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Identification and molecular characterization of SIV Vpr R50G mutation associated with long term survival in SIV-infected morphine dependent and control macaques

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ABSTRACT

Viral protein R (Vpr) is an accessory protein of HIV and SIV involved in the pathogenesis of viral infection. In this study, we monitored SIV evolution in the central nervous system and other organs from morphine-dependent and control animals by sequencing *vpr* in an attempt to understand the relationship between drug abuse, disease progression, and compartmentalization of viral evolution. Animals in the morphine group developed accelerated disease and died within twenty weeks post-infection. A unique mutation, R50G, was identified in the macaques that survived regardless of morphine exposure. Functional studies revealed that the R50G mutation exhibited altered cellular localization and decreased the expression levels of both IL-6 and IL-8. Our results, therefore, suggest that sequence changes within the SIV/17E-Fr *vpr* occur regardless of drug abuse but correlate with survival, and that they alter disease progression rates by affecting Vpr functions.

Introduction

Viral protein R (Vpr) is an accessory gene of 14k-Da and 96 amino acids present in both HIV and SIV (slightly larger at 15k-Da and 101 amino acids) that is produced late in the virus replication and is also found within the viral particle. Vpr is dispensable for viral replication in $CD4^+$ T-cells but is required for infection of primary macrophages (Eckstein et al., 2001; Rey et al., 1998). Vpr is a multifunctional protein that facilitates many processes in HIV-1 infection, such as evasion of the immune system and persistence in the host, thus contributing to the morbidity and mortality of acquired immunodeficiency syndrome (AIDS). Inside infected cells, Vpr is mainly a nuclear protein that has been demonstrated to play various roles in viral infection. Vpr supports nuclear import of the pre-integration complex (PIC) and has also been shown to participate in the transactivation of long terminal repeat – LTR (Andersen et al., 2008; Andersen and Planelles, 2005; Goh et al., 1998; Tungaturthi et al., 2004). Vpr protein induces G_2 cell cycle arrest, modulates T-cell apoptosis, co-activates host genes, and regulates nuclear factor kappa B (NF-κB) activity (Ayyavoo et al., 1997; Conti et al., 2000; Li et al., 2010).

Although much effort has been placed in answering major questions related to HIV-1 quasispecies evolution, the effect that these mutations have on the response to antiretroviral treatment, and the interactions of the newly emerged viruses with the immune system remains only partially understood. In addition, few studies have focused on the effect that viral diversity of the non-structural genes (i.e. *vif*, *vpr*, *vpu*, *tat*, and *rev*) has on disease outcomes. Disease progression and infection in vivo is attenuated in patients with Vpr defects at the C terminus, indicating the importance of the role that Vpr plays in viral pathogenesis (Caly et al., 2008; Jacquot et al., 2009; Lum et al., 2003; Mologni et al., 2006; Noel and Kumar, 2007; Wang et al., 1996). However, other studies found an association between increased amino acid variation over time and rapid disease progression (Cali et al., 2005).

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More recently, a picture is emerging wherein mutations in *vpr* are associated with antiretroviral-experienced patients failing therapy. Viruses harboring a mutation in *vpr* (E17A) combined with thymidine analog mutations in the reverse transcriptase exhibit decreased susceptibility to didanosine and thus imply a novel role for Vpr in HIV-1 drug resistance (Fourati et al., 2012).

We have followed SIV virus evolution in morphine-dependent and control animals by sequencing *env* (Rivera-Amill et al., 2010a; Rivera-Amill et al., 2007; Rivera-Amill et al., 2009; Tirado and Kumar, 2006), *tat* (Noel and Kumar, 2006; Noel et al., 2006a), *vpr* (Noel and Kumar, 2007) and *nef* (Noel et al., 2006b) from cell-free virus in plasma and cerebrospinal fluid samples in an attempt to understand the relationship between drug abuse, disease progression and viral evolution. In terms of *vpr* evolution, our studies have shown an inverse correlation of SIV *vpr* evolution and disease progression (Noel and Kumar, 2007). In the current study we wanted to examine the evolution of *vpr* from proviral sources in brain tissue, PBMCs and several peripheral tissue compartments to determine whether there is an association between virus evolution and disease progression. We also wanted to determine whether there is a correlation between disease-progression rate and viral compartmentalization. We have computationally analyzed the structure of SIV Vpr in the context of a change detected in some of the compartments (R50G) that appears to be linked to the progression rate. These studies were complemented with functional analyses of the SIV Vpr R50G in the context of inflammatory cytokine expression.

Results

A unique change, R50G, in Vpr is associated with survival

Initial studies using the SIV/SHIV macaque model revealed a remarkably consistent pattern of inverse correlation between disease progression and SIV *vpr* evolution in circulating virus from both plasma and cerebrospinal fluid (CSF) (Noel and Kumar, 2007). In the current study, we analyzed proviral *vpr* gene variants from brain tissue and peripheral blood mononuclear cells (PBMCs) from morphine-dependent rapid progressors, morphine-dependent normal progressors and control macaques. Phylogenetic analyses using SIV *vpr* sequences derived from brain DNA and PBMC DNA were carried out to determine the relatedness of variants between these compartments in 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. As previously reported for *vpr* clones derived from plasma and CSF, the resulting phylogenetic trees of proviral brain- and PBMC-derived clones show that *vpr* evolution and the degree of compartmentalization are inversely correlated with disease progression (data not shown) (Noel and Kumar, 2007). In the brain, morphine-dependent rapid progressors, had roughly 20–25% less sequence diversity as the morphine-dependent normal progressors and approximately 10% less sequence diversity as the control animals, although not statistically significant (data not shown).

We next examined the deduced amino acid sequences for all *vpr* clones to identify specific changes that could lead to altered disease progression. As shown in Fig. 1, a unique mutation, R50G, was consistently detected as the predominant form in brain-derived sequences of macaques that survived 20 weeks or longer post-infection. This change was not present in the inoculum, nor did it constitute a major form in the rapid progressors.

Analyses of proviral sequences form other compartments including kidney, lung, spleen, stomach, and testes revealed that the R50G change was markedly decreased in macaques that died within 19 weeks post-infection (Fig. 2). Analysis of the frequency of amino acid mutations in all compartments revealed a consistent pattern of fewer R50G mutations in the morphine-dependent rapid progressors as compared to the morphine-dependent normal progressors and control macaques, reaching significance in all compartments analyzed. These results suggest that among proviral forms, SIV *vpr* evolution is independent of the presence of morphine, inversely related to the disease progression rate and correlates with survival. This trend agrees with our previous study of circulating virus in plasma and CSF (Noel and Kumar, 2007).

The R50G mutation results in structural and cellular distribution differences

The 3D models were initially built based on the amino acid sequences for wild type (WT) SIV Vpr and SIV Vpr R50G using multiple-threading alignments and assembly simulations within I-TASSER. Protein function insights were then derived by matching the predicted models with protein function database (Roy et al., 2010; Zhang, 2008). Fig. 3 shows the 3D models with the highest confidence (*C*-score) and TM score, estimates of the quality of the predicted models, for SIV Vpr and SIV Vpr R50G as determined by I-TASSER. The *C*-score is calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. *C*-score is typically in the range of $[-5, 2]$, where a *C*-score of higher value signifies a model with a high confidence and vice-versa. The TM-score is the estimated accuracy of the model where a TM-score >0.5 indicates a model of correct topology and a TM-score <0.17 means a random similarity. On comparison of the *C*-scores and TM-scores of predicted 3D structures, both SIV Vpr WT and SIV Vpr R50G exhibit a similar 3D model (Zhang and Skolnick, 2005). In context with the secondary structure, both the SIV Vpr and SIV Vpr R50G are composed of three α-helices separated by loops of exposed residues as is observed for HIV Vpr (Morellet et al., 2003). The R50G change falls within the loop that separates α-helices two and three and is predicted to disrupt the spatial relationships of the three helices and both the N- and C-termini.

We next wanted to determine whether R50G mutation results in differences in SIV Vpr localization. Using the 3D models generated in I-TASSER, COFACTOR threaded the structure through three comprehensive function libraries by local and global structure matches to identify functional sites and homologies. Based on the gene ontology (GO) terms in I-TASSER server, the putative cellular localization of modeled SIV Vpr R50G differs from SIV Vpr WT (data not shown). In order to corroborate these findings V5-Vpr mammalian cell expression vectors were used to transfect U87 MG astrocytoma cells (Fig. 4, panel A) to investigate Vpr's subcellular localization (Fig. 4, panel B). Wild-type SIV Vpr localized to both the nucleus and the cytoplasm of transfected cells, whereas SIV Vpr R50G was localized mainly in the nucleus. To determine whether this difference in cellular distribution was specific to the R50G mutation, we also analyzed other SIV Vpr mutants. SIV Vpr L24Q had a similar distribution as WT Vpr and Vpr E29G, that also contains the R50G, localized mainly to the cell nucleus. These results corroborate that the R50G mutation alters the cellular distribution of SIV Vpr.

Differential regulation of pro-inflammatory factors in WT SIV Vpr, R50G and mock transfected astrocytes

Astrocytes are abundant cells in the central nervous system and their physiological roles are essential for normal brain function. In the setting of HIV infection, these cells play a central role in the generation of inflammatory mediators in response to HIV-1 presence in the brain (Cota et al., 2000; Dou et al., 2006;