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651

Abstract

Many patients with suppression of viremia on HAART occasionally have higher plasma virus measurements, or blips, following which virus levels fall back below 50 c/ml. There is evidence that patients who experience blips have higher levels of viral replication leading to concerns about the emergence of drug resistance. The nature of this intermittent viremia and the long-term consequences of blips on viral suppression have not been established. Here, we determine the frequency, magnitude, and duration of blips in 10 well suppressed patients on HAART by monitoring viral loads 3 times a week over a period of 3 months. The presence of drug resistance was determined by genotypic analysis at each time point. Blips were detected in 9 of the 10 patients, with a mean frequency of 0.67 blips/month (range 0–2) and a mean magnitude of 97.5 c/ml (range = 51–352). The mean duration of the blips detected was 131.3 hours. Genotypic analysis showed no new drug resistance mutations. Our results suggest that most, but not all, patients experience intermittent viremia in a setting of sustained suppression on HAART. Genotypic analysis both during and after the blip shows no emergence of new resistance, suggesting that these episodes of viremia above 50 c/ml do not necessarily result from partially resistant virus. In summary, patients may experience blips without evolution of drug resistance.

Introduction

Treatment of HIV-1 infection with HAART can reduce viremia to below 50 copies of HIV-1 RNA/ml. However, many patients on HAART occasionally have higher measurements, or blips, following which virus levels fall back below 50 c/ml. There is evidence that patients who experience blips have higher levels of viral replication leading to concerns about the emergence of drug resistance. The nature of this intermittent viremia and the long-term consequences of blips on viral suppression have not been established. Additionally, the management of HIV-1 infection could be substantially improved by determining what blips actually represent in terms of the dynamics of viral replication *in vivo*. Here, we examine the frequency, magnitude, and duration of blips in 10 well suppressed patients on HAART by monitoring viral loads 3 times a week over a period of 3 months. The presence of drug resistance during these blips was determined by genotypic analysis before, during, and after each blip.

Methods

Patient Population: We studied asymptomatic adults with HIV-1 infection who had excellent responses to HAART with suppression of viremia to below 50 copies/ml. Participants were on a stable HAART regimen for at least 6 months prior to study entry and remained on this regimen throughout the study period. After written informed consent, volunteers in our study donated small volumes of blood (17 ml) thrice weekly (Monday, Wednesday, Friday) for 12 weeks.

HIV-1 plasma RNA quantification: Viral RNA from the plasma of well suppressed patients on HAART was quantified using the ultrasensitive Roche Amplicor Monitor System Version 1.5 (detection limit of 50 c/ml) in two independent laboratories.

Sequence analysis and quality control: Genotypic analysis was carried out by nested RT-PCR amplification of regions of the protease and RT genes. Sequences were analyzed for known drug resistant mutations using the Los Alamos HIV Sequence Database of drug resistance mutations.

Results

Plasma viral RNA levels: Viral loads were measured in two independent, approved laboratories using the same assay with a detection limit of 50c/ml. A positive value above 50c/ml in either laboratory was considered a blip, although results typically did not correlate. Using this definition of a blip, a total of 20 blips were detected. Blips occurred in 9 out of the 10 patients. The mean frequency was 2 blips during the 3 month study period (range 0–6). The duration of 18 of the 20 blips detected was no more than 96 hours, while the other 2 blips lasted a maximum of 216 and 240 hours (Table 2). Due to variability in viral load results from independent labs we recalculated the blip dynamics using the following, more stringent definition of a blip: a detectable value corroborated in two different laboratories, or a value above 150c/ml (the upper bound for the standard error of the assay at its detection limit), or a detectable value at two consecutive time points. Using this new definition, a total of 4 blips were detected, occurring in 2 of the 10 patients. One patient had 2 blips with a maximum duration of 96 hours and the other patient had 2 blips of 216 and 240 hours (Table 3).

Results

Genotypic analysis: The reverse transcriptase region of the viral genome was successfully amplified from 3 milliliters of plasma and sequenced in 9 of the 10 patients. An average of 3 independent clones were obtained at baseline and at each of the 36 time points. Sequence analysis showed no evidence for the development of new drug resistance mutations. Virus obtained from one patient showed no drug resistance, while the other 8 patients retained both wildtype and 1–2 different drug resistant variants that were present at baseline. Patient 154 had the greatest number of positive viral load measurements. The only mutation observed (K103N) conferred NNRTI resistance and mostly likely arose during prior nonsuppressive therapy with a regimen containing efavirenz (Figure 2). The resistant variant was seen at baseline and at 3 other time points throughout the study. In all other patients, similar patterns were seen, with drug resistance being attributed to previous nonsuppressive regimens.

Table 1: Patient Characteristics

Patient ID	Age	Sex/Race	HAART
99	42	M/B	d4T, 3TC, TDF, LPV/r
113	42	M/B	ABC, NFV, 3TC
134	48	M/B	TDF, EFV, 3TC
135	49	F/W	AZT, 3TC, SQV/r
136	50	M/B	AZT, 3TC, EFV
139	47	F/B	EFV, APV, LPV/r
140	59	M/W	AZT, 3TC, NFV
147	43	M/W	TDF, EFV, SQV/r
148	39	F/B	ddI, 3TC, TDF, LPV/r
154	48	M/B	AZT/3TC/ABC, LPV/r, TDF

Table 2: Frequency, Magnitude, and Duration of Blips
(defined as any positive value >50c/ml)

N = 20 blips	Frequency (#/month)	Magnitude (RNA copies/ml)	Duration (max) (Hours)
Mean	0.67	97.5	131.3
Median	0.67	77.5	120
Range	0 – 2	51 – 352	48 – 168

Blips were observed in 9 out of the 10 patients

Table 3: Frequency, Magnitude, and Duration of Blips
 (defined as >50c/ml in 2 independent labs, >50c/ml on 2 consecutive time points, or magnitude >150c/ml)

N = 4 blips	Frequency (#/month)	Magnitude (RNA copies/ml)	Duration (max) (Hours)
Mean	0.13	191	162
Median	0	172.5	156
Range	0 – 0.67	67 – 352	96 – 240

Blips were observed in 2 out of the 10 patients

Patient Viral Loads

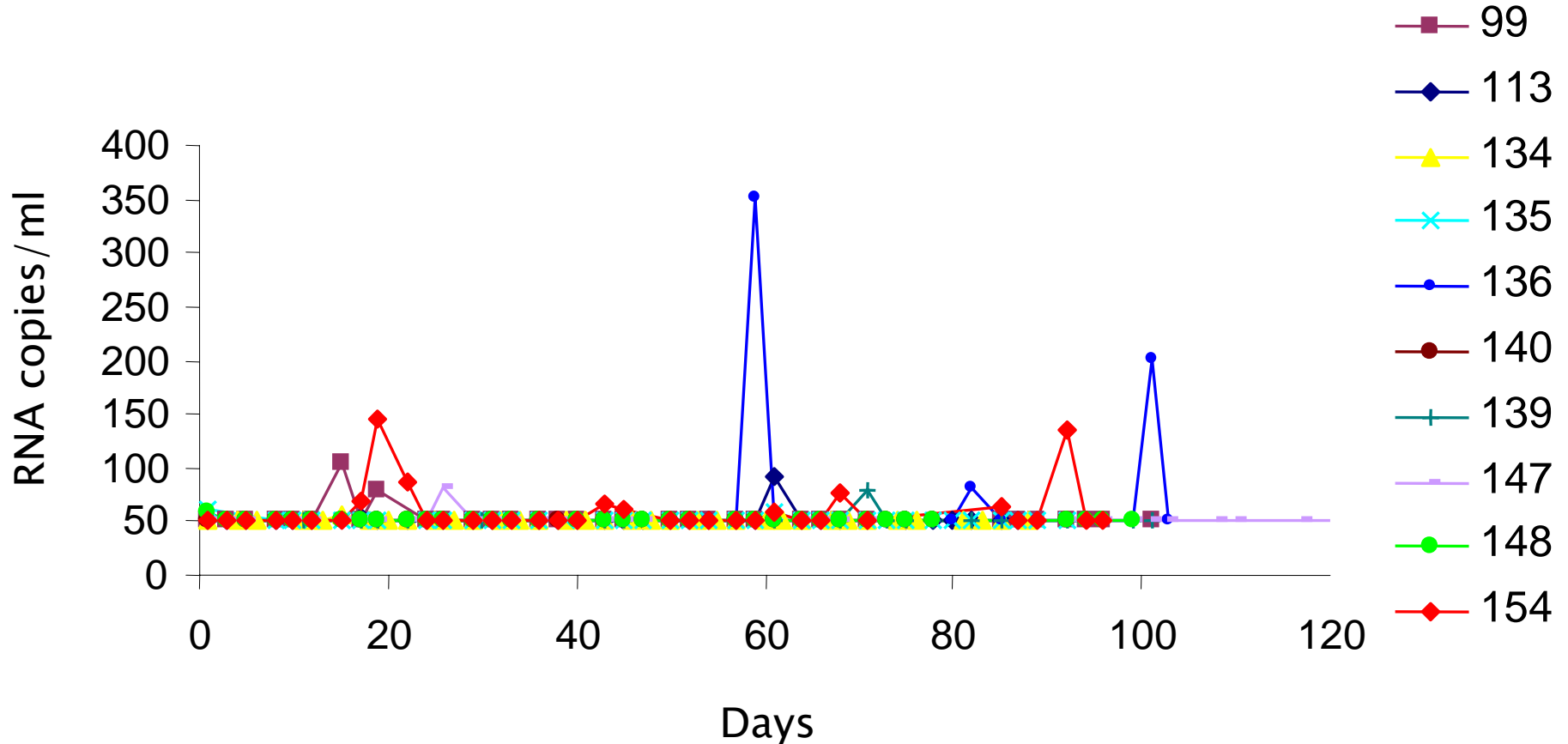
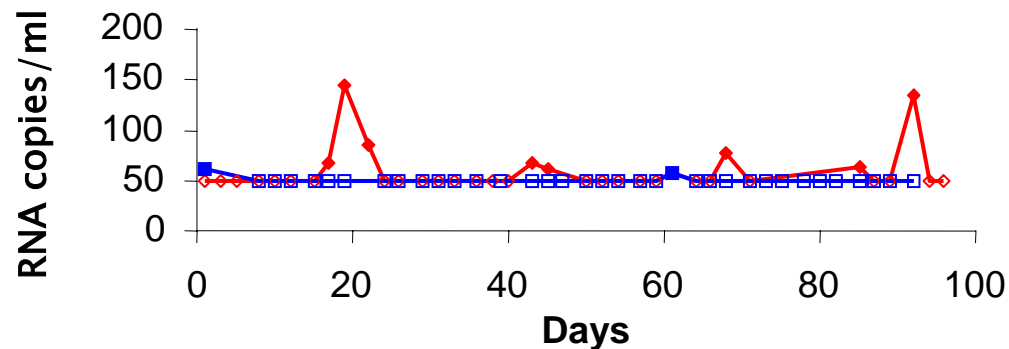


Figure 1: Plasma HIV-1 RNA levels sampled 36 times over 87–120 days in 10 well suppressed patients on HAART. The symbols at 50 copies/ml indicate a value below the detection limit of the assay.

Figure 2:



Patient	HAART	Previous ART	Genotypes
154	<div style="display: flex; flex-wrap: wrap; gap: 5px;"> <div style="background-color: red; color: white; padding: 2px;">AZT</div> <div style="background-color: blue; color: white; padding: 2px;">3TC</div> <div style="background-color: pink; color: white; padding: 2px;">TDF</div> <div style="background-color: yellow; color: black; padding: 2px;">LPV</div> <div style="background-color: purple; color: white; padding: 2px;">ABC</div> </div>	<div style="background-color: red; color: white; padding: 2px; display: inline-block;">AZT</div> 2 years <div style="background-color: green; color: white; padding: 2px; display: inline-block;">d4T</div> <div style="background-color: yellow; color: black; padding: 2px; display: inline-block;">ddl</div> <div style="background-color: orange; color: white; padding: 2px; display: inline-block;">EFV</div> 1 year	Wildtype : 172/177 = 97% K103N : 5/177 = 3%
135	<div style="display: flex; flex-wrap: wrap; gap: 5px;"> <div style="background-color: red; color: white; padding: 2px;">AZT</div> <div style="background-color: blue; color: white; padding: 2px;">3TC</div> <div style="background-color: green; color: white; padding: 2px;">RTV</div> <div style="background-color: purple; color: white; padding: 2px;">SQV</div> </div>	<div style="background-color: red; color: white; padding: 2px; display: inline-block;">AZT</div> 2 years <div style="background-color: green; color: white; padding: 2px; display: inline-block;">d4T</div> 1.5 years	Wildtype : 15/90 = 17% T215Y : 75/90 = 83%

Figure 2: Genotypic analysis of patient HIV *RT* sequences of viruses isolated from the plasma of representative patients (154, 135). Plasma HIV-1 RNA levels over time are shown, with open symbols representing values less than 50 copies/ml. Current and previous treatments are given by color coded bars and genotypes are expressed by percentage of wildtype and drug resistant variants observed in independent clones. All drug resistant sequences were found at baseline and throughout the study period, with no temporal structure or increase in resistance over time.

Conclusions

Frequent sampling has allowed delineation of the frequency, magnitude, and duration of blips. Our results suggest that most, but not all, patients experience intermittent viremia in a setting of sustained suppression on HAART. However, a majority of these blips are not reproducible in independent laboratories and may represent random fluctuations near the limit of detection of the ultrasensitive assay. We suggest a more stringent definition of a blip as a positive value ($>50\text{c/ml}$) obtained in two independent laboratories, positive values on two consecutive time points, or a value above 150 c/ml (upper limit for the standard error). The source of these blips is unknown. They may result from assay variability, immune activation (illness or vaccination), reduced drug concentrations (poor adherence, altered absorption or metabolism of the drug), or multidrug interactions. Genotypic analysis both during and after the blips show no emergence of new resistance, suggesting that these episodes of viremia above 50 c/ml do not necessarily result from new partially resistant virus. In summary, patients may experience blips without evolution of drug resistance.