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Elevated hypermutation levels in HIV-1 natural viral suppressors ☆

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ABSTRACT

Mutations in the HIV-1 proviral genomes delay the progression of the disease. We compared the mutation status in full-length proviral genomes of 23 HIV-infected patients with undetectable viral loads in the absence of therapy named natural viral suppressors (NVS) or Elite Controllers with 23 HIV-infected controls (10 patients on HAART treatment and 13 untreated patients). Provirus DNA was extracted from PBMC for amplification and sequencing to determine the mutation status. Nine (39 %) of the 23 NVS patients had defective proviral genomes, compared to 4 of the treated controls (40%, $p=0.96$) and only one of the untreated controls (8%, $p=0.059$). Most of the defective genomes resulted from Gto-A hypermutation. Among patients with hypermutation, the rate ratio for mutation was significantly higher for the NVS compared to treated controls ($p=0.043$). Our data suggests that inactivation of the virus through the APOBEC3G system may contribute to the NVS phenotype.

Introduction

In recent years, there have been important advances in the understanding of innate immunity and the role it plays in the control of HIV-1 infection. Various patterns of disease progression have been associated with host genetics, immunological and virological factors (Grabar et al., 2009; Okulicz et al., 2009). A subset of HIV-infected patients who are able to suppress circulating virus naturally, without the use of anti-retroviral drugs, have been studied extensively. Often referred to as "Elite Controllers" or "Elite Suppressors," these individuals have very low to undetectable plasma HIV-1 RNA levels and relatively normal CD4+ T-cell counts (Wang et al., 2003; Walker, 2007; Sajadi et al., 2007; Blankson et al., 2007). In our Baltimore patient population, this phenotype is present in 1.5% of all HIV-1 seropositive individuals and they are referred to as "natural viral suppressors", or "NVS" (Sajadi et al., 2009). Thus far there has been an intensive effort by us and others to identify the mechanisms by which these individuals suppress their virus and multiple host factors have been identified (Okulicz et al., 2009; Hunt, 2009; Salgado et al., 2010; Baker et al., 2009; Blankson, 2010).

In addition to host factors, viral factors that might be associated with the NVS phenotype have been investigated. In a recent study, viruses from controllers displayed significantly lower replication capacity compared to those from progressors ($p<0.0001$) (Brumme et al., 2011). Others suggest that inhibitory mutations in *vpr, nef,* and *rev* could be associated with the NVS phenotype (Lum et al., 2003; Mologni et al., 2006; Iversen et al., 1995; Malim et al., 1991; Wang et al., 2003; Tolstrup et al., 2006; Caly et al., 2008). It has also been suggested that some individuals are infected with virions that are less capable of evading the host immunological response or are impaired. One common cause of impairment is due to viral genomes that contain APOBEC3G (A3G) or APOEC3F (A3F) induced hypermutation (Wang et al., 2003; Sandonis et al., 2009; Gandhi et al., 2008). In addition, mutations in epitopes for B27, B57, B58 recognition and the number of NFkappaB sites have also been correlated with long-term control (Migueles et al., 2000; Bailey et al., 2007; Goulder et al., 2001).

The current study investigates this unique group of NVS individuals to test the hypothesis that durable viral suppression may be caused in part by viral inactivation. Using both partial and full genome sequencing, others have found no significant mutations or deletions in the viruses with which these patients are infected (Blankson et al., 2007; Gandhi et al., 2008). This study presents 23 nearly full genome sequences of NVS patients, spanning most of the viral genome, for examination and comparison with genomes from two control groups. The control groups consist of 10 patients with undetectable viral loads on HAART treatment and 13 untreated patients with detectable viral loads.

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Table 1
Characteristics of NVS patients, controls on HAART, and untreated controls.

Subject ID	Age	Sex	Race	Year of HIV Diagnosis	Year of Sample	Risk Factor	Last Viral Load (copies/ml)	ART Treatment	REV (L78I)	Hypermutation Identified	Hypermutation Rate Ratio ^	APOBEC Ratio	Genes Analyzed								
													Gag	Pol	Vif	Vpr	Vpu	Env	Nef	Rev	Tat
05US.SAJ.NVS3	62	M	AA	1991	2005	IDU	<75	no	L	no		1.0									
05US.SAJ.NVS5	50	M	AA	1991	2005	MSM	93	no	L	no		1.9									
05US.SAJ.NVS7	60	M	AA	1994	2005	IDU	<75	no	L	yes	4.88 [p=7.68e-60]	8.1									
05US.SAJ.NVS8	58	M	AA	1989	2005	IDU	<40	no	L	no		1.3									
05US.SAJ.NVS9	53	M	AA	2003	2005	IDU	<40	no	L	no		1.4									
05US.SAJ.NVS12	40	M	AA	1997	2005	IDU	<40	no	L	no		1.3									
05US.SAJ.NVS15	36	F	AA	2002	2005	S	<50	no	L78I	yes	2.97 [p=1.18e-20]	5.0									
05US.SAJ.NVS16	43	F	AA	1988	2005	S	<40	no	L	no		1.7									
05US.SAJ.NVS19	58	M	AA	1997	2005	S	359	no	L	yes	7.04 [p=7.08e-69]	11.2									
06US.SAJ.NVS20	54	F	AA	1995	2006	IDU	120	no	N/A	yes	4.86 [p=4.88e-51]	7.1									
06US.SAJ.NVS22	60	M	AA	1994	2006	S	163	no	L	no		1.6									
06US.SAJ.NVS23	36	F	AA	1995	2006	S	<75	no	L	no		1.6									
06US.SAJ.NVS27	55	M	AA	1989	2006	IDU	475	no	L	no		1.7									
06US.SAJ.NVS31	49	M	AA	1992	2006	S	<48	no	L	no		1.5									
06US.SAJ.NVS32	50	F	AA	2000	2006	IDU	<48	no	L	no		2.0									
06US.SAJ.NVS35	53	M	AA	1997	2006	IDU	<75	no	L	no		1.6									
06US.SAJ.NVS39	30	F	AA	1995	2006	S	184	no	L78I	no		1.6									
07US.SAJ.NVS40	35	F	AA	1995	2007	IDU	<48	no	L	yes	3.66 [p=3.85e-36]	5.3									
07US.SAJ.NVS42	57	M	AA	1990	2007	IDU	<400	no	L	no		1.3									
07US.SAJ.NVS48	44	F	AA	1986	2007	S	328	no	L78I	no		1.4									
07US.SAJ.NVS53	52	F	AA	1986	2007	IDU	256	no	L	yes	5.64 [p=3.01e-71]	9.4									
07US.SAJ.NVS54	57	F	AA	2004	2007	S	<48	no	L	no		1.6									
07US.SAJ.NVS55	49	M	AA	1994	2007	IDU	<48	no	L	no		1.2									
CONTROLS ON HAART																					
07US.SAJ.C154	40	M	AA	1998	2007	S	<50	yes	L	no		1.5									
07US.SAJ.C155	45	M	AA	1996	2007	S	<50	yes	L	yes	2.90 [p=1.50e-18]	4.2									
07US.SAJ.C156	23	M	AA	2005	2007	S	<50	yes	L	no		1.3									
07US.SAJ.C157	52	M	AA	2002	2007	S	<50	yes	L	yes	3.32 [p=9.08e-30]	5.5									
07US.SAJ.C158	44	F	AA	1998	2007	S	<50	yes	L	yes	3.53 [p=2.0e-39]	5.4									
07US.SAJ.C159	51	M	AA	2003	2007	IDU	<50	yes	L	yes	2.52 [p=1.19e-23]	4.6									
07US.SAJ.C161.H1	36	M	W	2003	2007	S	<50	yes	L	no		1.6									
07US.SAJ.C162.H2	46	F	W	2005	2007	S	<50	yes	L	no		1.5									
07US.SAJ.C.163.H3	27	M	AA	2004	2007	S	<50	yes	L	no		1.4									
07US.SAJ.C.166.MS	31	M	AA	2006	2007	S	<48	yes	L	no		1.2									
UNTREATED CONTROLS																					
06US.SAJ.C164.SC	30	F	AA	1999	2006	IDU	94,379	no	L	no		1.6									
06US.SAJ.C164.MS	48	M	AA	2006	2006	N/A	2,630	no	L	yes	2.19 [p=5.74e-10]	3.4									
06US.SAJ.C165.TJ	34	F	AA	1995	2006	N/A	>750,000	no	L	no		1.7									
06US.SAJ.C166.SG	45	F	AA	2003	2006	S	>750,000	no	L	no		1.6									
06US.SAJ.C167.LH	45	F	AA	1990	2006	S	>750,000	no	L	no		1.4									
06US.SAJ.C168.LS	39	F	AA	2004	2006	IDU	164,000	no	L	no		1.8									
06US.SAJ.C169.JS	52	M	AA	2004	2006	IDU	94,000	no	L	no		1.1									
06US.SAJ.C170.JP	58	M	AA	1996	2006	IDU	99,000	no	L	no		1.7									
07US.SAJ.C200	24	M	AA	2005	2007	S	208,000	no	L	no		1.3									
08US.SAJ.C202	42	M	AA	1992	2008	IDU	57,800	no	L	no		1.9									
08US.SAJ.C203	46	F	AA	1997	2008	IDU	43,000	no	L	no		1.8									
08US.SAJ.C204	45	F	AA	2007	2008	IDU	>100,000	no	L	no		1.2									
08US.SAJ.C205	43	F	AA	2007	2008	IDU	205,418	no	L78I	no		1.6									

^ Fisher Exact *p*-value where less than 0.05 is indicative of a hypermutant. The APOBEC Ratio is defined as all GG and GA substitutions compared to all GC and CT substitutions. Analysis of all genes: color grey shows defective due to hypermutation; orange defective due to incorrect start amino acid and hypermutation, yellow defective due to incorrect start amino acid, blue defective due to deletion/frameshift, red defective due to frameshift, incorrect starting codon and hypermutation.