

A Case of Apparent Recent Infection with a Multi-Drug-Resistant and Dual-Tropic HIV-1 in Association with Rapid Progression to AIDS.

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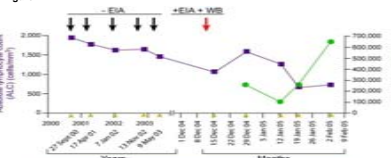
SUMMARY

We report a case of apparent recent HIV-1 infection by a viral variant that is resistant to multiple classes of antiretroviral drugs, including nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitors (PI). In addition, this virus is dual-tropic for cells expressing CCR5 or CXCR4 coreceptor. This infection has resulted in progression to symptomatic AIDS with documented CD4+ T-cells of 230 cells/mm³ over a course of 4 to, at most, 20 months. The intersection of multi-drug resistance and rapid development of AIDS in this patient is worrisome, particularly in light of a case history that includes extensive high-risk sexual contacts and methamphetamine use.

CASE HISTORY

The patient is a man in his late forties who has sex with men. He had repeatedly tested negative for HIV-1 antibodies between September 2000 and May 2003 (Figure 1). His absolute lymphocyte counts throughout this period were within normal limits (Figure 1). In early November 2004, the patient developed the onset of fever, pharyngitis, weakness, and fatigue of approximately one week in duration. These symptoms abated, but intractable nose throat, fatigue and malaise recurred, prompting a visit to his private physician in mid-December 2004, when he was found to be HIV-1 antibody positive. On his follow-up visit in late December 2004, his CD4+ T-cell count was 80 cells/mm³, CD8+ T-cell count was 1012 cells/mm³ and the level of HIV-1 RNA in plasma was 280,000 copies/ml. He was then referred to the Aaron Diamond AIDS Research Center for evaluation as a possible case of recent HIV-1 infection. When he was seen in mid-January 2005, laboratory evaluation confirmed the positive HIV-1 serology, and a detailed enzyme immunoassay was positive, indicating that his infection is likely beyond the acute or primary phase. A number of viral load and CD4/CD8 T-cell measurements were taken serially, and the results are summarized in Figure 1. Collectively, the results suggested that his man has already progressed to symptomatic AIDS with profound CD4+ T-cell depletion. By early February 2005, the patient had lost an additional 4 kilograms of body weight. A more detailed history was pursued given the dramatic course seen in this patient. He reported that he had been sexually active with countless male partners over the years, often in conjunction with methamphetamine use. In particular, he believed he was infected while having risky sex with multiple partners, including both insertive and receptive and intercourse without the use of condoms, in the third week of October 2004. He has stopped methamphetamine use since November 2004, but continued to have sex with approximately 10 partners until the end of December 2004, when sexual activities ceased due to deteriorating health.

Figure 1



DRUG RESISTANCE

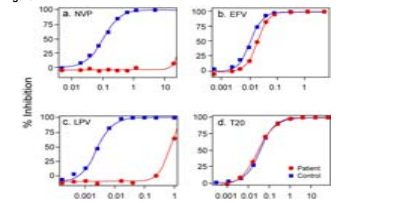
His HIV-1 was examined for susceptibility to antiretroviral drugs. Sequencing of the viral pol gene and the gag1 envelope open reading frame were performed on a plasma sample from mid-January 2005. These genotyping results, summarized in Table 1, revealed broad resistance to NRTI, NNRTI, and PI. The genotype was confirmed by further testing carried out at ViroLogic, with one notable difference: the detection of a mixture of M184V/I in reverse transcriptase (RT). We interpret the collection of mutations to confer resistance to thymidine analogues, lamivudine and emtricitabine, reduced susceptibility to abacavir and tenofovir, high-level resistance to nevirapine, possibly an attenuated response to efavirenz, and broad resistance to protease inhibitors.

A phenotyping assay for drug susceptibility (PhenoSense, ViroLogic) was also performed. Susceptibility (fold change in IC50) of the patient's virus was comparable to a drug-susceptible reference virus for a number of drugs: abacavir 0.81, didanosine 0.82, stavudine 0.98, zidovudine 0.50, and tenofovir 0.38. Low levels of reduced susceptibility to lamivudine and emtricitabine, 3.27 and 3.83 fold, respectively, were observed. Superficially, these findings suggest little evidence of drug resistance to these agents. However, given the presence of mixtures of viral species detected at amino-acid positions 184, 210, and 215 in RT (Table 1), all well-established resistance-conferring substitutions for NRTI, a discordance between the genotype and phenotypic results is expected. The minor drug-resistant HIV-1 species may not have been "scored" in the phenotypic assay, although they surely will be important in vivo when drugs are administered. Additionally, a replication assay showed that this virus was highly resistant to nevirapine and all commercially available protease inhibitors (examples shown in Figure 2). The virus tested sensitive to two NNRTI, efavirenz and delamanid, and to efavirenz, an inhibitor that blocks HIV-1 entry into cells (Figure 2). Of note, the replication capacity of this patient's HIV-1 as determined in a modified PhenoSense assay was measured at 136%, compared to a median value of 100% derived from a large number of wild-type viruses. This finding indicates that, as measured in an *in vitro* assay, this multi-drug resistant virus replicates as well as most wild-type drug-susceptible viruses.

Table 1
Resistance Genotype

RTI	NNRTI	PI
M41L	K101E	L10I
DE7/DN	Y181I	L33F
V118I		E34Q
M184V/I		M46I
L210L/G/M/R/W		E44M
T215C/Y		L63P
K219E		G71V
		V77I
		I84V
		L89V
		L90M

Figure 2



CHARACTERIZATION OF CORECEPTOR USAGE

HIV-1 was isolated from this patient's peripheral blood mononuclear cells using a standard protocol. Although normal kinetic analyses are yet to be done, it is already clear that this virus replicates to high titers, and readily forms syncytia in both MT-2 cells and normal-donor PBMC (Figure 3a, b). Formation of the giant cells in a MT-2 culture strongly indicates that the presence of X4 viruses (3). Coreceptor usage was determined using a PhenoSense tropism assay. RT-PCR was performed to amplify the envelope gene from this patient's plasma virus, which is then used to generate pseudoviruses for analysis of viral tropism in U87 cells expressing either CCR5 or CXCR4. Data shown in Figure 3c demonstrates that the viral quasiparticles in this patient are able to infect cells using both CCR5 and CXCR4. Based on this assay, we do not know whether we have a mixture of RS and X4 viruses or dual-tropic population that uses both CCR5 and CXCR4. Nevertheless, the swarm of viruses in this patient is, collectively, dual-tropic.

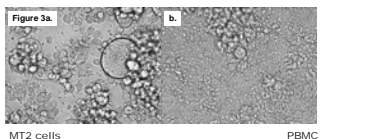
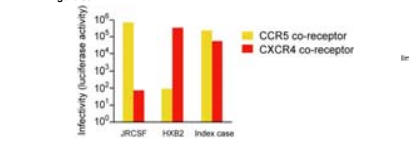


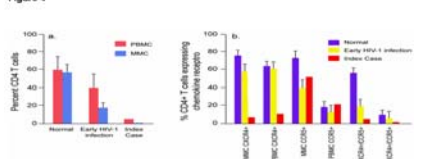
Figure 3c



FLOW CYTOMETRIC ANALYSES OF PBMC AND MUCOSAL MONONUCLEAR CELLS (MMC)

In early February 2004, the patient underwent flexible sigmoidoscopy with biopsy to assess the impact of his infection on the MMC population as compared to PBMC. Both MMC and PBMC were stained by select monoclonal antibodies and analyzed by flow cytometry. His results, in relation to previously published data on uninfected (N=10) and newly infected (N=19) individuals, are summarized in Figure 4. Panel a shows CD4+ T-cell depletion in the index case to be severe in both PBMC (5%) and MMC (1%), and much more significant than results seen in other newly infected patients. Panel b further demonstrates that the depletion is most prominent in subpopulations of cells that express either CXCR4 alone or CXCR4 in conjunction with CCR5. Taken together, these results document the marked depletion of CD4+ T-cells in his gastrointestinal tract as well as in blood. Moreover, the profound loss of CXCR4+ T-cells from blood and gut is not only striking, but also suggestive of the functional dominance of X4 viral variants *in vivo* in this case.

Figure 4

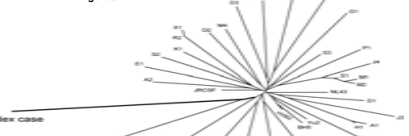


VIRAL SEQUENCE ANALYSIS

We next characterized the genomic diversity of HIV-1 in this case. DNA extracted from his PBMC was subject to limiting-dilution PCR to amplify a region of gag p17 (nucleotides 814 to 1279) and env V3 (nucleotides 702-7336). Ten clones for each DNA fragment were sequenced. The virus in this case belongs to subtype B. The average intra-sample diversity for the p17 sequences was quite small (0.4%), and slightly higher for the V3 sequences (1.7%). The observed relative frequency of the viral population is consistent with early HIV-1 infection. However, it is difficult to use this sort of genetic data to determine when this patient became infected by the virus.

A nucleotide sequence from the viral pol gene of the patient was subjected to phylogenetic analysis, together with sequences derived from 30 newly infected individuals identified in 2004 and five reference HIV-1 strains. Figure 5 shows that this viral sequence is indeed unique, thus eliminating the possibility of contamination. In fact a search of our entire sequence database did not yield a match. Given its unique features, this pol sequence is now being compared to those in the Database at the Los Alamos National Laboratory and in various commercial laboratories, with the hope of finding a closely related HIV-1 that might provide an epidemiological linkage to this case.

Figure 5



Rapid progression to AIDS following acute HIV-1 infection has been described previously, as has the transmission of multi-drug-resistant viruses. The unique feature in this case is the convergence of the two phenomena: the transmission of a remarkably multi-drug-resistant HIV-1 variant and the extremely rapid clinical course to AIDS.

It is uncontroversial that the duration of infection in this case cannot be longer than 20 months given his five negative HIV-1 antibody tests and normal absolute lymphocyte counts in the period prior to May 2003. It is possible that the transient febrile illness in early November 2004 was the manifestation of his primary HIV-1 infection, occurring approximately two weeks after a series of high-risk sexual contacts with multiple partners. If this were the case, then the duration of his infection would be about 4-5 months. That the detected antibody test was positive is in line with an infection beyond the acute phase. Likewise, the relative sequence homogeneity in gag p17 and env gp120 V3 is consistent with, although not diagnostic of, recent infection. Thus, the totality of the evidence allows us to confidently say that this man has been infected for as little as 4 and not more than 20 months.

This patient has been symptomatic with severe fatigue and weight loss, and his CD4+ T-cell counts have been consistently below 80/mm³. He would therefore be classified as an AIDS patient. But his rate of deterioration over 4-20 months considered remarkable? A couple of comparisons are quite revealing. First, an analysis of the data generated on acute seroconvertors in the Multicenter AIDS Cohort Study suggests that likelihood of progression to AIDS in 6 and 12 months to be 71/1000 and 4510/1000, respectively (A. Munoz, personal communication). Thus, by comparison, the index case would be in the top 0.5-percentile in terms of rapidity of disease. Second, an initial analysis of the database in the NIH Acute Infection and Early Disease Research Program revealed only 6 of 1709 cases with persistently low CD4 cell counts in early infection. Again, this comparison highlights our case as exceptional.

Could the rapid clinical course be explained by the properties of the patient's unique HIV-1 variant? It is well documented that the presence of X4 variants of HIV-1 is associated with a more aggressive clinical course. This patient clearly has an X4 virus based on the 5% phenotype in MT-2 cells, and phenotypic studies unequivocally show viral tropism for both CCR5 and CXCR4. Despite the multitude of drug-resistance mutations, his virus grows well *in vitro*, and an assessment in the PhenoSense assay shows a replication capacity of 136% compared with wild-type viruses. These *in vitro* characteristics, together with the profound depletion of CXCR4+ T-cell populations *in vivo* raise the specter that this might be a particularly aggressive HIV-1. That said, we are still unable to discount host factors that could have contributed to the clinical course.

Treatment options for this patient are limited. His virus is resistant to all PI and to nevirapine. It is sensitive to efavirenz, and phenotypic test results indicate that it is sensitive to efavirenz despite the marked resistance to nevirapine. What about the profile for NNRTI? A potential discordant genotype and phenotype results merit some discussion. The phenotype data for NRTI show susceptibility to a number of drugs. However, viral mixtures with amino-acid substitutions at positions 184 (conferring resistance to lamivudine and emtricitabine), and with TAMs at 210 and 215 (conferring resistance to abacavir and thymidine analogues) suggests that most NRTI are unlikely to be effective *in vivo*. Furthermore, the presence of M41L together with mixtures reflected at positions 210 and 219 in RT predicts an attenuated response to tenofovir. It is therefore our interpretation that this case cannot be readily treated with a standard antiretroviral regimen. Efavirenz and efavirenz are the only two antiretroviral drugs that can provide full activity against the virus in this patient. Given his low CD4+ T-cell counts and high viral load, a multi-drug regimen, including efavirenz and efavirenz, has been initiated.

Another worrisome feature of this case is this man's history of a large number of high-risk sexual contacts and methamphetamine use. It was the convergence of his sexual history with multi-drug resistance and rapid progression led us to bring this case to the special attention of the New York City Department of Health and Mental Hygiene, which in turn issued a health alert to physicians in the area on 11 February 2005, in which the clinical course of this patient's infection was discussed. Only additional investigations will reveal whether this is an isolated case or not. Irrespective of the outcome, the public health implications of this single patient should not be minimized. Interfection of HIV-1 prevention efforts must be reinforced, with particular emphasis on the epidemic that is being propelled by the use of methamphetamine. However, in so doing, care must be taken to avoid punitive measures against the populations most vulnerable to HIV-1.

DISCLOSURE: D.D. has been a paid advisor to ViroLogic since its inception in 1995, and has a brother who is an employee at the company.

Table 2
Host Genetics

HLA alleles	Class II	CCR5:
Class I:	DR*01:01	no evidence of D32 deletion
A*3002	DR*13:02/01	
A*3201		
B*58:01		
B*40:001		
CW*03:01		
DR*16:01		