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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

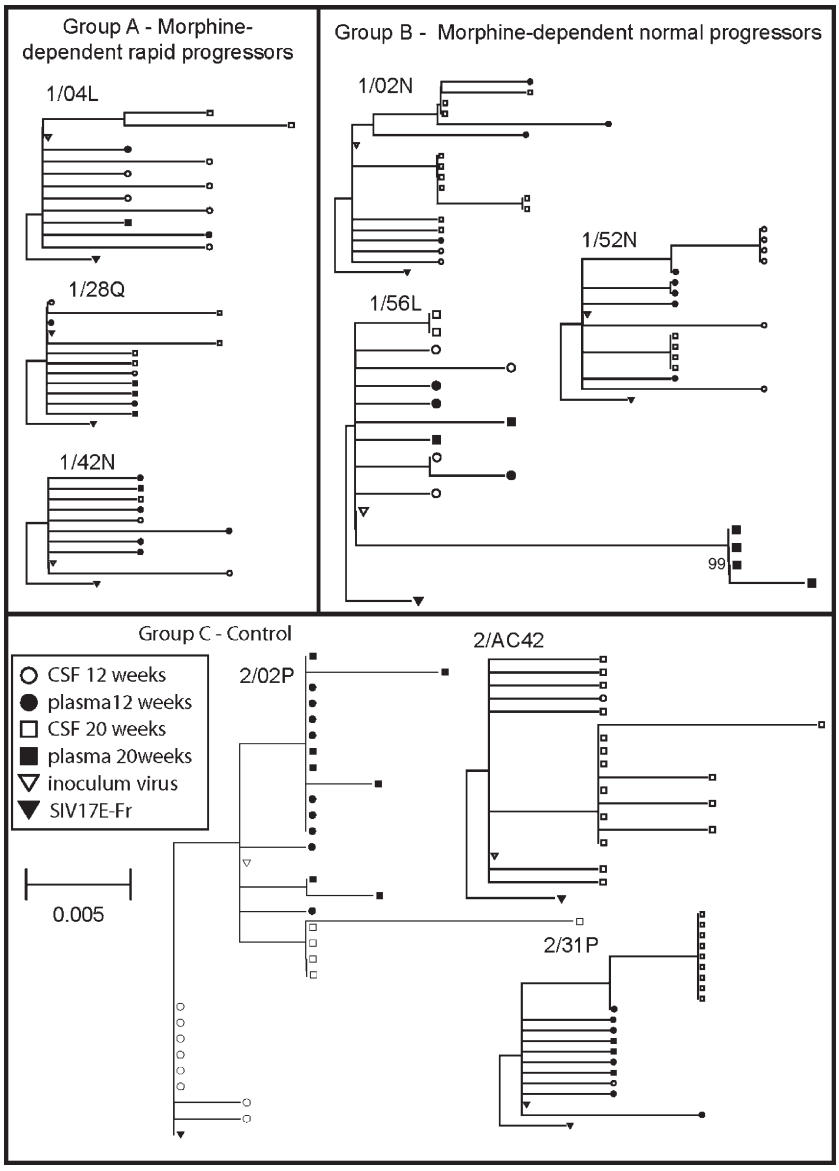

Fig. 1. Phylogenetic trees were made for each individual animal and included plasma (filled symbols) and CSF (open symbols) clones that were divergent from the inoculum clone (open triangle). Group A are morphine-dependent rapid progressors; Group B are morphine-dependent normal progressors; and Group C are control, non-morphine normal progressors. Clones are from 12 weeks (circles) or 20 weeks (squares) post infection. All trees were drawn to the same scale provided in the legend. The sequence for the original SIV17E-Fr (accession #AY033146) was used to root each tree (filled triangle). The scale bar in each tree represents 0.5% distance, or 1 change per 200 nucleotides. Bootstrap values, based on 100 replicates, that are greater than 70 are included. All sequences in this study were deposited into Genebank (DQ839744–DQ840024).

among the rapid progressors, but modest responses by 20 weeks in normal progressors (Kumar et al., 2006), are suggestive of a role for immune pressure in the enhanced compartmentalization we detected for both *tat* (earlier) and *vpr* in this work.

SIV vpr diversity and divergence are less in morphine-dependent rapid progressors

Similar to what has been reported for *tat* and the 5′ part of *env* (Noel and Kumar, 2006; Noel et al., 2006a, 2006b; Tirado and Kumar, 2006), the SIV *vpr* gene had the lowest overall diversity in Group A (Fig. 2). The average diversity of all clones was significantly lower in the rapid progressors than in all normal progressors (Fig. 2A, 0.31% Group A vs. 0.59% Group B+ C, $p<0.05$) as well as compared to the normal progressor, morphine dependent group alone (0.31% Group A vs. 0.64% Group B, $p<0.05$). Diversity was the lowest in Group A animals in both plasma and CSF when compared only in those compartments (Figs. 2B and C); however, the trend did not achieve statistical significance.

The *vpr* divergence in rapid progressors was also significantly less than in all normal progressors (Group A 0.16% vs. Groups B+ C 0.34%, $p<0.05$, Fig. 3A). Further, the divergence was significantly less than either Group B (0.16% vs. 0.35%, $p<0.05$, Fig. 3A) or Group C (0.16% vs. 0.32%, $p<0.05$, Fig. 3A) when all samples from both plasma and CSF were considered; as well as versus Group C specifically in the CSF (0.19% vs. 0.39%, $p<0.05$, Fig. 3C). Divergence in plasma

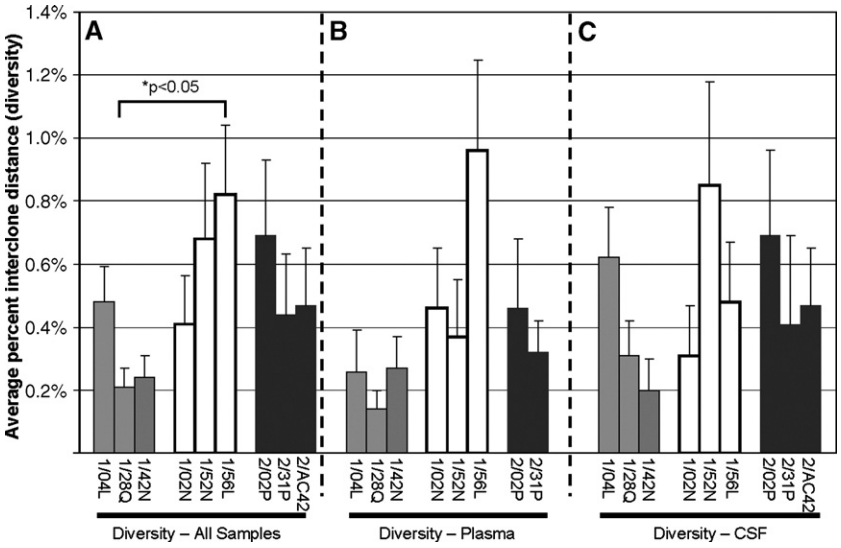

Fig. 2. For each animal, the sequenced clones for all samples (A), plasma only (B), or CSF only (C) were aligned (BioEdit) and a distance matrix was calculated (Mega, 3.0). The means of these distances for each time is plotted versus time for Group A: 1/04 L, 1/28Q, 1/42N , Group B: 1/02N, 1/52N, 1/56 L, and Group C: 2/02P, 2/31P, 2/AC42 to show the overall diversity of clones within each macaque including all times. Standard errors are indicated for each animal. Statistical differences in diversity between Groups were calculated by Student's *t*-test (Noel and Kumar, 2006; Noel et al., 2006a, 2006b). A value of 0.05 was set as the level of significance.

alone was lowest in Group A, but was not statistically less than in normal progressors (Fig. 3B).

Compartmentalization of virus occurs only in normal disease progression

The relationship between the full array of *vpr* sequence changes within an individual animal (the total diversity) and the evolution of the viral population from the origin sequence (divergence from the inoculum) was characterized by two measures. First, we compared the ratio between diversity to divergence within each monkey. This ratio was always the greatest (near 2.0) in the rapid progressors, including all samples (Fig. 4A), plasma only (Fig. 4B) and CSF only (Fig. 4C). In comparisons with rapid progressors, the ratio of diversity to divergence is statistically less for Groups B or C, with the single exception of Group C plasma for which data from one animal, 2/AC42, was unavailable. As a second measure, we assessed the divergence between the plasma and CSF populations in all three groups (Fig. 3D). The divergence was significantly greater in both Groups B and C as compared to Group A for all samples (Fig. 3D, $p<0.05$). This indicates a greater degree of specific selection of virus in these compartments in normal disease progression. Although we did not find evidence of different immune pressure by comparing the rates of synonymous/non-synonymous mutations (data not shown) as was the case for *tat* previously (Noel and Kumar, 2006; Noel et al., 2006a, 2006b), the greater divergence between plasma and CSF in Groups B and C suggests a different level of selective pressure, including the possibility of the emergence of a functional immune response. In fact, none of the Group A animals developed detectable virus-specific immunity, while both Group B and C macaques showed

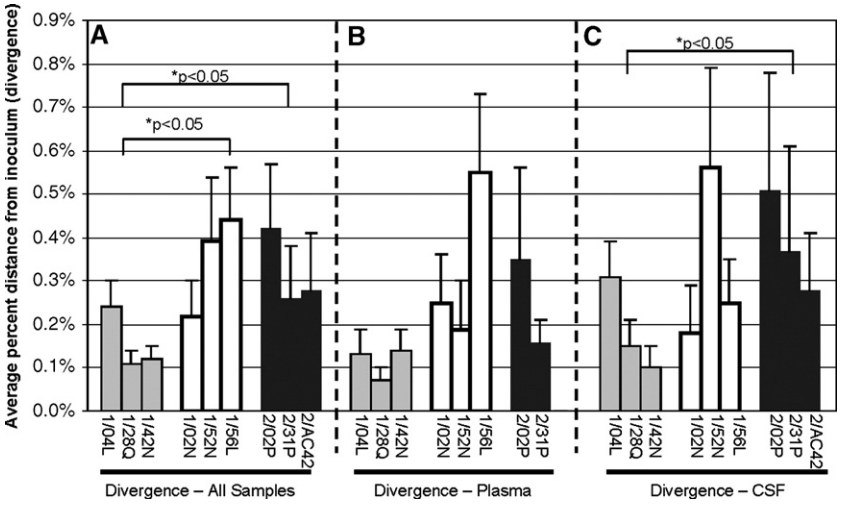

Fig. 3. The divergence of the clones within each animal from the inoculum for all samples (A), plasma only (B), or CSF only (C) were calculated using Mega 3.0. Standard error values are indicated by the error bars and significance of differences in divergence (Student's *t*-test) are indicated in each graph.

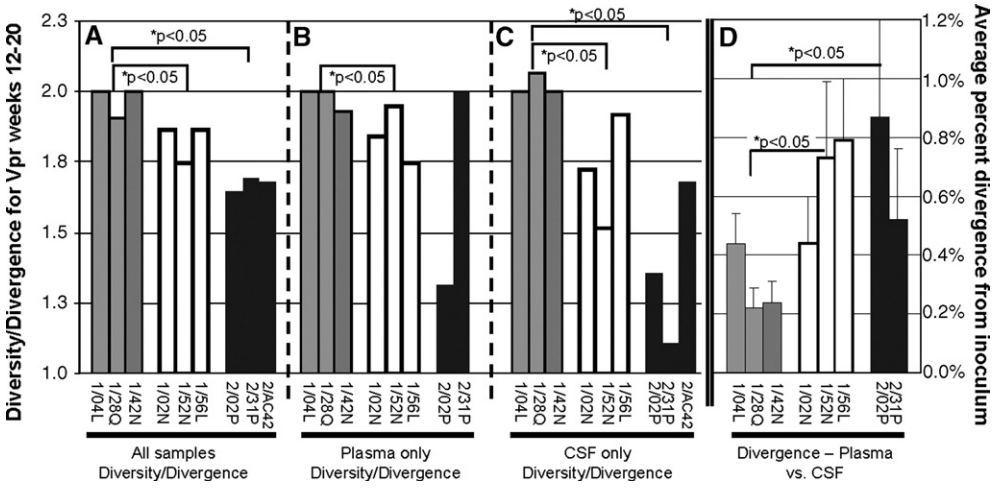

Fig. 4. The ratio of diversity to divergence for each macaque was calculated by dividing the mean diversity by the mean divergence as calculated for Figs. 3 and 4. These ratios were calculated for all samples (A), plasma only (B) and CSF only (C) and significant difference between the means of each group were calculated by Student's *t*-test. In (D), the divergence between all plasma clones and all CSF clones within each individual macaque was calculated using Mega 3.0. Standard errors are also graphed and statistical significance is indicated. Please note that the axis for (D) is to the right of the graph and that the scale is different than for (A–C).

evidence of a specific immune response to SIV, including binding and neutralizing antibodies as well as virus-specific CTL (Kumar et al., 2006).

Changes at the amino acid level do not appear to predict different rates of disease progression

Work reported previously by a number of groups have indicated the functional regions of Vpr responsible for a variety of the biological roles of this conserved lentiviral protein. For example, both the transactivation and cell cycle arrest functions are compromised by a change from arginine to serine at position 73(Sawaya et al., 2000). Cell cycle arrest can also be compromised by alteration of the c-terminus (amino acids 83–89) and at least once such mutations have been linked to long-term non-progression (Wang et al., 1996). We examined the deduced amino acid sequences for all *vpr* clones with unique, non-synonymous mutations (Fig. 5) to determine if we could find evidence of early changes in known functional domains leading to slowed progression. No qualitative differences are readily evident among the three groups in terms of absolute number nor distribution of amino acid substitutions. Furthermore, R73 described previously by Sawaya et al. is universally conserved in our clones. Interestingly, only monkeys in Groups B and C show any changes between 83 and 89 amino acids, that may be involved in G2 arrest (Wang et al., 1996), however, these mutants were not a majority for any macaque.

Conclusions

This work examines, for the first time, the evolution of the SIV *vpr* gene during rapid progression to simian AIDS in the setting of morphine abuse. Much like the SIV *tat* gene in both plasma and CSF or SIV *env* in plasma, evolution of *vpr* shows a significant inverse correlation with disease progression. As with *tat*, the virus appears to show compartmentalization only when disease progression occurs at a normal rate, regardless of the morphine status. This is supported both in the trees and in a direct comparison of the divergence between plasma and CSF viral populations which was significantly less in the rapid than in the normal progressors. In addition, in this report we provide the first direct comparison of the ratio of diversity to divergence for rapid and normal progressors. In all cases the total population diversity was greater than the average evolution away from the inoculum (divergence); however, the ratio was statistically greater in the rapid progressors, perhaps suggesting that biological selection had begun to promote evolution of particular branches from among the viral quasispecies only in the normal progressors. In fact, only in normal progressors was there any evidence of a modest immune response by 20 weeks post-inoculation (Kumar et al., 2006), indicating that immune selection may have contributed to the reduced diversity to divergence ratio. In contrast to the accessory gene product Nef (Noel et al., 2006a, 2006b), this study provides the first indication that the evolution is related to rapid disease progression during drug abuse for a gene product that is dispensable *in vitro*.

Previous work with HIV-infected humans has indicated that the evolution of Vpr is related to long-term nonprogression (Cali et al., 2005). Some long-term survivors have shown predominantly intact and functional vpr sequences, while lack of progression in an injecting drug user was associated with subtle changes in *nef* and the G2 arrest domain in the c-terminus of *vpr* (Saksena et al., 1996; Zhang et al., 1997). Thus, the role of *vpr* evolution in slow disease progression in humans may be distinct from that of rapid disease in our model, although the study of an individual drug user may lend a parallel to our system as we carry out analysis beyond 20 weeks. Perhaps a better comparison can be made with studies of the role of *vpr* sequence changes on HIV transmission. In this case, viral *vpr* sequences showing greater hetereogeneity proved less infectious during vertical transmission (Yedavalli and Ahmad, 2001), perhaps due to greater likelihood of loss of important functional components of the Vpr protein (Yedavalli et al., 1998). In these studies as well as

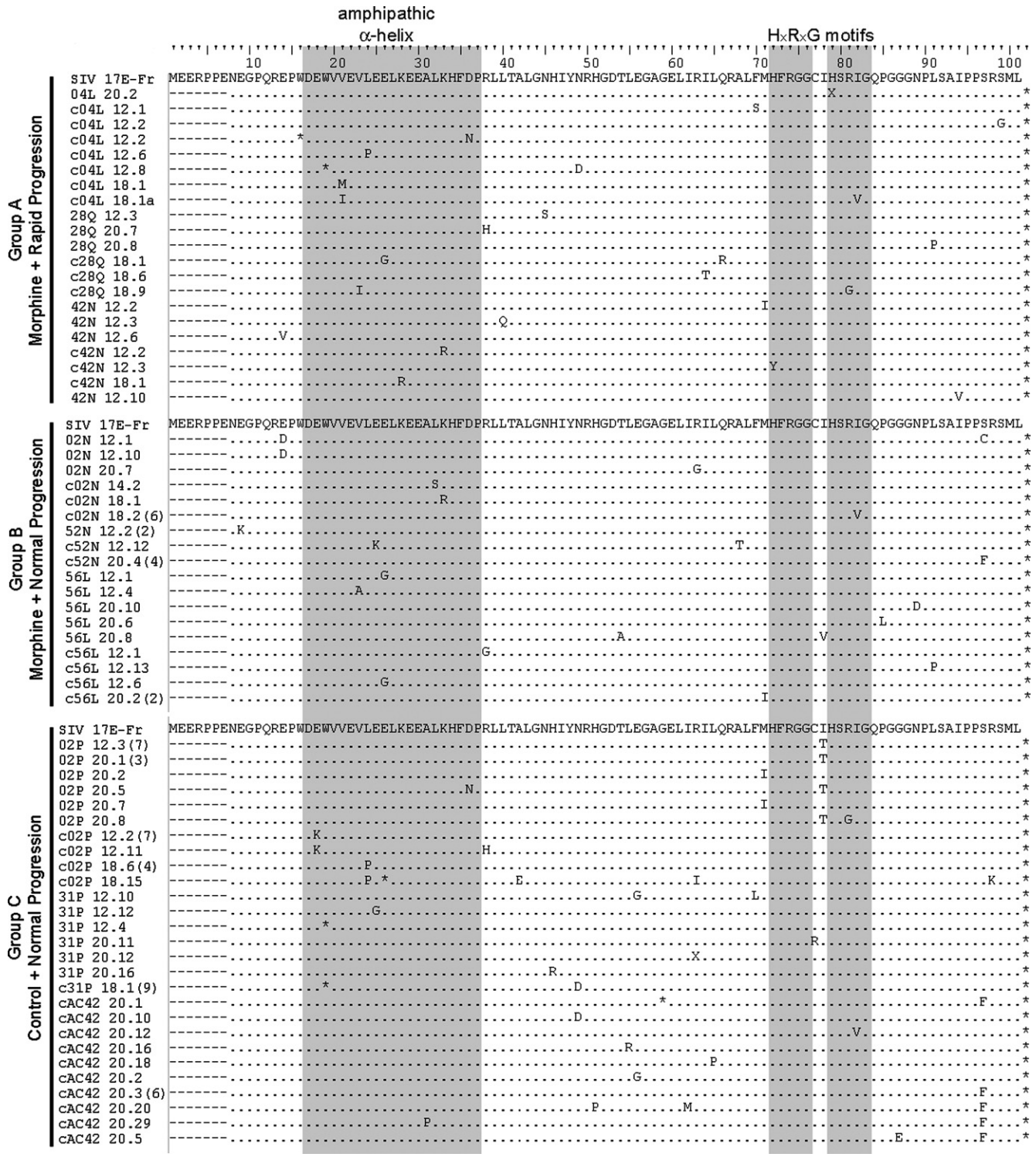

Fig. 5. The *vpr* coding sequences were translated to amino acids using BioEdit. Only sequences with nucleotide changes causing an amino acid substitution (non-synonymous change) are included in each group. SIV7E-Fr and serves as the reference within each group. Each sequence is represented by *animal week-clone* in the left column and all changes from 17E are indicated in the right column. The number of clones for each unique amino acid sequence is indicated in parenthesis to the right of each sequence name. Positions of identity are indicated by a dot (.), frameshift mutations are indicated by (X) and stop codons by an asterisk.

our own work, greater virulence is associated with greater conservation of the infecting form.

The lack of humoral responses to Vpr have also been implicated in rapid progression (Richardson et al., 2003). Thus the well known roles in immune interference (Muthumani et al., 2002a)and T cell apoptosis (Muthumani et al., 2002b)played by Vpr *in vivo* may be involved in our system, even though we are looking predominantly at rapid pathogenesis. We do see greatly depleted levels of CD4+ T cells and, predominantly in the rapid progressors, we have reported a failure to mount any detectable

immune response (Kumar et al., 2006). Although, these data do not clearly define the spectrum of amino acid changes that differentiate our rapid from our normal progressors, based on the pathogenicity of the inoculum virus and the more rapid rate of change in the normal progressors, it is possible that the mutations that occur within the first 20 weeks of infection in normal progressors contribute to the slower progression in their respective hosts beyond this time period.

Finally, although we still cannot describe the full role of morphine in our system, we do find that only morphine-dependent monkeys progress rapidly, and at a rate of 50%. A picture is emerging that the evolution of some (*tat, env, vpr)* but not all (*nef)* viral genes is directly related to the rate of pathogenesis in the setting of drug abuse, but the cause effect relationship is unclear. Morphine has well characterized effects on cells of the immune system, but in spite of years of study, there remains the question of the role of morphine in HIV progression – whether it advances or prolongs the time to (and severity of) AIDS. Our current work does suggest, as with *tat* and *env*, that *vpr* evolution is a potentially important component of early determination of disease progression and rapid pathogenesis in morphine-dependent AIDS.

Materials and methods

Animal model

This study used a previously described rhesus macaque model of drug abuse and AIDS (Kumar et al., 2004a; Noel and Kumar, 2006). Nine male monkeys (*Macaca mulatta*) were divided into morphine-dependent ($N=6$) or non-morphine controls ($N=3$). Prior to viral infection, morphine was administered for 18 weeks (5 mg/kg, T.I.D.) following a period of morphine introduction with gradual dose increase from 1 to 5 mg/kg over 2 weeks (Kumar et al., 2004a). Morphine administration was maintained throughout the study to avoid withdrawal effects. Infection was by intravenous route with a 2 mL inoculum containing 10^4 50% tissue culture infective doses each of simian-human immunodeficiency virus KU-1B ($SHIV_{KU-1B}$), (Singh et al., 2002) $SHIV_{89.6P}$(Reimann et al., 1996) and SIV 17E-Fr (SIV/17E-Fr)(Flaherty et al., 1997). This three-virus combination produces uniform disease leading to clinical AIDS in a relatively short time (Kumar et al., 2002). In this group of animals, the SIV 17E-Fr virus shows the greatest tissue distribution and generates the predominant immune response (Kumar et al., 2006). All animal protocols were approved by the local animal care committee (IACUC) in accordance with the Guide for the Care and Use of Laboratory Animals.

Amplification, cloning and sequencing of 5′ exon of tat

Viral RNA was extracted from cell-free fluids (plasma and CSF) at approximately 12 and 20 weeks post infection. In cases where samples were unavailable, samples at 14 or 18 weeks served as substitutes. Primers (specific for SIV 17E-Fr) and PCR conditions were as reported previously (Noel and Kumar, 2006; Noel et al., 2006a, 2006b). Briefly, an RT-PCR reaction (primers F1 5′-GGCAGGGGGATGGAGACCAGG and R1 5′-GCACAAAAAAGGGGAATTGTCGC) was followed by a secondary PCR reaction (primers F2 5′-AAATGAAGGACCA-CAAAGGGAACC and R2 5′-CCCATAGACACTTAAAAG-CAAGATGGC) to generate a 491 base pair DNA fragment. The 5′ end of this product, that encodes the C-terminal 94 amino acids of the SIV Vpr protein, was sequenced after cloning into pPCR2.1 (Invitrogen, Carlsbad, CA). A target of 6–10 positive clones per time point were sent for sequencing to the DNA Sequencing Facility of Florida State University, Department of Biological Sciences. In general, two or more independent PCR/cloning reactions were used to generate sufficient clones. All sequences in this study were deposited into Genebank (DQ839744–DQ840024).

Sequence editing and alignment and statistics

Sequence files (*.ab1 format) were decoded and edited using BioEdit version 7.0.1(Hall, 1999). Alignments were performed using BioEdit after removal of primer sequences and included the 282 nucleotides encoding the final 94 amino acids of SIV Vpr. Amino acid sequences were inferred by translation of the nucleotide alignments using the standard genetic code. Intraindividual diversity for all clones as well as divergence from the inoculum clone were calculated using MEGA3.1(Kumar et al., 2004b). Phylogenetic trees were formed using a neighbor-joining method (Saitou and Nei, 1987). Group diversity and divergence were compared statistically using an unpaired *t*-test. The statistical cut-off for significance in these analyses was $p=0.05$.

Acknowledgments

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References

Aldrovandi, G.M., Zack, J.A., 1996. Replication and pathogenicity of human immunodeficiency virus type 1 accessory gene mutants in SCID-hu mice. J. Virol. 70, 1505–1511.

Cali, L., Wang, B., Mikhail, M., Gill, M.J., Beckthold, B., Salemi, M., Jans, D., Piller, S., Saksena, N., 2005. Evidence for host-driven selection of the HIV type 1 vpr gene in vivo during HIV disease progression in a transfusion-acquired cohort. AIDS Res. Hum. Retrovir. 21, 728–733.

Chen, R., Le Rouzic, E., Kearney, J.A., Mansky, L.M., Benichou, S., 2004. Vpr-mediated incorporation of UNG2 into HIV-1 particles is required to modulate the virus mutation rate and for replication in macrophages. J. Biol. Chem. 279, 28419–28425.

Chuang, R.Y., Suzuki, S., Chuang, T.K., Miyagi, T., Chuang, L.F., Doi, R.H., 2005. Opioids and the progression of simian AIDS. Front. Biosci. 10, 1666–1677.

Desrosiers, R.C., Lifson, J.D., Gibbs, J.S., Czajak, S.C., Howe, A.Y., Arthur, L.O., Johnson, R.P., 1998. Identification of highly attenuated mutants of simian immunodeficiency virus. J. Virol. 72, 1431–1437.

Di Marzio, P., Choe, S., Ebright, M., Knoblauch, R., Landau, N.R., 1995. Mutational analysis of cell cycle arrest, nuclear localization and virion packaging of human immunodeficiency virus type 1 Vpr. J. Virol. 69, 7909–7916.

Donahoe, R.M., 2004. Multiple ways that drug abuse might influence AIDS progression: clues from a monkey model. J. Neuroimmunol. 147, 28–32.

Dull, T., Zufferey, R., Kelly, M., Mandel, R.J., Nguyen, M., Trono, D., Naldini, L., 1998. A third-generation lentivirus vector with a conditional packaging system. J. Virol. 72, 8463–8471.

Flaherty, M.T., Hauer, D.A., Mankowski, J.L., Zink, M.C., Clements, J.E., 1997. Molecular and biological characterization of a neurovirulent molecular clone of simian immunodeficiency virus. J. Virol. 71, 5790–5798.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows95/98/NT. Nucleic Acids Symp. 41, 95–98.

Kapadia, F., Vlahov, D., Donahoe, R.M., Friedland, G., 2005. The role of substance abuse in HIV disease progression: reconciling differences from laboratory and epidemiologic investigations. Clin. Infect. Dis. 41, 1027–1034.

Kondo, E., Mammano, F., Cohen, E.A., Gottlinger, H.G., 1995. The p6gag domain of human immunodeficiency virus type 1 is sufficient for the incorporation of Vpr into heterologous viral particles. J. Virol. 69, 2759–2764.

Kumar, A., Mukherjee, S., Shen, J., Buch, S., Li, Z., Adany, I., Liu, Z., Zhuge, W., Piatak Jr., M., Lifson, J., McClure, H., Narayan, O., 2002. Immunization of macaques with live simian human immunodeficiency virus (SHIV) vaccines conferred protection against AIDS induced by homologous and heterologous SHIVs and simian immunodeficiency virus. Virology 301, 189–205.

Kumar, R., Torres, C., Yamamura, Y., Rodriguez, I., Martinez, M., Staprans, S., Donahoe, R.M., Kraiselburd, E., Stephens, E.B., Kumar, A., 2004a. Modulation by morphine of viral set point in rhesus macaques infected with simian immunodeficiency virus and simian-human immunodeficiency virus. J. Virol. 78, 11425–11428.

Kumar, S., Tamura, K., Nei, M., 2004b. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief. Bioinform. 5, 150–163.

Kumar, R., Orsoni, S., Norman, L., Verma, A.S., Tirado, G., Giavedoni, L.D., Staprans, S., Miller, G.M., Buch, S.J., Kumar, A., 2006. Chronic morphine exposure causes pronounced virus replication in cerebral compartment and accelerated onset of AIDS in SIV/SHIV-infected Indian rhesus macaques. Virology 354, 205–219.

Le Rouzic, E., Benichou, S., 2005. The Vpr protein from HIV-1: distinct roles along the viral life cycle. Retrovirology 2, 11.

Luciw, P., 1996. Human immunodeficiency viruses and their replication. In: Fields, B., Knipe, D., Howley, P., Chanock, R., Melnick, J., Monath, T., Roizman, B., Straus, S. (Eds.), Fields Virology. Lippincott-Raven Publishers, Philadelphia, PA, pp. 1881–1975.

Mahalingam, S., Ayyavoo, V., Patel, M., Kieber-Emmons, T., Weiner, D.B., 1997. Nuclear import, virion incorporation, and cell cycle arrest/differentiation are mediated by distinct functional domains of human immunodeficiency virus type 1 Vpr. J. Virol. 71, 6339–6347.

Mueller, S.M., Lang, S.M., 2002. The first HxRxG motif in simian immunodeficiency virus mac239 Vpr is crucial for G2/M cell cycle arrest. J. Virol. 76, 11704–11709.

Muthumani, K., Hwang, D.S., Dayes, N., Kim, J., Weiner, D.B., 2002a. The HIV-1 accessory gene vpr can inhibit antigen-specific immune function. DNA Cell Biol. 21, 689–695.

Muthumani, K., Hwang, D.S., Desai, B.M., Zhang, D., Dayes, N., Green, D.R., Weiner, D.B., 2002b. HIV-1 Vpr induces apoptosis through caspase 9 in T cells and peripheral blood mononuclear cells. J. Biol. Chem. 277, 37820–37831.

Noel Jr., R.J., Kumar, A., 2006. Virus replication and disease progression inversely correlate with SIV tat evolution in morphine-dependent and SIV/SHIV-infected Indian rhesus macaques. Virology 346, 127–138.

Noel Jr., R.J., Marrero-Otero, Z., Kumar, R., Chompre-Gonzalez, G.S., Verma, A.S., Kumar, A., 2006a. Correlation between SIV Tat evolution and AIDS progression in cerebrospinal fluid of morphine-dependent and control macaques infected with SIV and SHIV. Virology 349, 440–452.

Noel, R.J., Toro-Bahamonde, A., Marrero-Otero, Z., Kumar, R., Kumar, A., 2006b. Lack of correlation between SIV Nef evolution and rapid disease progression in morphine-dependent non-human primate model of AIDS. AIDS Res. Hum. Retrovir. 22, 817–823.

Patel, C.A., Mukhtar, M., Harley, S., Kulkosky, J., Pomerantz, R.J., 2002. Lentiviral expression of HIV-1 Vpr induces apoptosis in human neurons. J. NeuroVirol. 8, 86–99.

Paxton, W., Connor, R.I., Landau, N.R., 1993. Incorporation of Vpr into human immunodeficiency virus type 1 virions: requirement for the p6 region of gag and mutational analysis. J. Virol. 67, 7229–7237.

Philippon, V., Matsuda, Z., Essex, M., 1999. Transactivation is a conserved function among primate lentivirus Vpr proteins but is not shared by Vpx. J. Hum. Virol. 2, 167–174.

Piller, S.C., Ewart, G.D., Jans, D.A., Gage, P.W., Cox, G.B., 1999. The amino-terminal region of Vpr from human immunodeficiency virus type 1áForms ion channels and kills neurons. J. Virol. 73, 4230–4238.

Planelles, V., Jowett, J.B., Li, Q.X., Xie, Y., Hahn, B., Chen, I.S., 1996. Vpr-induced cell cycle arrest is conserved among primate lentiviruses. J. Virol. 70, 2516–2524.

Purcell, D.W., Metsch, L.R., Latka, M., Santibanez, S., Gomez, C.A., Eldred, L., Latkin, C.A., 2004. Interventions for seropositive injectors—research and evaluation: an integrated behavioral intervention with HIV-positive injection drug users to address medical care, adherence, and risk reduction. J. Acquired Immune Defic. Syndr. 37 (Suppl. 2), S110–S118.

Reimann, K.A., Li, J.T., Veazey, R., Halloran, M., Park, I.W., Karlsson, G.B., Sodroski, J., Letvin, N.L., 1996. A chimeric simian/human immunodeficiency virus expressing a primary patient human immunodeficiency virus type 1 isolate env causes an AIDS-like disease after in vivo passage in rhesus monkeys. J. Virol. 70, 6922–6928.

Richardson, M.W., Mirchandani, J., Duong, J., Grimaldo, S., Kocieda, V., Hendel, H., Khalili, K., Zagury, J.F., Rappaport, J., 2003. Antibodies to Tat and Vpr in the GRIV cohort: differential association with maintenance of long-term non-progression status in HIV-1 infection. Biomed. Pharmacother. 57, 4–14.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Saksena, N.K., Ge, Y.C., Wang, B., Xiang, S.H., Dwyer, D.E., Randle, C., Palasanthiran, P., Ziegler, J., Cunningham, A.L., 1996. An HIV-1 infected long-term non-progressor (LTNP): molecular analysis of HIV-1 strains in the vpr and nef genes. Ann. Acad. Med. Singapore 25, 848–854.

Sawaya, B.E., Khalili, K., Gordon, J., Srinivasan, A., Richardson, M., Rappaport, J., Amini, S., 2000. Transdominant activity of human immunodeficiency virus type 1 Vpr with a mutation at residue R73. J. Virol. 74, 4877–4881.

Singh, D.K., McCormick, C., Pacyniak, E., Griffin, D., Pinson, D.M., Sun, F., Berman, N.E., Stephens, E.B., 2002. Pathogenic and nef-interrupted simian-human immunodeficiency viruses traffic to the macaque CNS and cause astrocytosis early after inoculation. Virology 296, 39–51.

Tirado, G., Kumar, A., 2006. Evolution of SIV envelope in morphine-dependent rhesus macaques with rapid disease progression. AIDS Res. Hum. Retrovir. 22, 114–119.

Vogt, V., 1997. Retrovrial virions and genomes. In: Coffin, J., Hughes, S., Varmus, H. (Eds.), Retroviruses. Cold Spring Harbor Laboratory Press, Plainville, NY.

Wang, B., Ge, Y.C., Palasanthiran, P., Xiang, S.H., Ziegler, J., Dwyer, D.E., Randle, C., Dowton, D., Cunningham, A., Saksena, N.K., 1996. Gene defects clustered at the C-terminus of the vpr gene of HIV-1 in long-term nonprogressing mother and child pair: in vivo evolution of vpr quasispecies in blood and plasma. Virology 223, 224–232.

Yedavalli, V.R., Ahmad, N., 2001. Low conservation of functional domains of HIV type 1 vif and vpr genes in infected mothers correlates with lack of vertical transmission. AIDS Res. Hum. Retrovir. 17, 911–923.

Yedavalli, V.R., Chappey, C., Ahmad, N., 1998. Maintenance of an intact human immunodeficiency virus type 1ávpr gene following mother-to-infant transmission. J. Virol. 72, 6937–6943.

Zhang, L., Huang, Y., Yuan, H., Tuttleton, S., Ho, D.D., 1997. Genetic characterization of vif, vpr, and vpu sequences from long-term survivors of human immunodeficiency virus type 1 infection. Virology 228, 340–349.

Zhu, Y., Gelbard, H.A., Roshal, M., Pursell, S., Jamieson, B.D., Planelles, V., 2001. Comparison of cell cycle arrest, transactivation, and apoptosis induced by the simian immunodeficiency virus SIVagm and human immunodeficiency virus type 1 vpr genes. J. Virol. 75, 3791–3801.