Cerebrospinal fluid response to structured treatment interruption after virological failure

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Objective Structured antiretroviral treatment interruption (STI) has been advocated as a therapeutic strategy for HIV-1 infection. We report initial observations of cerebrospinal fluid (CSF) HIV-1 infection in five patients undergoing serial lumbar punctures (LPs) during STI undertaken following virological failure.

Design and methods In this prospective observational study we quantified HIV-1 RNA concentrations and assessed both phenotypic drug susceptibility profiles and genotypic antiviral drug resistance mutations in CSF and plasma during the period of treatment interruption. CSF white blood cells were also counted, and patients' neurological status monitored.

Results In four of the patients, CSF HIV-1 concentration increased more rapidly than that of the plasma, with consequent reduction in the ratio between plasma and CSF viral loads (pVL : cVL). Three individuals developed robust, though asymptomatic CSF lymphocytic pleocytosis. In all patients the predominant HIV-1 quasispecies shifted simultaneously in CSF and plasma from a drug-resistant to a more drug-susceptible phenotype with identical and simultaneous changes in genotypes associated with drug resistance.

Conclusions STI may be accompanied by previously unrecognized changes in tissue viral exposures and lymphocyte traffic. Hence, despite 'virological failure' as evidenced by persistent plasma viremia, ongoing antiretroviral treatment prior to its interruption appeared to suppress CSF HIV-1 infection (indeed more effectively than that of plasma) and restrain lymphocyte traffic into the CSF. Simultaneous change of resistance mutations in CSF and plasma was likely due to re-emergence and overgrowth of pre-existing strains with ready exchange of virus between these two compartments, either facilitated by or provoking a local CSF lymphocytosis.

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Introduction

Despite the remarkable impact of highly active antiretroviral therapy (HAART) on the course of HIV-1 infection, there remain important limitations to its long-term efficacy. Many patients develop antiviral drug resistance, and viral eradication now appears a distant prospect [1–8]. Structured treatment interrup-

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tion (STI) has been proposed as a strategy to prolong the effectiveness of antiviral therapy [9–14]. For those experiencing virological failure, interruption of treatment may allow drug-susceptible ('wild-type') virus to overgrow resistant variants, thereby restoring therapeutic susceptibility in preparation for re-initiation of therapy. In patients with sustained HIV-1 suppression, STI has been advocated as a way to boost antiviral immunological responses, and as a consequence increase the likelihood of longer lasting viral suppression. Treatment interruption also affords patients a respite from the rigors of strictly adhering to a medication regimen. However, the effect of STI on potential viral reservoirs in chronically infected individuals is unknown.

Sampling cerebrospinal fluid (CSF) provides an accessible window to an important sequestered tissue compartment [15]. We performed serial lumbar punctures (LPs) to assess the effects of STI on CSF HIV-1 infection and host responses. We report the results of initial observations from five patients which show that this therapeutic strategy can be accompanied by dynamic changes in CSF infection and lymphocyte traffic that are not appreciated by monitoring blood or patients' symptoms alone.

Methods

Study protocols and lumbar punctures

Patients participated in a University of California San Francisco (UCSF) Committee on Human Research (CHR)-approved protocol evaluating the CSF responses to changes in HAART, including STI. Repeated LPs with accompanying blood sampling were performed at approximately 3–6 week intervals over the scheduled 12 weeks of treatment interruption. Additional LPs were performed if participants continued off therapy after 12 weeks. Baseline LPs for all participants were carried out within 2 weeks before stopping therapy, and the results carried forward as day 0. CSF sampling was performed as previously described [16].

Laboratory methods

Cell-free CSF and plasma HIV-1 RNA levels were measured using the Amplicor HIV-1 Monitor assay (Roche Diagnostic Systems, Inc., Branchburg, New Jersey, USA) in all but one patient. Samples with viral loads below 500 copies/ml were re-assessed using the Ultra Sensitive modification of the Monitor assay with a dynamic range down to 50 copies/ml and a detection limit of approximately 20 copies/ml; results below this were assigned a value of 19 copies/ml. In the exception, patient 6001, HIV-1 RNA levels were determined using the bDNA assay (Quantiplex, Version 3.0; Chiron, Emeryville, California, USA) with a lower detection limit of 50 copies/ml. Paired CSF and plasma specimens from each patient were run in batch. Antiviral drug resistance was assessed by both phenotypic and genotypic analyses. Phenotypic resistance was measured for all approved antiretroviral drugs using the PhenoSense^{TM} HIV assay (ViroLogic, South San Francisco, California, USA) with results reported as fold-change in the 50% inhibition concentration (IC_{50}) relative to a wild-type virus reference standard [17]. Genotypic analysis of drug resistance mutations in reverse transcriptase (RT) and protease inhibitor (PI)-resistant used an automated dye-primer cycle sequencing method based on the TruGene^{TM} HIV-1 drug resistance kit (Visible Genetics, Inc., Toronto, Canada) with results compared to consensus NL4-3 sequence.

Routine CSF assessments included CSF white blood cell (WBC) and differential counts, and total protein determination; the ratio between CSF and serum albumin ($\times 10^{-3}$) was used as an index of integrity of the blood–brain barrier to albumin and compared to age-adjusted norms from the literature. [18]. Participants also underwent standardized neurological assessments, including AIDS dementia complex (ADC) staging [19] and brief quantitative neurological performance testing incorporating four tasks and yielding an aggregate scaled z-score (QNPZ-4) [20].

Results

Participants

The baseline characteristics of the five male patients are summarized in Table 1. Four patientts (6001, 6004, 6005 and 6006) were recruited from a larger CHR-approved study investigating the systemic consequences of STI in individuals experiencing virological failure [14]. The fifth patient, 4026, was studied in the context of an unanticipated treatment interruption preceding a longitudinal study of the kinetics of CSF viral reduction following new or changed therapy. The median CD4+ T-lymphocyte count at the start of STI was 196×10^6 cells/l, whereas pre-therapy nadir CD4+ counts were all lower than 200×10^6 cells/l. All were highly experienced and had been classified clinically as virological failures with plasma HIV-1 RNA concentrations > 2500 copies/ml for more than 6 months. The median HIV-1 RNA was 4.29 $\log_{10}$ copies/ml in plasma and 2.36 $\log_{10}$ copies/ml in CSF with a median plasma : CSF ratio (pVL : cVL; $\log_{10}$ copies plasma HIV-1 RNA minus $\log_{10}$ copies CSF HIV-1 RNA) of 1.71 $\log_{10}$ copies/ml (range, 1.09–3.04). Baseline CSF cell counts were normal in all, although the CSF total protein was elevated in four and the albumin ratios within the normal range in all but one (6005). Four patients were neurologically normal (ADC Stage = 0),

Table 1. Baseline CD4+ cell counts, AIDS dementia complex (ADC) staging, cerebrospinal fluid (CSF) findings, viral loads and antiretroviral therapies of patients.

Patient ID	Age years	CD4+ count (nadir) cells $\times 10^6$/L	QNPZ-4	ADC Stage	Baseline CSF[a] WBC cells $\times 10^6$/L	Baseline CSF[a] Protein mf/dl	Alb. Ratio	Baseline Viral DNA Plasma	Baseline Viral DNA CSF	Ratio[b]	Antiretroviral therapies Stopped at baseline	Antiretroviral therapies Other previous exposures
								$\log_{10}$ copies/ml				
6001	38	282 (181)	ND	0	0	22	2.61	4.70	2.99	1.71	ritonavir, zidovudine/lamivudine (as combivir)	nelfinavir, saquinavir, zidovudine, didanosine, abacavir, nevirapine
6004	50	275 (110)	0.37	0	0	52	5.51	4.11	1.28	2.83	inidinavir, stavudine, lamivudine	saquinavir, zidovudine
6005	53	143 (14)	0.42	0	0	66	8.88	4.79	1.76	3.04	indinavir, stavudine, lamivudine	saquinavir, zidovudine
6006	39	196 (126)	−0.08	0	2	64	6.02	3.45	2.36	1.09	nelfinavir, adefovir, abacavir, ephavirenz	indinavir, zidovudine, lamivudine, stavudine
4026	52	164 (4)	−1.16	1	3	59	4.91	4.29	2.90	1.39	nelfinavir, stavudine, didanosine	indinavir, saquinavir, zidovudine, lamivudine, zalcitabine

[a]Normal values: CSF white blood cells (WBCs) < 5 cells $\times 10^6$/l; CSF protein ⩽ 45 mg/dl; Alb. Ratio (CSF albumin/serum or plasma albumin $\times 10^{-3}$) from literature approximately 4.6 ± 1.3 SD [18].

[b]The plasma: CSF ratio = $\log_{10}$ plasma HIV-1 RNA copies/ml − $\log_{10}$ CSF HIV-1 RNA copies/ml.

whereas one was diagnosed as ADC Stage 1 (4026). QNPZ-4 scores substantiated this classification in the four in which formal testing was performed; the three normal individuals scored near or above 0.0 whereas patient 4026 exhibited impaired performance with a QNPZ-4 score of -1.16. One additional individual (designated 6002) was studied under the STI CSF protocol. As he stopped only one medication, indinavir, but continued treatment with zidovudine and lamivudine, the details of his results are omitted from this report. He experienced minimal change in his plasma viral load (baseline 10 265 copies/ml HIV-1 RNA), and his CSF HIV-1 remained below or at the limit of detection and hence too low to consistently analyze by either phenotypic or genotypic methods. He also exhibited normal CSF cell counts throughout 12 weeks of STI.

Changes in HIV-1 RNA

Figure 1 shows the changes in plasma and CSF HIV-1 RNA concentrations during the period of STI. Patient 6001 exhibited little change in either plasma or CSF viral loads, and his pVL : cVL remained constant (median difference of 1.65 $\log_{10}$ copies/ml between the two fluids, with a range of 1.58 to 1.71). In the remaining four patients, increases in the plasma HIV-1 viral load were variable, but in each case the CSF HIV-1 RNA concentrations rose more rapidly and to a proportionally greater extent. This resulted in a ten-fold reduction of the pVL : cVL, from a median baseline value of 2.11 $\log_{10}$ copies/ml (range, 1.09–3.04) to 1.16 $\log_{10}$ copies/ml (range, 0.13–1.79) at the last sampling point. In three cases there appeared to be re-divergence of these ratios toward the end of the study interval, away from a point of minimal difference (the median minimal pVL : cVL for the four patients was 0.29 $\log_{10}$ copies/ml (range, 0.03–1.58).

CSF cell counts and neurological status

Three patients (6004, 6005 and 4026) with increases in CSF viral loads also developed substantial CSF lymphocytic pleocytosis, with peak WBC counts of 46, 46 and 31×10^6 cells/l, respectively (> 90% lymphocytes) (Fig. 1). Small changes in the CSF cell counts in the other two patients occurred at the peak of their CSF viral loads, raising the possibility that subthreshold increases in lymphocytes (from below the linear range of routine CSF cell counts) might also have developed in them. CSF cell and viral changes were entirely asymptomatic; none of the participants experienced headache, stiff neck or other neurological symptoms. Likewise, examinations remained unchanged, and in the four who underwent serial QNPZ-4 testing, there was no decline in performance (not shown). Both CSF total protein (mean increase 10.4 mg/dl, 2.9 mg/dl SD) and the CSF : serum albumin ratio (mean increase 0.78, SD 0.27) rose during the STI period.

Antiviral drug susceptibility

Phenotypic susceptibility profiles for the drugs in each patient's pre-STI treatment regimen are plotted in Figure 1. Baseline drug susceptibility varied among individuals: patient 6001 had nearly 100-fold decreases in susceptibility to all three drugs in his pre-STI regimen; 6004 and 6005 had decreased susceptibility (> 10-fold resistance) to two of three drugs in their regimens; and 6006 and 4026 had decreased susceptibility to only one of their prescribed drugs. In only one specimen (baseline CSF of 6005) was the HIV-1 concentration too low to amplify for phenotypic testing. Susceptibility profiles were nearly identical in paired CSF and plasma samples at baseline and throughout the course of STI. Fold-changes in IC_{50} values were within 2.5-fold of each other for 98.7% of the 315 paired CSF-plasma sample comparisons carried out in the course of evaluating susceptibilities to 15 drugs at each time interval for the five patients.

Following treatment interruption, CSF and plasma viruses changed from drug-resistant to more drug-susceptible phenotypes in all five patients. These changes in drug susceptibility occurred simultaneously in CSF and plasma after an initial delay following treatment interruption. Moreover, where major reversions in susceptibility to more than one drug developed, these also occurred during the same time interval. The mean time of change, estimated by using the half-way point within the assessed interval of the change, was 61.2 (± 26.2, SD) days after interruption. The increases in CSF viral load occurred before or during this reversion interval. After reversion to a more drug-susceptible phenotype, CSF viral loads either leveled or decreased (resulting in the previously discussed late widening of the pVL : cVL). Patient 6001 was the only participant with high-level resistance to all three drugs in his regimen and experienced the smallest increase in viral load among the study patients.

The RT and PI-resistant genotypes (Table 2) not only were consistent with the phenotypic profiles for all five patients, but showed concordance in the mutations between the matched CSF and plasma samples. Multiple drug resistance mutations were present in the CSF and plasma at baseline in all patients. In four of five patients, multiple resistance mutations present at baseline disappeared simultaneously following treatment interruption, indicating replacement of the baseline drug-resistant virus with a drug-sensitive viral variant. Phylogenetic analysis of PI-resistant codons 4–99 and RT codons 41–250 indicated no evidence of viral compartmentalization between CSF and plasma in any of the individuals at any time point. Hence, both genotypic and phenotypic data indicate that the drug-resistant viral variants predominating at baseline first expanded and then were superseded in both CSF and plasma during treatment interruption.

Discussion

The eventual role of STI in the long-term management of HIV-1 infection remains to be defined. The findings in this preliminary study provide a unique view of virus exposure and altered lymphocyte traffic during STI within an anatomically-sequestered body compartment, the leptomeninges, as reflected in its fluid contents, the CSF. Four of five patients experienced a steeper and proportionally greater rise of the HIV-1 concentration in CSF than in plasma, with consequent reduction in the pVL : cVL, over the course of STI. The increase in HIV-1 RNA in both compartments following treatment interruption suggests that despite 'virological failure' as judged by blood analysis, the prior treatment was indeed having a salutary effect. Plasma viral load was partially suppressed, and there was an even greater suppressive effect on infection in the CSF. In all five participants, the virus populations in both compart-

Fig. 1. Results of HIV-1 RNA and phenotypic antiretroviral drug resistance. Each panel shows data from one study patient. Virological and cerebrospinal fluid (CSF) cell count results are plotted in the upper (larger) graphs and phenotypic drug susceptibility data are presented in the lower (and shorter) graphs within each outlined panel. Plasma (open blue squares and broken line) and CSF (solid red circles and solid line) HIV-1 RNA concentrations (left *y*-axis) and CSF WBC counts (green open triangles/broken line and right *y*-axis) are shown for the five participants. The limits of detection of viral RNA are depicted by the thin black horizontal broken line in these graphs (bDNA for patient 6001 and polymerase chain reaction for the others). The lower graphs plot the fold change in the IC_{50} concentrations (i.e. drug resistance) to the baseline drugs each patient was taking prior to treatment interruption. CSF values are represented by closed circles and solid lines, whereas the matching plasma values are represented by open squares and dashed lines in paler shades of the same colors. The broken horizontal line indicates the susceptible strain reference value (unity). In patient 6006, adefovir is omitted from the figure, but values were near 1.0 for all points in both fluids. 3TC, lamivudine; ZDV, zidovudine; d4T, stavudine; ddI, didanosine; ABC, abacavir; EFV, efavirenz; RTV, ritonavir; IDV, indinavir; NFV, nelfinavir. For two of the drugs, maximum drug resistance values were obtained in more than one patient (values were above the maximum IC_{50} concentration that is measurable in the assay) and plotted as such: 3TC, 300-fold; EFV, 320-fold.

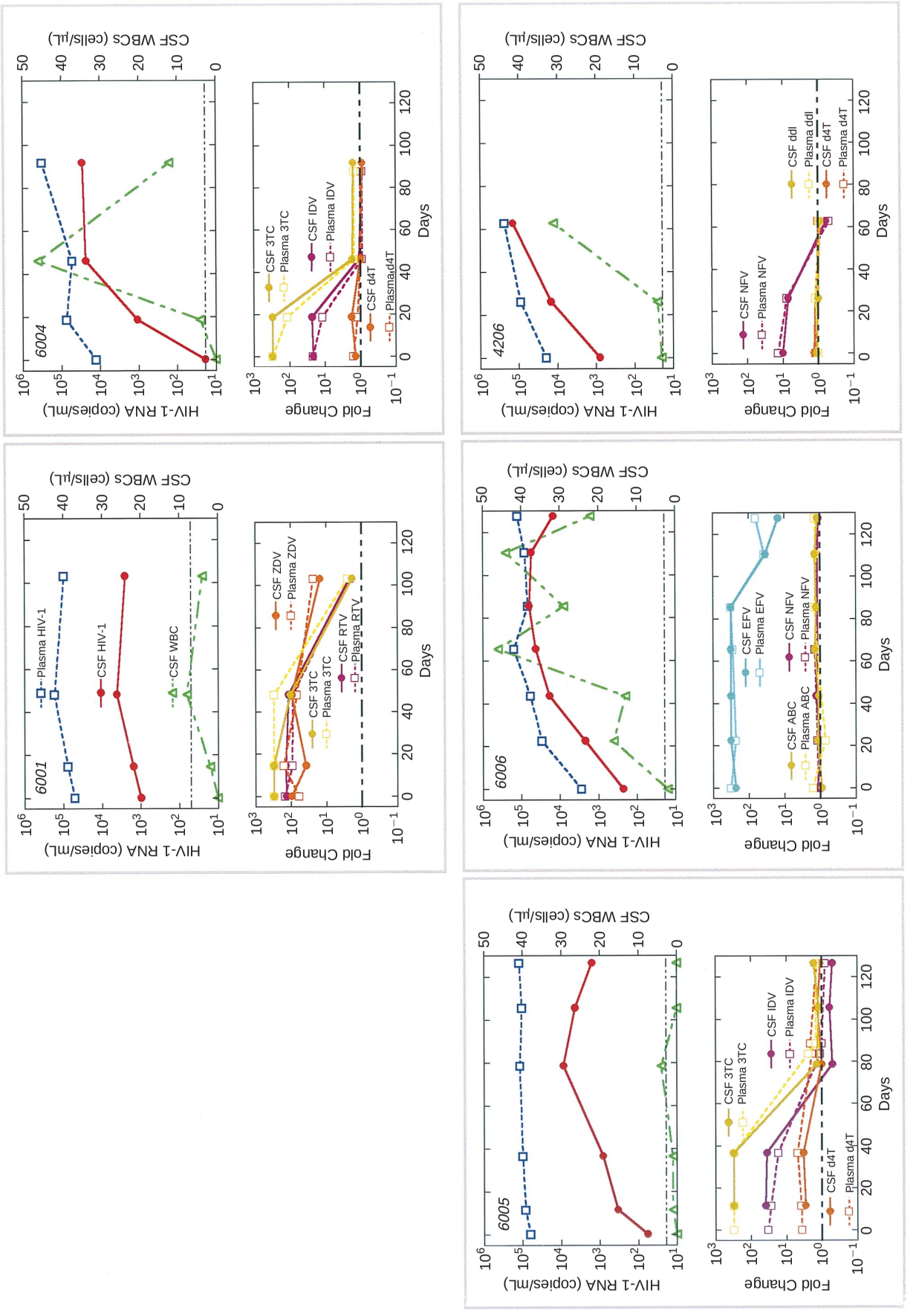
6004
HIV-1 RNA (copies/mL)
CSF WBCs (cells/μL)
Fold Change
Days
CSF 3TC
Plasma 3TC
CSF IDV
Plasma IDV
CSF d4T
Plasma d4T
4206
CSF NFV
Plasma NFV
CSF ddI
Plasma ddI
6001
Plasma HIV-1
CSF HIV-1
CSF WBC
CSF ZDV
Plasma ZDV
CSF RTV
Plasma RTV
6006
CSF ABC
Plasma ABC
CSF EFV
Plasma EFV
6005

Table 2. HIV-1 genotypic changes in plasma and cerebrospinal fluid (CSF) samples during structured antiretroviral treatment interruption (STI) in the five patients in which resistance genotyping was performed. The table includes changes at all sites where mutations known to influence resistance in the protease inhibitor (PI)-resistant and reverse transcriptase (RT) genes were noted in the group. Where genotyping was not possible the line is blank. Consensus wild-type sequences are listed with sequence numbers. Mutations are indicated by letter substitutions and concordance with consensus is noted by a dash. Functional interpretations are listed on the right.

		Plasma																														
		PR															RT																
Codon:																																
Wildtype sequences:		10	20	33	36	46	48	54	63	71	73	77	82	84	90		41	67	69	70	98	103	108	181	184	2008	210	215	219				
Subject	nterval (days)	L	K	L	M	M	G	I	L	A	G	V	V	I	L		M	D	T	K	A	K	V	Y	M	H	L	T	K				
6001	0	I	-	L/F	-	-	-	V	P	V	-	I	A	-	M		L	-	SSG	-	-	-	-	-	V	-	-	Y	-				
	14	I	-	L/F	-	-	-	V	P	V	-	I	A	-	M		L	-	SSG	-	-	-	-	-	V	-	-	Y	-				
	48	-	-	-	-	-	-	V	P	V	-	I	A	-	M		L	-	SSG	-	-	-	-	-	V	-	-	Y	-				
	103	-	-	-	-	-	-	-	P	-	-	I	-	-	-		L	-	-	-	-	-	-	-	-	-	-	Y	-				
6004	0	I	-	-	-	-	-	V	-	V	-	-	A	-	-	M/L		-	-	-	-	-	-	-	V	-	W	Y	-				
	19	I	-	-	-	-	-	I/W	-	V	-	-	A	-	-		L	-	-	-	-	-	-	-	V	-	W	Y	-				
	46	-	-	-	I	-	-	-	-	-	-	-	-	-	-		L	-	-	-	-	-	-	-	-	-	W	D	-				
	92	-	-	-	I	-	-	-	-	-	-	-	-	-	-		L	-	-	-	-	-	-	-	-	-	W	D	-				
	105	-	-	-	I	-	-	-	-	-	-	-	-	-	-		L	-	-	-	-	-	-	-	-	-	W	D	-				
6005	0	I	I	-	-	-	-	-	P	V	S	I	-	V	M		L	N	-	K/	-	-	-	-	V	Y	W	Y	-				
	11	I	I	-	-	-	-	-	P	V	S	I	-	V	M		L	N	-	K/	-	-	-	-	V	Y	W	Y	-				
	36	I	M	-	-	I/M	-	-	P	V	S	I	-	V	M		L	N/D	-	K/	-	-	-	-	V	-	W	Y	-				
	78	-	R	-	-	-	-	-	P	-	-	-	-	-	-		-	N	-	-	-	-	-	-	-	-	W	Y	-				
	105	-	R	-	-	-	-	-	P	-	-	-	-	-	-		L	N	-	-	-	-	-	-	-	-	W/	Y*	-				
	126	-	R	-	-	-	-	-	P	-	-	-	-	-	-		L	N	-	-	-	-	-	-	-	-	W/	Y*	-				
6006	0	-	-	-	-	-	-	-	-	-	-	I	-	-	-		-	-	-	-	-	N	I/W	-	-	-	-	-	-				
	22	-	-	-	-	-	-	-	-	-	-	I	-	-	-		-	-	-	-	-	N	I/W	-	-	-	-	-	-				
	43	-	-	-	-	-	-	-	-	-	-	I	-	-	-		-	-	-	-	-	N	I/W	-	-	-	-	-	-				
	65	-	-	-	-	-	-	-	-	-	-	I	-	-	-		-	-	-	-	-	N	-	-	-	-	-	-	-				
	85	-	-	-	-	-	-	-	-	-	-	I	-	-	-		-	-	-	-	-	N	-	-	-	-	-	-	-				
	110	-	-	-	-	-	-	-	-	-	-	-	I	-	-		-	-	-	-	-	-	K/N	-	-	-	-	-	-				
4026	0	I	-	-	-	-	V	V	-	-	-	I/V	A	-	-		-	-	-	R	-	-	-	-	-	-	-	-	Q				
	26	-	-	-	-	V	V	-	-	-	I/V	A	-	-		-	-	-	K/R	-	-	-	-	-	-	K/Q							
	63	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	K/R	-	-	-	-	-	-	-	-	K/Q				

Table 2. continued

CSF																											
PR															RT												
10 L	20 K	33 L	36 M	46 M	48 G	54 I	63 L	71 A	73 G	77 V	82 V	84 I	90 L	41 M	67 D	69 T	70 K	98 A	103 K	108 V	181 Y	184 M	208 H	210 L	215 T	219 K	Interpretation (Predicted resistence)
I/F	-	L/F	-	-	-	V	P	V	-	I	A	-	M	L	-	SSG	-	-	-	-	-	V	-	-	Y	-	PI^r, nRTI^r
I	-	L/F	_	-	-	V	P	V	-	I	A	-	M	L	-	SSG	-	-	-	-	-	V	-	-	Y	-	PI^r, nRTI^r
-	-	-	-	-	-	V	P	V	-	I	A	-	M	L	-	SSG	-	-	-	-	-	V	-	-	Y	-	PI^r, nRTI^r
-	-	-	-	-	-	-	P	T/A	-	I	-	-	-	L	-	-	-	-	-	-	-	-	-	-	Y	-	ZDC^r
																											PI^r, SDV^r, 3TC^r
I	-	-	-	-	-	V	-	V	-	-	A	-	-	L	-	-	-	-	-	-	-	V	-	W	Y	-	PI^r, ZDV^r, 3TC^r
-	-	-	I	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	-	-	-	W	D	-	possible ZDV^r
-	-	-	I	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	-	-	-	W	D	-	possible ZDV^r
-	-	-	I	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	-	-	-	W	D	-	possible ZDV^r
																											PI^r, ZDC^r, 3TC^r
																											PI^r, ZDC^r, 3TC^r
I	I/V	-	-	I/M	-	-	P	V	S	I	-	V	M	L	N	-	K/	-	-	-	-	V	-	W	Y	-	PI^r, ZDV^r, 3TC^r
-	R	-	-	-	-	-	P	-	-	-	-	-	-	L	N	-	-	-	-	-	-	-	-	W	Y	-	ZDV^r
-	R	-	-	-	-	-	P	-	-	-	-	-	-	L	N	-	-	-	-	-	-	-	-	W	Y*	-	ZDV^r
-	R	-	-	-	-	-	P	-	-	-	-	-	-	L	N	-	-	-	-	-	-	-	-	W/	Y*	-	ZDV^r
-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	N	I/V	-	-	-	-	-	-	nnRTI^r
-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	N	I/V	-	-	-	-	-	-	nnRTI^r
-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	N	I/V	-	-	-	-	-	-	nnRTI^r
-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	N	-	-	-	-	-	-	-	nnRTI^r
-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	N	-	-	-	-	-	-	-	nnRTI^r
-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	K/N	-	-	-	-	-	-	-	possible nnRTI^r
I	-	-	-	-	V	V	-	-	-	I	A/V	-	-	-	-	-	R	-	-	-	-	-	-	-	-	Q	PI^r, ZDV^r
I	-	-	-	-	V	V	-	-	-	I/V	A/V	-	-	-	-	-	R	-	-	-	-	-	-	-	-	Q	PI^r, ZDV^r
I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	K/R	-	-	-	-	-	-	-	-	Q	ZDV^r

PR^r, PI resistant; nRTI^r, nucleoside RT resistant; mRT^r, non-nucleoside RT resistant; ZDV, zidovudine.

ments underwent simultaneous reduction of resistance following interruption of therapy, probably reflecting overgrowth by more fit, drug-sensitive HIV-1.

A striking lymphocytic pleocytosis, with peak WBC counts between 31 and 46×10^6 cells/l, accompanied the increases in CSF HIV-1 RNA in three patients. This provides further evidence of a biological effect of partially suppressive treatment in the face of drug resistance; in this case the suppression of lymphocyte traffic into or proliferation within the leptomeninges. The development of overt pleocytosis in these three patients and the suggestion of small increases in WBCs in the other two accompanying the rise in CSF viral load, also provides further evidence linking CSF lymphocytosis with higher CSF viral loads and smaller plasma : CSF ratios. This relationship has also been noted in cross-sectional observations [21,22] and agrees with our own broad experience. Additionally, we have found that in viremic patients with CSF pleocytosis not attributable to other causes, the elevated cell counts resolve following initiation of HAART [16]. The current study provides a complementary observation – cessation of HAART led to an increase in CSF cells and virus. The concomitant increases in CSF protein and CSF : serum albumin ratios also show that STI was accompanied by alteration of the blood–brain or blood–CSF barrier to protein.

Although the disproportionate rise in CSF viral load is probably linked to the increase in CSF lymphocytes, the nature of the interaction is uncertain. Two hypotheses can be suggested to explain the increase in CSF lymphocytes and its relationship to the CSF viral load. A *push* hypothesis proposes that resurgent systemic infection (even if not clearly evident from plasma sampling) augments lymphocyte activation, leading to non-antigen-specific entry of lymphocytes (including CD4+ T lymphocytes) into tissues, including the nervous system [23]. As some of these cells are infected, they can release virus into the CSF. Specifically, this hypothesis proposes that lymphocyte influx, containing virus-infected cells, causes the increase in CSF HIV-1. The recent observation of increased numbers of activated lymphocytes in the lymph nodes of patients undergoing STI [24] supports this hypothesis, and the pleocytosis might be viewed teleologically as a host effort to defend extra-vascular tissue compartments as CD8+ T-lymphocytes participate in this pleocytosis [25].

Conversely, a *pull* hypothesis proposes that interruption of treatment-related suppression of local nervous system infection and subsequent increase in the viral burden, recruits lymphocytes into the CSF. In this second scenario, the influx of lymphocytes is a secondary response to local infection, and does not contribute to elevated HIV-1 RNA in the CSF. Possibly both of these mechanisms contribute and, indeed, reinforce one another.

In all five participants, phenotypic drug susceptibility profiles were highly concordant between CSF and plasma samples obtained at the same time throughout the course of the study. When drug susceptibility was restored, it occurred in parallel in both fluids. Moreover, genotypic analysis showed that the similar phenotypes within individuals were associated with identical mutation patterns. These observations strongly suggest ongoing interchange between blood and CSF rather than independent evolution of virus strains in the two compartments in these patients. Thus, the predominating type of CSF infection in this setting is probably the transitory rather than more autonomous type [16,26]. The high CSF lymphocyte counts probably facilitate this rapid interchange between blood and CSF. Infection in this setting may differ from that predominating in patients with low blood CD4+ cells and chronic CSF infection in whom slower CSF than plasma responses to therapy suggest the predominance of more autonomous infection.

Earlier in the epidemic, development of meningoencephalitis was reported in some patients upon stopping zidovudine monotherapy. It is possible that these early observations provide precedent for the findings in our patients, although in the most detailed report of this phenomenon, Helbert and colleagues described recrudescence or exacerbation of ADC without pleocytosis after patients stopped zidovudine monotherapy [27]. None of the individuals in the current study developed symptoms or signs of meningitis, or indeed any other changes in neurological status as assessed by neurological history, physical examination and QNPZ-4 scores. This may imply that the perturbations in virus and lymphocyte exchange between blood and CSF in this particular setting are without clinical consequences, and therefore clinically benign. However, this study included only a small number of patients who were not severely immunosuppressed and who were, with one exception, neurologically normal, and the period of STI and subsequent follow-up may have been too short to witness development of more serious sequelae. Hence, the potential neurological implications of resurgence of virus and associated inflammation in the CSF, or in parallel within the brain, remain uncertain, and it is possible that neurological impairment would be more likely in individuals with active ADC or who harbour different, more 'neuropathic' viral strains. The lack of brain or meningeal disturbance in our patients who experienced CSF viral recrudescence and pleocytosis may be due to the character of their viruses, to limited macrophage infection or activation, or to the absence of neurotoxic cytokine production in this setting [28]. The benign clinical course in our patients underscores the fact that development of CSF HIV-1 infection does

not, in isolation, predict development of HIV-related neurologic disorders, including ADC.

If suppression of local infection can be achieved in the brain, as in the CSF, HAART may have substantial therapeutic benefit for ADC despite continued viremia. This benefit may relate to compartment-specific suppression of HIV-1 replication, which may be due to tissue-specific differences in drug efficacy, altered tropism or fitness of drug-resistant viruses, or other undefined factors.

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