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SIV Vpr evolution is inversely related to disease progression in a morphine-dependent rhesus macaque model of AIDS

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Abstract

Three of six morphine-dependent monkeys progressed rapidly to AIDS and died by 20 weeks in our SIV/SHIV non-human primate model of drug addiction and AIDS. We studied the evolution of the SIV *vpr* gene in both cerebrospinal fluid (CSF) and plasma in these rapid progressors, in their normal progressor counterparts and in infected, drug-free controls at 12 and 20 weeks post infection. Viral RNA was amplified, cloned, and sequenced to permit phylogenetic analyses of diversity and divergence of the *vpr* locus. As we found for SIV *tat* and *env,* the *vpr* gene evolves inversely to the rate of disease progression. Further, we found evidence that compartmentalization of the virus in plasma and CSF is significantly greater in the normal progressors than in the morphine-dependent, rapid progressors. Interestingly, although our previous work with the accessory gene *nef* indicated no association between disease progression and evolution, the accessory factor, *vpr*, behaves similarly to the essential lentiviral genes *tat* and *env*.

Keywords: SIV; SHIV; Morphine; Pathogenesis; Phylogenetics; Virus evolution

Introduction

All retroviruses share common elements in genomic structure including the *gag*, *pol*, and *env* genes. In addition to these universally essential genes the primate lentiviruses, including both HIV-1 and SIV have a well-characterized set of accessory gene products (Vogt, 1997). Only two of these accessory genes, *tat* and *rev*, are absolutely required both *in vivo* and *in vitro* for viral replication (Dull et al., 1998; Luciw, 1996). Another accessory gene, *vpr* (encoding the viral protein R), is common to both HIV-1 and SIV, yet unlike the *tat* and *rev* gene products, *vpr* has been shown to be dispensable for viral replication in a number of cell culture and animal model settings (Le Rouzic and Benichou, 2005; Aldrovandi and Zack, 1996). In *in vivo* studies, however, a *vpr*$^-$SIV is only half as virulent as the wild type SIVmac239, while a deletion of two accessory gene products, *vpr* and *nef*, is 99.5% less virulent and does not lead to simian AIDS (Desrosiers et al., 1998), indicating the importance of this ~100aa protein.

Vpr is packaged in the virus via an association with the p6 component of the viral *gag* gene (Kondo et al., 1995; Paxton et al., 1993). During viral replication, Vpr has been attributed a variety of roles including increasing the accuracy of reverse transcription, assisting in the nuclear import of the preintegration complex, transactivation of the viral promoter, regulation of cell cycle and in some cells, regulation of apoptosis (Le Rouzic and Benichou, 2005; Mueller and Lang, 2002; Patel et al., 2002; Chen et al., 2004; Mahalingam et al., 1997; Muthumani et al., 2002b; Piller et al., 1999; Di Marzio et al., 1995). In the specific setting of SIV, some of the functions of Vpr (in HIV) are shared or overlapping with SIV Vpx. However, at least the transactivation, apoptosis and cell cycle regulation appear to be conserved in Vpr proteins of primate lentiviruses (Planelles et al., 1996;

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Zhu et al., 2001; Philippon et al., 1999). Thus Vpr protein has demonstrable roles in a variety of aspects of viral infection and pathogenesis. Still, there is little information of the possible pathogenic role of Vpr during the setting of drug abuse regardless of the large impact of drugs of abuse on the HIV/AIDS epidemic both in the US and worldwide.

In the US alone, drug abuse is a co-factor in the acquisition and or pathogenesis in nearly 1/3 of the HIV-1 infection cases (Purcell et al., 2004). Although this clearly presents a considerable burden on the health care system and the economy, studies of the impact of drug abuse (morphine) have provided mixed message of harm (Chuang et al., 2005) versus reduced pathogenesis (Donahoe, 2004; Kapadia et al., 2005). In part to address this ongoing controversy, we have developed a non-human primate model of AIDS/drug abuse using rhesus macaques addicted to morphine and then infected with a combination of viruses (SIV17E-Fr, $SHIV_{89.6P}$, $SHIV_{KU-1B}$) to more rapidly induce simian AIDS (sAIDS), including a precipitous loss in CD4+T cells characteristic of AIDS in humans (Kumar et al., 2004a). In this model, we have observed that half of the morphine addicted animals progress rapidly to AIDS and death by 20 weeks post-infection (Table 1) (Kumar et al., 2004a; Noel and Kumar, 2006). While all animals in the study showed a rapid peak in virus and severe drop in CD4+T-cells during the acute phase of infection, the rapid progressors never regained CD4+T-cells; nor did they establish control of the viral replication in either the plasma or the cerebrospinal fluid (CSF) (Kumar et al., 2004a). Macaques that did not show rapid progression, including half of the morphine group and all members of the morphine-free cohort, established some recovery of CD4+T-cells and control over plasma and CSF viral load. One goal of our work with these cohorts has been to evaluate the impact of viral evolution in rapid progression in the setting of drug abuse. We have already established a relationship between viral evolution and the rate of disease progression for a number of critical viral genes in this system. Both the essential accessory gene *tat* (in plasma and in CSF) and the structural gene *env* (plasma) have shown an inverse correlation between evolution and disease progression in the setting of morphine-dependence (Noel and Kumar, 2006; Noel et al., 2006a, 2006b; Tirado and Kumar, 2006). However, our experience to date with an *in vitro* dispensable accessory gene (*nef*) showed no correlation between evolution rate and disease progression (Noel et al., 2006a, 2006b). This suggested that some non-essential accessory genes could prove less influential to rapid pathogenesis. We have now extended this analysis to the *vpr* gene where we find that unlike the dispensable accessory gene *nef*, and like the essential accessory gene *tat*, evolution is inversely correlated to disease progression both in plasma and CSF, and furthermore, that compartmentalization of virus does not occur in rapid progression.

Table 1
CD4+, viral load in plasma and cerebrospinal fluid

Monkey	Sampling week	$CD4^+T$ (cells/mL)	plasma viral load (10^4 copies/mL)	CSF viral load (10^4 copies/mL)
Group A [a]				
1/04L	12	16	401	37.3
	18	2.9	3340	226
1/28N	12	21	2030	1150
	20	6	7660	204
1/42N	12	39	2590	17.8
	19	10	10,700	111
Group B				
1/02N	14	31	3.63	0.052
	18	10	6.62	0.012
1/52N	14	707	0.144	below limit
	20	1365	0.022	below limit
1/56L	12	31	44.2	0.023
	20	13	147	0.024
Group C				
2/02P	12	113	3.14	0.140
	18	447	3.35	0.008
2/31P	12	334	3.14	0.008
	18	214	0.457	0.008
2/AC42	12	154	1.41	0.140
	18	44	0.186	0.008

[a] No Group A monkeys survived beyond week 20 1/04L was euthanized at week 18, 1/28Q at week 20 and 1/42N at week 19.

Results and discussion

Rate and complexity of evolution of vpr correlate inversely with disease progression

As with our previous studies, viral nucleic acids were extracted for two times points roughly 12 and 20 weeks post infection from cell-free fluids and subjected to RT-PCR amplification, cloning and sequencing (Noel and Kumar, 2006; Noel et al., 2006a, 2006b; Tirado and Kumar, 2006). Phylogenetic comparisons were made of all clones in both plasma and CSF within each individual monkey. The resulting phylogenetic trees (Fig. 1) show that *vpr* evolution appears to be inversely correlated with disease progression. Each tree includes the sequence of the SIV virus used in the initial infection (inoculum) as well as the original SIV17E-Fr sequence (Genebank #AY033146). Although all three inoculum virus have identical Vpr amino acid sequences there are silent nucleotide changes present in the SHIVs. We found no evidence in our clones for amplification of *vpr* from either SHIV, indicating that our primers were specific for SIV as was the case for *tat* (Noel and Kumar, 2006; Noel et al., 2006a, 2006b). We found no evidence of recombination among the three viral forms as we did for *nef* (Noel et al., 2006a, 2006b), thus we did not include the SHIVs in our trees. Interestingly, as we observed for *tat*, the clustering patterns of the trees were more evident in normal progressors (Fig. 1, Groups B and C), and in particular with CSF samples as opposed to the plasma (Noel et al., 2006a, 2006b). Thus, not only does evolution appear to be inversely correlated with rate of disease progression, but the complexity of the evolution and the ability of a variant to start a persistent evolutionary path appear to be related to slower progression rather than the presence of morphine itself (compare Group A vs. B and C, Fig. 1). Recent studies that show no detectable binding or neutralizing antibodies nor virus specific CTL