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Accelerated evolution of SIV *env* within the cerebral compartment in the setting of morphine-dependent rapid disease progression

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ABSTRACT

Human immunodeficiency virus-1 (HIV-1) and simian immunodeficiency virus (SIV) have been shown to compartmentalize within various tissues, including the brain. However, the evolution of viral quasispecies in the setting of drug abuse has not been characterized. The goal of this study was to examine viral evolution in the cerebral compartment of morphine-dependent and control macaques to determine its role in rapid disease progression. To address this issue, we analyzed the envelope (*env*) gene from proviral DNA in our SIV/SHIV macaque model of morphine dependence and AIDS. Analyses of proviral DNA revealed a direct correlation between total genetic changes and survival time. However, the rate of evolution during disease progression was higher in morphine-dependent and rapid-progressor macaques than was the rate of evolution in the control animals. This study provides additional insight into SIV envelope variation in the CNS of morphine-dependent macaques and genotypes that may have evolved in the brain and contributed to disease progression.

Introduction

Drug abuse and dependence are widespread in the general population in many parts of the world and intravenous drug use is thought to play a major role in spread of the human immunodeficiency virus-1 (HIV-1) through engagement in a number of high-risk behaviors, including sharing needles and sexual contact.

The occurrence of HIV-1 infection in the context of drug abuse raises important questions about the potential interactions between the two phenomena. This is particularly significant considering that a large proportion of drug abusers are also HIV-1-infected individuals (2002; 2003; Alcabes and Friedland, 1995; Chu and Levy, 2005; Cohn, 2002; Des, 1999). As of 2006, the Centers for Disease Control had estimated that there were 540,436 deaths in the United States of persons with AIDS, of which approximately 28% of the total were injection drug users (IDUs) (Centers for Disease Control and Prevention, 2008). The most widely abused drugs in the United States include heroin, cocaine and methamphetamine. However, the majority of IDUs report using multiple drugs at different times, thus it is difficult to directly link a specific drug to the observed clinical health parameters. Although numerous studies have documented that HIV-1-infected IDU experience substantial pre-AIDS morbidity, the natural history and progression of HIV-1 infection among IDU remains uncertain. Regardless, both *in vitro* and *in vivo* studies have demonstrated a correlation between drugs of abuse and increased viral replication (Chuang et al., 1993; Nyland et al., 1998; Guo et al., 2002; Suzuki et al., 2002; Kumar et al., 2006).

HIV-1 and simian immunodeficiency virus (SIV) infections lead to neurological complications, and drug abuse is thought to adversely affect this occurrence in humans. Although antiretroviral therapy has proven effective in reducing the viral load in the central nervous system (CNS) with a resulting improvement in neurological function, viral reservoirs persist in the brain (reviewed in: (Dunfee et al., 2006). Understanding viral evolution in the brain will help determine the viral factors that contribute to neurological disease.

The SIV/macaque model is particularly valuable for evaluating information that is usually impossible to obtain from human studies, such as the genotypic and phenotypic properties of the infecting virus, knowledge of the exact time of virus exposure, assessment of disease progression, and the characteristics of viral variants in the infected host. Whereas compartmentalization has been well demonstrated for both HIV-1 and SIV, few studies have characterized the impact of drugs of abuse on the degree of SIV *env* evolution and the role such viral dynamics may play in the rate of disease progression. Our model of SIV/ SHIV infection and drug abuse offers a unique opportunity to address these important questions. We have previously shown a negative correlation between evolution of viral accessory genes in

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plasma and cerebrospinal fluid (CSF) and opiate-mediated disease progression (Noel and Kumar, 2007; Noel et al., 2006; Tirado and Kumar, 2006; Noel and Kumar, 2006). In addition, we have also shown a positive correlation between the evolution of virus envelope and opiate-mediated disease progression (Rivera-Amill et al., 2007). In this study we wanted to investigate the pathogenic and biologic consequences of SIV envelope sequence variation within the brain in morphine-dependent and control macaques infected with SIV/SHIV. In this report, the sequences of SIV/17E-Fr envelope gene recovered from the brain of morphine-dependent macaques are described. The results indicate that the highest levels of nucleotide substitution in sequences isolated from the brain were observed in animals that survived for longer periods, regardless of whether they were morphine dependent or not. Although the total diversity was most closely related to duration of infection, the rate of evolution was fastest for the morphine-dependent rapid disease progressors, who showed a rapid accumulation of changes early during infection.

Results

Clinical disease parameters of the six morphine-dependent and three control macaques used in this study are shown in Table 1. Half of the morphine-dependent macaques developed rapid disease progression (1/04L, 1/42N and 1/28Q). These macaques maintained high viral loads both in plasma and CSF, and low $CD4^+$ T cell counts. In contrast, morphine-dependent normal progressors (1/56L, 1/02N and 1/52N) and control macaques (2/02P, 2/AC42 and 2/31P) exhibited lower viral loads, and higher $CD4^+$ T cell counts. Two normal progressors and two control macaques exhibited CSF viral loads below the level of detection (Table 1). In order to determine whether viral DNA persisted in these macaques, genomic DNA from brain tissue was isolated and used for Real Time PCR to quantitate SIV/17E-Fr in the brain. Using *env* specific primers and probe, we demonstrated the presence of SIV/17E-Fr in the brain tissue from morphine-dependent and control macaques despite significant down-regulation of viral RNA in the CSF (Table 1). Viral DNA in the brains of morphine-dependent and control animals was detected at similar levels and there were no statistically significant differences among the groups. These results, are in agreement with a previous study of SIV encephalitis by Clements et al. (2002), where they revealed that despite significantly lower levels of viral RNA in the brain after the acute phase, the DNA levels remained constant. Therefore, even though viral load in the CSF of two morphine-dependent normal progressors and two control macaques at the time of death were below the level of detection, virus-infected cells were not cleared. We were able to detect SIV RNA in brain tissue albeit at low levels and there were no significant differences between morphine dependent and control macaques (Table 1). Pathological changes in the brains of morphine-dependent macaques with neurological symptoms were characterized by accumulation of macrophages in the perivascular region (Fig. 1A), focal histiocytic nodules (Fig. 1B) and multi-nucleated giant cells (Fig. 1C), characteristic of lentiviral encephalitis. Next we analyzed the SIV/17E-Fr *env* gene from the DNA isolated from morphine-dependent and control macaques in order to determine whether there are remarkable differences in the viruses that had established in the brain. Based on our previous study (Rivera-Amill et al., 2007), we focused this analysis on SIV *env* V3-V5. In the present study we analyzed the diversity, divergence, synonymous and non-synonymous substitutions, as well as amino acid mutations in key functional regions in order to characterize SIV env evolution within the CNS.

Phylogenetic analysis of env clones from brain tissue

Phylogenetic analyses using SIV *env* V3V5 sequences derived from genomic brain DNA were carried out to determine the relatedness of variants between morphine-dependent and control macaques. Five to ten clones from each macaque were sequenced, aligned and subjected to phylogenetic analysis using the distance-based (neighbor-joining) method. The SIV/17E-Fr *env* sequence was used as the root for the trees. Bootstrap resampling using 500 replicate trees was carried out to estimate the robustness of the observed groupings. Phylogenetic trees of the morphine-dependent and control macaque brain-derived clones revealed clustering of the majority of the sequences derived from rapid progressors and a mixed distribution for some of the sequences derived from normal progressors and control macaques (Fig. 2). Greater branch lengths were observed in the phylogenetic reconstruction for morphine-dependent normal progressors and control macaques, whereas rapid progressors exhibited less clonal diversity. When taking into consideration the average survival time for each group (rapid progressors 19 weeks; normal progressors 124 weeks; control macaques 90 weeks), we find that the rapid progressors exhibited a higher rate of mutations as compared to the other groups and that the sequences isolated from this group appear ancestral to the sequences isolated from the other macaques.

Table 1
Summary of clinical data.

Macaque	Survival[a] (weeks, pi)	$CD4^+$ T cells[b]	Viral load ($\times 10^4$ RNA copies/ml)		Brain proviral load ($\times 10^3$ copies/μg of DNA)	Brain RNA viral load ($\times 10^2$ copies/μg of RNA)	SAIDS[c]
			Plasma	CSF			
Morphine-							
Rapid							
1/04L	18	2.9	3340	226	0.007	12.3	+
1/42N[d]	19	10	10700	111	0.003	7.38	+
1/28Q[d]	20	6	7660	166	0.001	4.93	+
Normal							
1/56L[d]	51	23	436	382	0.0002	4.13	+
1/02N	161	43	2.87	ND[e]	0.03	ND[f]	−
1/52N	161	160	ND[e]	ND[e]	0.0001	4.75	−
Control							
2/02P	60	42	2.56	1.67	0.00003	5.0	+
2/AC42	64	67	0.038	ND[e]	0.0005	4.98	+
2/31P	147	503	0.236	ND[e]	0.0001	5.34	−

[a] A monkey surviving <24 weeks after infection was considered a rapid disease progressor.
[b] $CD4^+$ T cells are presented as number/ ml of blood at the time of necropsy.
[c] SAIDS: Simian AIDS.
[d] The animal developed neurological disorders.
[e] Not detectable. The viral load was <20 RNA copies/ml.
[f] Not done.