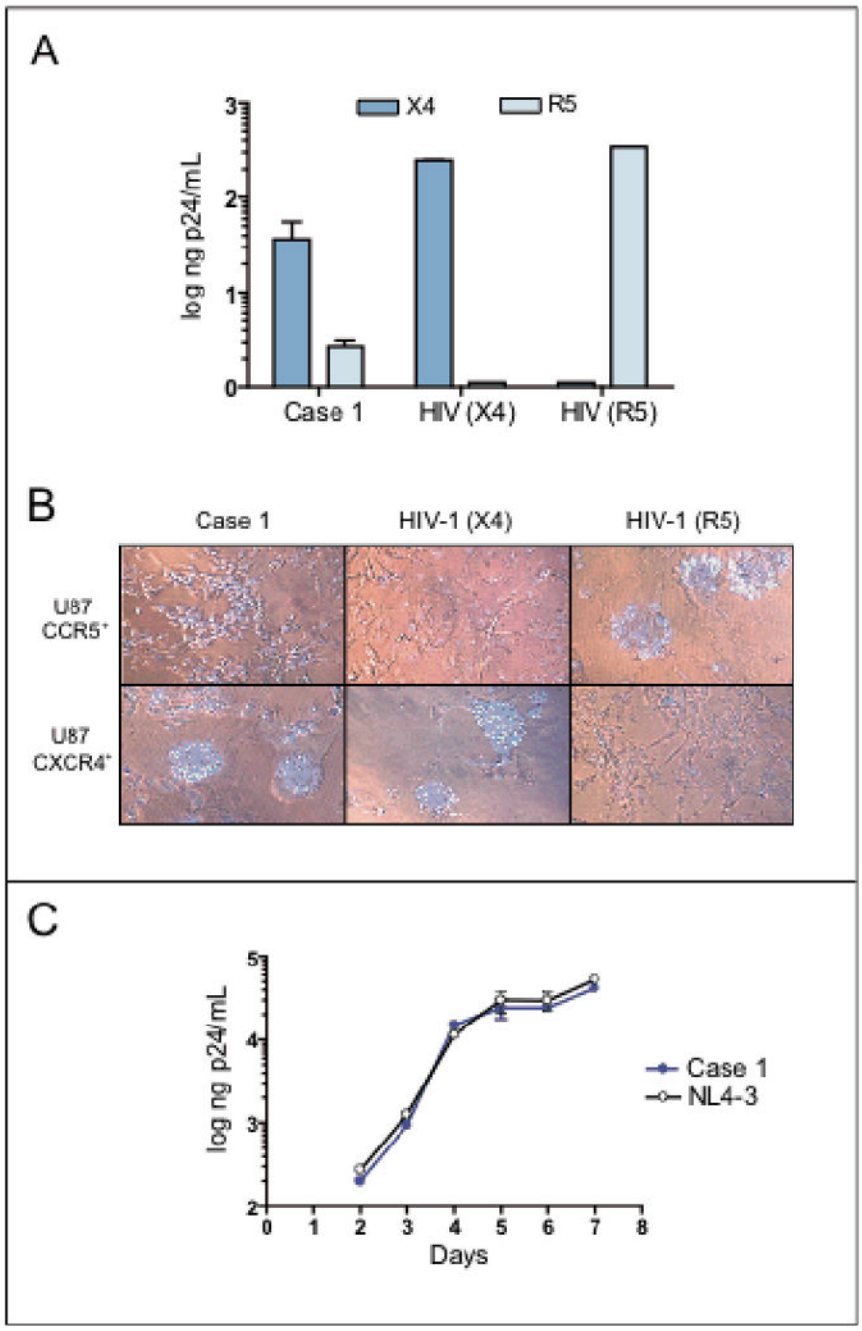

Figure 2.

Case 1 virological data. Viral coreceptor usage based on p24 production (A) and syncytia formation (B) in U87.CD4 cells expressing either CXCR4 or CCR5. Control viral strains HIV-1_{NL4-3} (CXCR4-tropic, syncytia inducer) and HIV-1_{NFN-SX} (CCR5-tropic, non-syncytia inducer) were included in both assays. Viral replication growth rates in PHA-stimulated primary donor PBMC infected with the patient's viral isolate (C). The laboratory-adapted HIV-1_{NL4-3} reference strain was grown in parallel. One representative experiment out of two with PBMCs from two different donors is shown.

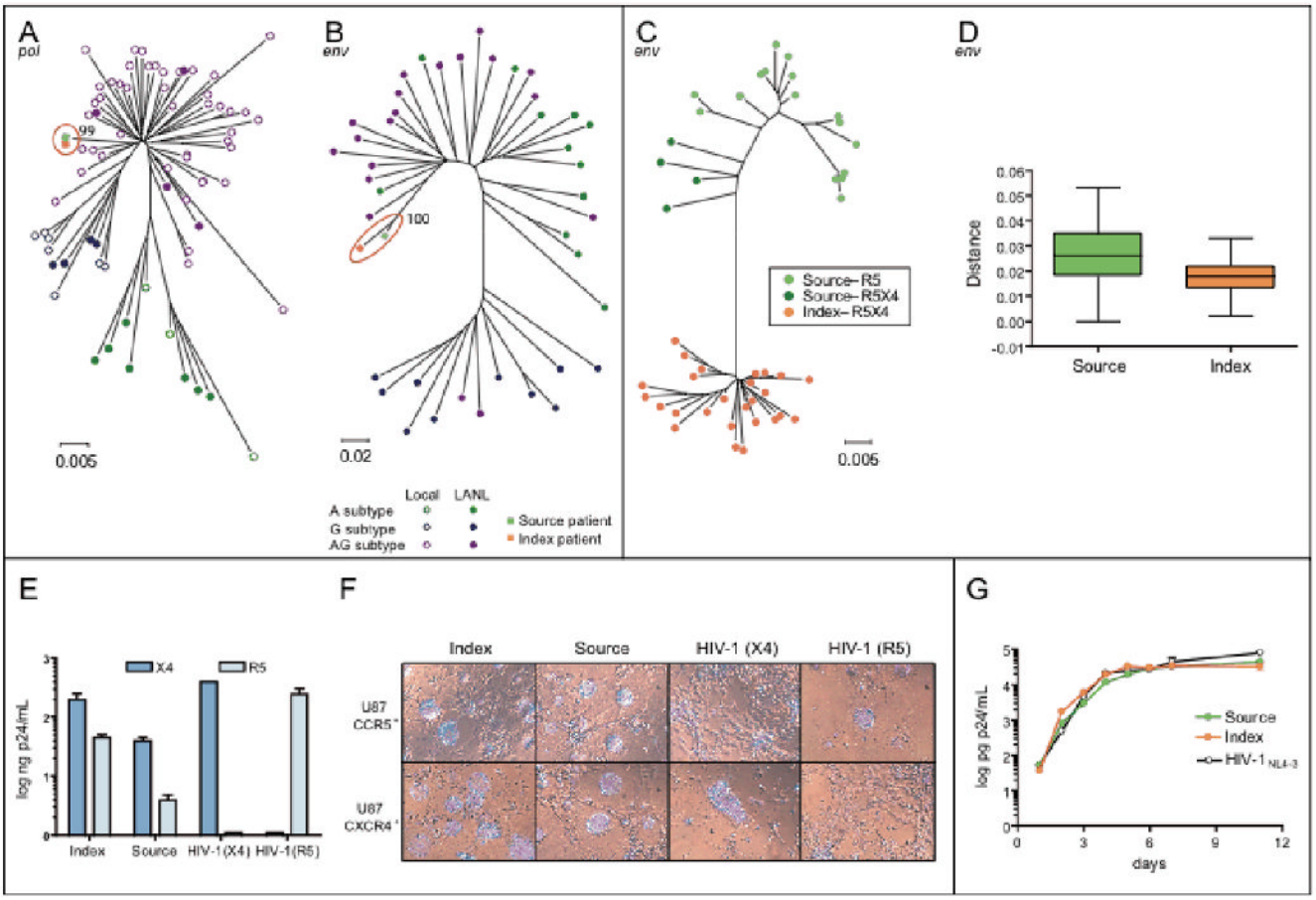

Figure 3.

Case 2 virological data, including source patient and index patient samples. Neighbour-joining phylograms of pol (A) and env (B) sequences derived from viral RNA in plasma. Case sequences are represented by an orange square (index) or a green square (source). The scale for the genetic distance, based on the Kimura 2-parameter method, is indicated below each phylogram. Phylogenetic analysis of the env C2V3 clonal sequences derived from the index and source viral isolates with different colour patterns represent the virtual tropism of each clone (C). Intra-patient diversity is shown (D). The scale for the genetic distance, based on the Kimura 2-parameter method, is indicated below the phylogram. Viral coreceptor usage based on p24 production (E) and syncytia formation (F) in U87.CD4 cells expressing either CXCR4 or CCR5. Control viral strains HIV-1NL4-3 (CXCR4-tropic, syncytia inducer) and HIV-1NFN-SX (CCR5-tropic, non-syncytia inducer) were included in both assays. Viral replication growth rates in PHA-stimulated primary donor PBMCs infected with the patient's viral isolate (G). The laboratory-adapted HIV-1NL4-3 reference strain was grown in parallel. One representative experiment out of three with different PBMC donors is shown.

Table 1

Laboratory assessment of the patients involved in 2 case reports of sexual transmission of severe HIV-1 infection

Variable	Case report 1	Case report 2	
		Index patient	Source patient
Plasma HIV-1 RNA level, copies/mL	32×10^4	2×10^6	4×10^4
$CD4^+$ T cell count, cells/mm^3	108	0	33
$CD4^+$ T cell percentage	27	0	4
$CD8^+$ T cell count, cells/mm^3	Not determined	203	481
$CD8^+$ T cell percentage	Not determined	59	59
Result of standard HIV Ab test	Negative	Weak[a]	Positive
HIV Western blot findings	Undetermined	Undetermined	Positive
Result of nucleic acid and/or viral load test	Positive	Positive	Positive
Plasma p24 level, pg/mL	6.3	72.6	12.5
Viral subtype	B	AG	AG
Drug resistance genotype			
Protease	K20M and M36I	L10V, I13V, G16E, M36I, H69K and L89I	L10V, I13V, G16E, K20I, M36I, H69K and L89I
Reverse transcriptase	None	None	None
Coreceptor useb	R5/X4	R5/X4	R5/X4
CCR5 Δ32 genotype	WT/WT	WT/WT	WT/WT
HLA alleles			
Class I[c]	A*0201 (A2), A*1101 (A3)	A*0201 (A2), A*0301 (A3)	A*6802 (A2), A*6801 (A3)
	B*3503 (B7), B*4001 (B44)	B*0702 (B7), B*1801 (B44)	B*5101 (B7), B*5301 (B7)
	Cw*0304, Cw*0401	Cw*0702, Cw*1203	Cw*0401, Cw*1502
Class II	DRB1*0701, DRB1*1302	DRB1*0405, DRB1*1201	DRB1*0101, DRB1*0701
	DQB1*0202, DQB1*0604	DQB1*0301, DQB1*0302	DQB1*0202, DQB1*0501

NOTE. WT, wild type.

[a] Different HIV antibody tests provided nonreactive or weakly reactive results.

[b] Viruses from all of the patients were syncytia-inducing viruses. Supertypes are shown in parenthesis.

[c] Supertypes are shown in parenthesis. A*68, B*53 (increased susceptibility), B*51 (increased protection).

Table 2

Optimal epitopes showing sequence diversity between viruses from the source and index patients on case report 2

Epitope	Protein	HXB2 position	Reference	Sequence
A02 AL9	Vpr	59-67	con B	AIIRILQQL
			source/index	---------
			ConAG	---------
A02 FK10	Gag	70-79	con B	FLGKIWPSYK
			source/index	-------- H-
			ConAG	----------
A02 GT9	Vpr	41-49	con B	GLGQHIYET
			source	------- N-
			index	------- D-
			ConAG	---------
A02 SL9	Gag	77-85	con B	SLYNTVATL
			source/index	-- F-- I---
			ConAG	-- F-- I---
A02 RI9	Vpr	62-70	con B	RILQQLLFI
			source/index	-------- T
			ConAG	-------- V
A02 RI10	Env	311-320	con B	RGPGRAFVTI
			source/index	I--- QT- Y--
			ConAG	I--- QT- YAT
A02 YV9	Pol	127-135	con B	YTAFTIPSV
			source/index	-------- L
			ConAG	---------
A03 AK9	Pol	158-166	con B	AIFQSSMTK
			source/index	---- A----
			ConAG	---- A----
A03 RK9	Gag	20-28	con B	RLRPGGKKK
			source/index	-------- Q
			ConAG	---------
A03 RY10	Gag	20-29	con B	RLRPGGKKKY
			source/index	--------- Q-
			ConAG	----------
A03 TN10	Vpr	19-28	con B	TLELLEELKN
			source/index	---------H
			ConAG	---------H
A68 DL9	Pol	30-38	con B	DTVLEEWNL
			source/index	------ I--

Epitope	Protein	HXB2 position	Reference	Sequence
			ConAG	----- I- L-
A68 IV9	Pol	3-11	con B	ITLWQRPLV
			source/index	-------- V-
			ConAG	---------
A*6801 DR11	Vpr	52-62	con B	DTWAGVEAIIR
			source	--- E-- M----
			index	--- E-- V----
			ConAG	--- E-------
A*6802 EV10	Vpr	48-57	con B	ETYGDTWAGV
			source	N------- E--
			Index	D------ E--
			ConAG	------- E--
B07 RI10	Env	298-307	con B	RPNNNTRKSI
			source	-- S----- N-
			Index	--------- GV
			ConAG	--------- V
B07 TL9	Gag	48-56	con B	TPQDLNTML
			source/index	------ M--
			ConAG	---------
B18 FK10	Gag	161-170	con B	FRDYVDRFYK
			source/index	-------- F-
			ConAG	--------F-
B51 LI9	Env	416-424	con B	LPCRIKQII
			source/index	- Q-------
			ConAG	---------
B51 TI8	Pol	128-135	con B	TAFTIPSI
			source/index	------- L
			ConAG	--------
B53 QW9	Gag	176-184	con B	QASQEVKNW
			source	-- T---- H-
			index	-- T------
			ConAG	-- T------
B53 TL9	Gag	48-56	con B	TPYDINQML
			source/index	-- Q- L- T--
			ConAG	---------
X-GL12	Vpr	9-20	con B	GPQREPHNEWTL
			source/index	----- F-----
			ConAG	----- F-----

NOTE. Changes in the sequences are represented by boldface type.

Table 3

Cellular immune responses in patients involved in 2 case reports of sexual transmission of severe primary HIV-1 infection

Patient, peptide	Protein	HXB2 position	Sequence	SFC per 1 × 10^6 PBMCs
Case report 1: OLP 83 con B	Nef	118-135	**tQGYFPDWQNY**TPGPGIRY	47
Case report 2				
Source patient				
OLP 42 con A	Gag	172-189	LRAE**QA*T*QEVK*G*W**MTETL	41
OLP 42 con B			LRAE**QA*S*QEVK*N*W**MTETL	72
B53 QW9 con B	Gag	176-184	**QA*S*QEVKNW**	97
B53 QW9 index/ con A			**QA*T*QEVKNW**	97
OLP 84 con A	Nef	126-143	NYTPGPGIRYPL*C*FGWCF	10
OLP 84 con B			NYTPGPGIRYPL*T*FGWCF	103
OLP 223 con A	Pol	583-600	QLEK*D*PI*A*GAETFYVDGA	52
OLP 223 con B			QLEK*E*PI*V*GAETFYVDGA	21
OLP 224 con A&B			GAETFYVDGAANRETKL	72
Index patient				
OLP 296 con A&B	Env	52-69	**LFCASDAKAY**DTEVHNVW	18
A24 LY10 con B&A/index	Env	52-61	**LFCASDAKAY**	24

NOTE. Sequence changes between peptides are shown in italics, and optimal peptides are shown in boldface type. OLP, overlapping peptide; SFC, spot-forming cells; con, consensus.