Sequence Note

Analysis of the V1V2 Region of the SIV Envelope in the Brains of Morphine-Dependent and Control SIV/SHIV-Infected Macaques

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Abstract

Six morphine-dependent and three control macaques were infected with a mixture of SIV/SHIV. Half of the animals in the morphine group developed accelerated disease (rapid progressor) and died within 20 weeks postinfection. The evolution of the envelope gene in the brain of the rapid progressor and morphine-dependent group along with that in the control group was assessed. Six to 10 clones from the brain of each macaque were sequenced and were compared against each other as well as against a challenge virus. Analysis of the sequences revealed that the diversity and divergence of the clones were higher in the control group as compared to the morphine-dependent macaques, although this difference was not statistically significant.

THE USE OF ILLICIT DRUGS ADMINISTERED INTRAVENOUSLY together with HIV/AIDS is a major public health problem in many parts of the world. According to a Centers for Disease Control (CDC) estimate, injection drug users (IDUs) comprise a large cohort among HIV-infected individuals. It was recently reported that approximately 28% of 540,436 AIDS-associated deaths belong to this group.¹ One of the many complications associated with the clinical study is that the majority of IDUs report using multiple drugs at different times, thereby making it difficult to directly link a specific drug to the observed clinical health parameters. Previous studies² have shown that heroin abusers exhibit reduced immune system responses and increased incidence of infections.³ However, the natural history and progression of HIV infection among IDUs remain uncertain. Conflicting reports of mortality rates among HIV-infected IDUs have demonstrated both a survival advantage and lower survival rates among HIV-infected IDUs.^{4,5} Nevertheless, there is ample evidence that HIV-infected IDUs experience substantial pre-AIDS morbidity, particularly from bacterial infections, suggesting that coinfection with other pathogens may enhance HIV expression and assist in the spread of the disease.^{2,3,6,7} In

addition, *in vitro* and *in vivo* studies have demonstrated a correlation between drugs of abuse and increased viral replication.^{8–14}

We have developed a reproducible nonhuman primate model of IDU and HIV disease that utilizes a neuropathogenic SIV (SIV/17E-Fr) and two SHIVs (SHIV_{KU1-B} and SHIV_{89.6P}) in Indian rhesus macaques.^{13,14} The use of these three viruses leads to massive CD4⁺ T cell loss and neurological disorders mimicking comparable clinical manifestations in humans. Briefly, morphine dependence was established by injecting increasing doses of morphine (1–5 mg/kg of body weight over a 2-week period) by the intramuscular route at 8-h intervals. The animals were then maintained at three daily doses for an additional 18 weeks. Control macaques received saline injections. All animals were then infected via the intravenous route with a 2-ml inoculum containing 10⁴ TCID₅₀ (50% tissue culture infective doses) of each virus. Blood and cerebrospinal fluid (CSF) were collected at regular intervals and tissues were collected at the time of necropsy. Using this model we have previously shown that both morphine and control groups showed precipitous loss of CD4⁺ T cells. However, the decline of CD4⁺ cells was more prominent in the morphine group.

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TABLE 1. SURVIVAL TIME, PLASMA/CSF VIRAL LOAD, AND CD4⁺ T CELL COUNT

Macaque	Survival ^a (weeks, pi)	Viral load (×10 ⁴ RNA copies/ml)		CD4 ⁺ T cells ^b
		Plasma	CSF	
Morphine-dependent				
1/04L	18	3,340	226	3
1/42N ^c	19	107,000	111	10
1/28Q ^c	20	7660	166	6
Control				
2/02P	60	2.56	1.67	42
2/AC42	64	0.038	ND ^d	67
2/31P	147	0.236	ND ^d	503

^aA monkey surviving <24 weeks after infection was considered a rapid disease progressor.

^bCD4⁺ T cells are presented as number/ μ l of blood at the time of necropsy.

^cThe animal developed neurological disorders.

^dNot detectable. The viral load was <20 RNA copies/ml.

The plasma and CSF values were significantly higher in the morphine-exposed group than in the control group (Table 1). Three morphine-dependent animals, rapid progressors, succumbed to SIV/SHIV-induced AIDS at week 18, 19, and 20, respectively. Among rapid disease progressors, two macaques exhibited severe neurological manifestations whereas none of three controls developed neurological symptoms.

We have previously reported SIV virus evolution in morphine-dependent and control animals by sequencing *env*,^{15,16} *tat*,^{17,18} *vpr*,¹⁹ and *nef*²⁰ from plasma and cerebrospinal fluid samples in an attempt to understand the relationship between drug abuse, disease progression, and viral evolution. These studies revealed no correlation for *nef* and an inverse correlation between *tat*, *vpr*, and *env* V1V2 evolution

	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	
SIV17/E-Fr env v1-v2	EDV	WOLFETSIKP	CVKLSPLCIT	MRQNKSETR	WGLTKSITT	ASTTSTASA	RVMVNETSS	CIAQNCAGL	BQEQMISCKF	NMIGLNDRKK	KEYNETWYSA	DLVCEQGNNT	GNESRCYMNH	CNTSVIKESC	DRHYWDAIRF	RYCA
1/04L Brain-(6)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/04L Brain-4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/04L Brain-5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/04L Brain-6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/04L Brain-8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/04L Brain-10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/04L Brain-11	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/42N Brain-1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/42N Brain-2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/42N Brain-3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/42N Brain-4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/42N Brain-5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/42N Brain-6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/42N Brain-8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/42N Brain-9	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/42N Brain-10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/42N Brain-11	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/42N Brain-12	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/28Q Brain-1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/28Q Brain-2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/28Q Brain-3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/28Q Brain-4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/28Q Brain-5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/28Q Brain-10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/28Q Brain-11	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
1/28Q Brain-12	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/02P Brain-3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/02P Brain-6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/02P Brain-(7)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/02P Brain-10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/02P Brain-16	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/AC42 Brain-1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/AC42 Brain-(2)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/AC42 Brain-6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/AC42 Brain-10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/AC42 Brain-11	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/AC42 Brain-12	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/31P Brain-1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/31P Brain-2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/31P Brain-3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/31P Brain-8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/31P Brain-13	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/31P Brain-20	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2/31P Brain-22	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

FIG. 1. Alignment of the deduced amino acid sequence of the SIV envelope from morphine-dependent rapid progressor and control rhesus macaques infected with SHIV_{KU1-B}, SHIV_{89.6P}, and SIV/17E-Fr. Brain tissue was collected from six animals at the time of necropsy. Genomic DNA was extracted and amplified. The PCR product was cloned into a pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA) and sequenced using an M13 primer. The first description indicates the name of the monkey, and the last number indicates the clone number. The number in parentheses is the number of identical clones from that monkey. The reference sequence on top denotes corresponding amino acids and the location in the SIV/17E-Fr envelope. Dashes represent amino acids similar to those found in the reference sequence and x denotes a gap in the sequence.

FIG. 2. Diversity and divergence of SIV/17E-Fr *env* V1V2 in brain from morphine-dependent and control macaques infected with SIV and SHIV. The *env* V1V2 sequences from morphine-dependent and control macaques were aligned and used to calculate diversity and divergence using MEGA 3.1. The mean percent diversity and divergence are plotted for each monkey. Error bars indicate standard error.

and disease progression, whereas *env* V4 evolution correlated directly with disease progression.²¹ In this study, we sought to determine whether there was a difference in genomic characteristics of SIV/17E-Fr *env* V1V2 between brain-derived sequences from morphine-dependent rapid progressors and control macaques.

Genomic DNA from brain tissue was extracted using a QIAmp DNA Mini kit (Qiagen, Inc., Valencia, CA). A 482-bp fragment comprising the V1V2 region of the envelope gene from SIV/17E-Fr was amplified by polymerase chain reaction (PCR). Primers and PCR conditions were as reported previously.¹⁶ The 482-base pair fragment was confirmed by agarose gel electrophoresis prior to cloning into pCR2.1 (Invitrogen, Carlsbad, CA). Positive clones were identified by colony screening with restriction enzyme digest (*EcoRI*, Promega) and agarose gel electrophoresis prior to plasmid prep using a QIAprep Spin Mini Kit (QIAGEN, Inc., Valencia, CA). Six to 12 clones of each sample were sent for sequencing to MCLAB (South San Francisco, CA). Raw sequence data were processed and aligned using BioEdit version 7.0.5.2 (Hall TA) and CLUSTAL W,^{22,23} and then subjected to phylogenetic analysis, including tree construction, divergence, and diversity calculations using Mega v. 3.1.²⁴ Finally, sequences were translated to amino acids using BioEdit.

Envelope gene V1V2 variants from brain samples were cloned and sequenced from macaques 1/04L, 1/42N, and 1/28Q (morphine-dependent rapid progressors) and 2/02P, 2/AC42, and 2/31P (control group). Amino acid changes among the clones were similarly distributed between the V1 and V2 regions in both rapid progressors and controls (Fig. 1). While our previous study of *env* V1V2 evolution in plasma showed a significant inverse correlation with disease progression,¹⁶ proviral sequences in brain showed no significant differences in the frequencies of amino acid mutations be-

tween the two groups (data not shown). The total number of unique amino acid changes was the same when comparing the morphine-dependent rapid progressors and control macaques. However, two-thirds of the amino acid changes occurred in macaque 2/31P who survived the longest, 147 weeks postinfection (Fig. 1). Analysis of the sequences revealed that the diversity and divergence of the clones were higher in the control group as compared to the morphine-dependent rapid progressor macaques. This phenomenon corroborates our previous study in plasma, although the difference in brain was not significant (Fig. 2). Our results, therefore, suggest that sequence changes within the SIV/17E-Fr *env* V1V2 region in the brain occur regardless of drug abuse or rapid disease progression.

In addition, the length of infection prior to disease progression or death does not correlate with the overall extent of fixed changes within V1V2. This suggests that the brain compartment may limit the extent of V1V2 changes that do not compromise viral fitness. In a previous study, we had shown that 50% of the morphine-dependent macaques had a rapid disease course after failing to resolve the initial viremia. Neutralizing antibodies in this group of animals were undetectable, whereas control animals developed neutralizing antibodies against the three viruses used, albeit lower neutralizing antibodies were generated against SIV17/E-Fr as compared to the SHIVs.¹⁴ These results also suggest that a high viral load associated with rapid disease progression blunted the initial humoral response, thus precluding the establishment of host-selective pressures that could drive *env* V1V2 diversification.

Sequence Data

Sequences in this report are available from GenBank with accession numbers from FJ480958 to FJ481013.

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Disclosure Statement

No competing financial interests exist.

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