Published in final edited form as:

J Neuroimmune Pharmacol. 2010 March ; 5(1): 122–132. doi:10.1007/s11481-009-9184-0.

Morphine and Rapid Disease Progression in Nonhuman Primate Model of AIDS: Inverse Correlation Between Disease Progression and Virus Evolution

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Abstract

HIV and simian immunodeficiency virus (SIV) have a formidable capacity for mutation and adaptation, a characteristic that has contributed to the extensive genetic variability. Evolutionary pressures imposed within the host and the viral capacity to mutate lead to the generation of such variants. To date, very little information is available regarding the evolution of HIV with drug abuse as a cofounding factor. Using our macaque model of drug dependency and AIDS, we have investigated the dynamics of SIV mutations in the genes *tat*, *vpr*, envelope, and *nef*. The results presented in this review, from our laboratory and others, contribute to the overall understanding of how drugs of abuse might influence immune selective pressure contribution to variation in different SIV genes. Additionally, the studies presented could help enlighten the development of HIV vaccines that take into consideration viral diversity.

Keywords

morphine; SIV; viral evolution; AIDS

Introduction

Injection drug use (IDU) is one of the main risk factors associated with contracting HIV-1. A recent Centers for Disease Control and Prevention estimate indicated that, as of 2006, approximately 28% of the total AIDS-related deaths in the USA were associated with IDU (Centers for Disease Control and Prevention 2006). However, the natural history and progression of HIV-1 infection among IDU remains ambiguous and complex. Heroin, cocaine, and methamphetamine are among the most widely abused drugs in the USA. However, because drug abusers tend to use multiple drugs at different times, researchers have difficulty in directly linking specific drugs of abuse and the observed clinical health parameters in HIV-1-infected individuals.

The best way to resolve these ambiguities is by the use of an appropriate animal model, such as the nonhuman primate model of HIV/AIDS. However, confounding differences between simian immunodeficiency virus (SIV) and HIV-1 have somewhat hindered the correlation of results obtained from macaques with human studies. First, although SIV infection of macaques causes an AIDS-like syndrome, it does not lead to a massive loss of CD4⁺ T cells, characteristic of HIV-1 infection, until late in infection. Another significant difference between HIV-1 and SIV infection is that the latter one does not have a homolog for the HIV-1 *vpu* gene. Both of these difficulties have been overcome by the introduction of the simian-human immunodeficiency virus (SHIV) macaque model of AIDS, wherein macaques not only develop a highly productive infection in the lymphoid system, similar to that seen in SIV infection (Daniel et al. 1985; Nathanson et al. 1999), but also exhibit the near-total elimination of the CD4⁺ T cells in blood and lymphoid tissue during acute infection (Igarashi et al. 1999; Joag et al. 1996; Karlsson et al. 1997; Luciw et al. 1995; Reimann et al. 1999). The animals developing rapid disease (i.e., AIDS within 6–8 months) caused by pathogenic SHIV do not develop antiviral immune responses (Kumar et al. 2006). However, those surviving for longer periods develop both cellular and humoral immune responses (Kumar et al. 2001, 2006; Silverstein et al. 2000; Stipp et al. 2000). Prolonged survival of the infected animal has been correlated with humoral immune responses mediated by neutralizing antibodies and cellular immune responses mediated by CD8⁺ T cells. Such responses are similar to those found in chronic HIV infection.

We are interested in the effects that morphine has on both host selective pressures as well as viral factors in controlling the susceptibility to infection and the accelerated form of disease progression. Numerous studies support the notion that morphine has deleterious effects on the immune system and increases the rate of HIV/AIDS disease progression (Arora et al. 1990; Beagles et al. 2004; Malik et al. 2002; Perez-Casanova et al. 2007). In our model of HIV/AIDS, we have previously shown that although chronic morphine dependence did not alter the peak viral loads in rhesus macaques, it caused significantly higher viral replication in the brain at 6 weeks postinfection as well as causing a similar effect in the blood at 8 weeks postinfection (Kumar et al. 2004). We have also shown that morphine dependence causes accelerated onset of clinical AIDS in 50% of the SIV-/SHIV-infected animals, and these animals—unlike the remainder of the morphine-dependent and control animals—did not develop virus specific immune responses (Kumar et al. 2006). The three animals that did not develop immune responses succumbed to SIV-/SHIV-induced AIDS within 20 weeks

after infection. None of the control animals had developed AIDS within 60 weeks postinfection.

Our results prompted the question of how morphine altered the interaction between the host and the virus that ultimately led to rapid progression to AIDS and death. Based on the importance of both viral replication and the immune response to drive viral evolution, we used this model to investigate the possible influence of viral sequence evolution on driving viral pathogenesis.

How do opiates influence HIV/AIDS disease progression?

Opiates have been shown to adversely affect the immune system. Opiates, for example morphine, can disrupt the innate immune system. In vitro and in vivo studies have shown that morphine modulates phagocytic cells and nonspecific cytotoxic T cells ($\gamma\delta$ T), natural killer cells, and dendritic cells, all of which are functionally important for establishing first line of defense against invading pathogens (Eisenstein and Hilburger 1998; Messmer et al. 2006; Saurer et al. 2006; Wang et al. 2008). Opiate use also produces indirect evidence of compromised immune function, including substantially slowed healing and promotion of sepsis (Hilburger et al. 1997; Wang et al. 2008, 2005).

Epidemiological studies that attempt to determine the effects of opiates on disease progression in HIV-1-infected individuals face numerous obstacles. As discussed in Donahoe (2004) and Ansari (2004), such studies are confounded by a number of factors. One difficulty in such studies is obtaining a representative sample population. Subjects in an early phase of the disease, who are not in particularly ill health, may not be disposed to participate in such a study. In addition, those who are enrolled may be using multiple drugs of abuse. As discussed by both Ansari and Donahoe, the viral clade (s) responsible for the infection must also be determined because different clades exhibit different biological properties. The presence of opportunistic infections (e.g., cytomegalovirus, tuberculosis, pneumocystis, etc.) and the susceptibility of drug abusers to such infections also confound meaningful data analyses.

Thus, the effect of opiates on HIV disease progression is still controversial. In order to fill the gaps in human studies, the nonhuman primate model of AIDS has been used extensively. Despite the advantages of utilizing animal models to investigate the role of opiates in the progression of HIV/AIDS, different models have produced substantially different results. Donahoe et al. infected six opiate-dependent rhesus monkeys with the sooty mangabey strain of SIV (SIVsmm9). In this study, they found that continuous administration of opiates was not associated with exacerbation of the course of infection and development of simian AIDS. Indeed, AIDS-like symptoms that normally develop in SIV-infected monkeys were expressed to a lower extent in the opiate-dependent animals. Most notably, no opiate-dependent animals died in the first 2 years of the study from AIDS-related symptoms (Donahoe et al. 1993). Although this study utilized small number of animals and no saline-injected controls, Donahoe et al. reevaluated the effects of opiate dependency on development of simian AIDS using a larger cohort of animals (Donahoe et al. 2009). Viral, immunological, clinical and pathological parameters were evaluated on rhesus macaques

prior to and after establishment of opiate dependency and SIV infection. Although there was no statistically significant difference in mean viral titers over the course of the study, there were significant differences between control and opiate-treated macaques with regard to viral kinetics and survival that indicated a protective effect of opiate treatment (Donahoe et al. 2009). Based on these results, it could be argued that opiates provide protection from HIV/AIDS disease progression. A relevant observation is that slow AIDS progression rate was attributed to a well-maintained, low-stress opiate dependency; thus, a shift in this balance may worsen progression.

In contrast, studies by Chuang et al. demonstrated that morphine increased SIV replication in T cells (Chuang et al. 1993a) and decreased cellular and humoral immune responses in rhesus macaques infected with SIVmac239 (Chuang et al. 1993b). In the 1993 study, Chuang et al. infected three morphine-dependent and three saline-injected macaques and evaluated the correlation between the chronic administration of opioids and its effects on both cellular and humoral immune responses (Chuang et al. 1993b). The CD8⁺ T cells from morphine-dependent macaques exhibited a transient reduction in their ability to control SIV replication. In addition, morphine-dependent macaques exhibited a lower titer of SIV neutralizing antibodies (Chuang et al. 1993b). In 2005, the group reported that chronic opioid use resulted in an increased virus replication and mutation rates and a shorter life span for the infected animals (Chuang et al. 2005).

Kapadia et al. attributed the conflicting effects of opioids on disease progression between the two groups to differences in study design, including use of a less pathogenic strain and a higher dose of morphine in the study by Donahoe (Kapadia et al. 2005). As stated above, another difference between these studies is the variation in stress levels associated with opiate dosing—5 mg/kg at 8 h intervals in Chuang and Kumar studies (see below) versus 3 mg/kg at 6 h intervals in the Donahoe study. The Chuang and Kumar studies more faithfully recapitulate the opiate pharmacokinetic parameters (e.g., withdrawal) experienced by IDU, whereas the Donahoe studies provide relevant considerations to the clinical management of opiate-dependent human subjects with AIDS.

In our model, we used six rhesus macaques that were made dependent on morphine over a period of 20 weeks prior to viral infection and three macaques that underwent saline injections (control animals). Morphine dependence was established by injecting increasing doses of morphine (1 to 5 mg/kg of body weight over a 2-week period) by the intramuscular route at 8-h intervals. Control animals received saline injections via the same route and schedule. The animals were then maintained at three daily doses of morphine (5 mg/kg) for an additional 18 weeks prior to infection. We then infected morphine-dependent and control macaques with a mixed virus inoculum, combining SHIV_{KU-1B}, SHIV_{89.6P}, and SIV/17E-Fr, and morphine dosing was maintained throughout the study. The use of the three virus mixture resulted in depletion of circulating CD4⁺ T cells early after infection, allowing the development of simian AIDS in an experimentally useful time period. This model also includes known neurovirulent viruses, SIV/17E-Fr and SHIV_{KU-1B}, thus allowing assessment of opioid effects on central nervous system (CNS) infection (Buch et al. 2002; Flaherty et al. 1997). Analysis of peripheral CD4⁺ T cells revealed that both morphine and control groups experienced massive CD4⁺ T cell loss; however, this loss was more

prominent by week 2 in the morphine-treated than in the control group. Both the plasma and cerebrospinal fluid (CSF) viral loads were initially indistinguishable between the two groups, but as infection progressed, the morphine-dependent macaques exhibited significantly higher viral loads in both compartments than the control group (Kumar et al. 2004). It was later reported that four of the six morphine-dependent and none of the control animals had died by week 51 postinfection. Peripheral monocyte counts in these animals increased concomitantly with the development of disease and was accompanied by elevation of CC chemokine ligand 2/monocyte chemotactic protein 1 (MCP-1) in plasma. This model system provides evidence for an accelerated disease progression in half of the morphine-addicted animals (rapid progressors)—a phenotype that was absent in the control infected counterparts. One third of morphine-dependent macaques, as well as all control animals, did not develop AIDS in the first year following infection.

Of the four morphine-treated animals that developed AIDS, three had dramatic elevations of virus in the CSF and presented neurological symptoms. Examination of viral genotype in the CSF revealed that both SHIV_{KU-1B} and SIV/17E-FR, but not SHIV_{89.6P}, were neuroinvasive (Kumar et al. 2006). There is very little information available regarding the effects of drug abuse on the evolution of viral quasiespecies and their function in promoting particular manifestations of AIDS such as neurological impairment. The extent to which genetic variation contributes to individual differences in neuro-AIDS progression is unknown. Given that the brain is a predominant site of action of drugs of abuse and that HIV-1 load in the CSF does not precisely reflect damage in the brain, understanding of host and viral genetic factors associated with drug abuse and neuroAIDS progression is of great interest. Consequently, we have developed a model in which not only the effects of opiates on SIV/SHIV can be studied reproducibly but which also allows the study of the effects of opiates on virus in the CNS with two viral genotypes capable of crossing into the CNS and replicating to high levels. Our model can certainly be used for continuing studies on the effects of opiates on AIDS progression, for a more in-depth investigation of neuro-AIDS, as well as for evaluation of other drugs of abuse.

Based on the model described above, we then wanted to determine which factors correlate with disease progression. Our goal has been to determine how morphine affects disease progression, tissue compartmentalization, and viral evolution. To date, we have analyzed the association between disease progression and viral evolution of SIV *tat*, *vpr*, envelope (*env*), and *nef* genes. We have identified several patterns of evolution related to disease progression, depending on the viral genome region, indicating the possibility of a unique role for each viral gene in morphine induced rapid disease progression to AIDS.

***tat* and *vpr* evolution slowed in rapid pathogenesis in our morphine-addicted macaques**

The transactivator of transcription (Tat) and the viral protein R (Vpr) are two of the proteins produced by HIV. The Tat protein, 14 kDa, has a primary role in the transactivation of HIV transcription. Tat interacts with cellular proteins and facilitates the transcription of viral RNA from the long terminal repeat via the transactivation response element (Laschia et al. 1989; Rice and Mathews 1988; Zhu et al. 1997). Tat has also been implicated in many other

biological processes, including neurotoxicity in both in vitro and in vivo systems, increased expression of adhesion molecules on endothelial cells, and induction of expression of MCP-1 and interleukin-8, among others (Buonaguro et al. 1992; Philippon et al. 1994; Pu et al. 2003; Rappaport et al. 1999; Rautonen et al. 1994). While Tat is essential for efficient transcription of viral genes and viral replication, Vpr is dispensable for viral growth in many in vitro systems (Aldrovandi and Zack 1996; Le Rouzic and Benichou 2005). However, in vivo studies have revealed the essential function of this protein in viral pathogenesis. The Vpr protein, 12–14 kDa, is produced late in the virus replication cycle and is also found within the viral particle. Vpr, like Tat, has been associated with multiple functions in the viral infection process, including import of the HIV pre-integration complex, induction of cell cycle G2 arrest, and induction of apoptosis in infected cells (Andersen et al. 2008; Di Marzio et al. 1995; Heinzinger et al. 1994; Jowett et al. 1995). Because of the numerous potential functions of both Tat and Vpr in HIV infection, it is likely that the specific functions of these proteins depend on the type of cell infected or treated and the level of protein expression.

Using the SIV/SHIV macaque model described above, we analyzed cloned plasma-derived sequences of SIV17E-Fr isolated from each animal. The results showed a remarkably consistent pattern of inverse correlation with disease progression (Noel and Kumar 2006, 2007; Noel et al. 2006a). Phylogenetic tree analysis revealed that the animals with fast disease progression exhibited the lowest overall sequence diversity for *tat* (Noel and Kumar 2006; Noel et al. 2006a) and *vpr* (Noel and Kumar 2007) as compared to morphine-addicted normal progressors and nonmorphine controls. As may be expected, the evolution showed some relationship to immune status, as the rapid progressors failed to mount any detectable immune response, while the morphine normal progressors and controls each showed virus specific T cells by 4 weeks postinfection (pi) and binding antibodies by 8 weeks pi. In CSF, for *tat* and *vpr*, rapidly progressing, morphine-addicted monkeys had roughly half the overall sequence diversity as the control animals. The *vpr* divergence was also significantly less in rapid progressors as compared to normal progressors and control monkeys. In addition, there did not appear to be an imbalance between synonymous and amino acid changing mutations among the groups nor were there regions of either protein that emerged as hot spots. Rather the amino acid changes were broadly distributed over the protein sequences derived from the nucleotide translations. This pattern was true even in controls and morphine normal progressors, all of which showed viral specific immune responses. In contrast, viral quasispecies compartmentalization between the plasma and CSF was evident in the clustering patterns of sequence clones from all animals with detectable immune responses but notably absent in the three rapid progressors.

Role of Env in pathogenesis and its conflicting pattern of evolution within variable regions

HIV and SIV deliver their genetic material into the cell by direct fusion of the viral membrane with the plasma membrane of the host cell. The fusion is mediated by Env glycoproteins, gp120, and gp41, which are organized into trimeric units and anchored in the viral membrane by the gp41 transmembrane protein (Wyatt and Sodroski 1998). The Env

glycoprotein, gp120, forms surface spikes, which are tightly but noncovalently associated with a gp41 trimer that is hidden and buried within the gp120 trimer. A comprehensive review of HIV-Env-mediated fusion reaction is described elsewhere (Gallo et al. 2003). Some studies suggest that activities of human lentiviral envelope proteins extend beyond their role in viral entry. For example, Env signals through receptor and coreceptor molecules after envelope binding, an activity that may alter target cell function to increase the cellular permissivity to virus infection (Cicala et al. 2002). In addition, the HIV-1 Env, like other retroviral envelope proteins, contains domains that interact with the intracellular signal transduction machinery to promote changes in cell function (Micoli et al. 2000). For example, HIV Env (Swingler et al. 2007) (1) regulates the prosurvival cytokine M-CSF, (2) restricts expression of death receptors on infected macrophages, and (3) regulates tumor necrosis factor-related apoptosis-inducing ligand sensitivity via M-CSF. While much attention has focused on gp120 binding of CD4 to either CXCR4 or CCR5 as an entry mechanism, this interaction may also affect signal transduction pathways in affected cells. In macrophages, gp120 has been shown to elicit tumor necrosis factor- α (TNF- α) production (Lee et al. 2005). In human monocyte-derived macrophages, TNF- α secretion could be blocked by inhibitors of the mitogen-activated protein kinase (MAPK) pathways, as well as inhibitors of phosphatidylinositol-3 kinase pathways. Furthermore, this induction was mediated by the binding of gp120 to CCR5. Subsequent work in human macrophages has also shown gp120-CCR5 binding to affect additional pathways, including those that involve Src kinases (Cheung et al. 2008; Tomkowicz et al. 2006). In neurons, gp120 has been shown to be toxic, and this toxicity appeared to be mediated, at least in part, by members of the MAPK signaling pathway (Yao et al. 2009). Although astrocytes are not a primary target for HIV-1 infection, these cells can be affected by HIV-1 proteins that are shed from infected cells. gp120 has been shown to also elicit TNF- α secretion by cells exposed to the protein (Ronaldson and Bendayan 2006). As is the case in macrophages, this was mediated by gp120 binding to CCR5. As described above, soluble viral proteins can affect the chemokine balance in the CNS leading to influx of cells and can contribute to the deterioration of the CNS. Since cells of the immune system can directly respond to morphine via opioid receptors (Madden et al. 1998; Sharp et al. 1998), it is feasible to imagine that morphine alone or in conjunction with gp120 (Mahajan et al. 2005), or other viral proteins like Tat (Turchan-Cholewo et al. 2009), can alter the chemokine balance and their receptors further exacerbating the neuro-degeneration process.

We have analyzed the SIV *env* V1V2 region and V3V5 region from plasma and CSF, and the results show two clear patterns. The V1V2 analysis was performed exclusively with plasma and produced a pattern consistent with *tat* and *vpr* (Tirado and Kumar 2006). The sequence analysis showed more mutations in the control animals compared to those seen in the morphine-dependent rapid progressors (Tirado and Kumar 2006). This study was followed by analysis of proviral variants from the brains of morphine-dependent rapid progressors and control macaques (Rivera-Amill et al. 2009). We found that the diversity and divergence of the clones were higher in the control group corroborating our previous study in plasma. However, analysis of the V3V5 amplicon showed a conflicting pattern of evolution with respect to morphine addiction and disease progression (Rivera-Amill et al. 2007). We found a consistent pattern both in plasma and CSF in the V4 region. Morphine-

dependent animals exhibited a higher percentage of diversity in plasma and CSF compartments within V4 when compared to controls. Divergence from the inoculum was significantly greater in the morphine group as compared to controls in CSF but not in plasma. Analysis of the overall frequency of amino acid mutations in plasma-derived clones from morphine-dependent macaques revealed a higher frequency of mutations in the morphine group within V4 as compared to control macaques. This difference was significant at 12 weeks pi but not at 18 weeks pi indicating that mutations accumulate in V4 early after infection. When comparing the morphine subgroups to control macaques, the rapidly progressing macaques exhibited a significantly higher frequency of mutations within V4 early in infection. The V3–V5 analysis was also performed from CSF samples, where the plasma pattern was reproduced. As with plasma-derived clones, analysis of the overall frequency of amino acid mutations in CSF-derived clones from morphine and control groups revealed a higher frequency of mutation in the morphine group within V4. However, over time, this difference was significant at 12 and 18 weeks pi. Comparison of the morphine subgroups with control macaques revealed significant differences between rapid progressors and control macaques within V4, with the rapid progressors exhibiting higher frequency of amino acid changes but only significant at 12 weeks pi. The normal progressors exhibited a significantly higher frequency of amino acid mutations within V4 at both 12 and 18 weeks pi (Rivera-Amill et al. 2007). Different patterns of virus evolution in morphine-treated animals may be reflective of different virus–host interactions in these animals. Consequently, we are interested in the characterization SIV *env* evolution in the setting of drug abuse to look more in depth at the possible causes of rapid disease progression and whether specific changes within *env* correlate with the accelerated onset of disease in morphine-dependent animals. Further experiments would allow dissecting the molecular mechanisms on how drugs of abuse affect viral molecular diversity and how the resultant changes impact disease progression.

Nef is critical for progression but evolution does not correlate with rapid pathogenesis in the setting of morphine addiction

The Nef protein is an essential pathogenicity factor encoded at the ends of the SIV and HIV genomes. Although early characterizations indicated little importance of Nef for viral replication in cell culture (Ahmad and Venkatesan 1988), the lentiviral Nef protein is widely recognized to influence disease progression through its interactions with cellular signaling and protein sorting systems (Geyer et al. 2001). The critical role of Nef in development of AIDS has been shown in human studies and in animal models. In a macaque model, infected by a cloned form of SIVmac239 containing a truncated form of Nef disease was avoided. However, under this in vivo selection, a reversion of the stop signal at amino acid 93 to provide functional Nef became the predominant form and was necessary to produce and maintain high viral loads and for the development of disease (Kodama et al. 1993). Interestingly, under in vitro conditions, there was no such selection for reversion, yet replication was uncompromised (Kestler et al. 1991). In a separate study, specific disruption of the CD4 downregulation function of Nef was sufficient to disrupt SIV replication in vivo (Iafrate et al. 2000). In humans, defects in Nef, predominantly deletions, were associated with reduced HIV pathogenesis (Deacon et al. 1995; Kirchhoff et al. 1995), again

demonstrating the importance of Nef in development of AIDS. In fact, although such cohorts are rare, large deletions of Nef represent the most common virological explanation for long-term nonprogression (Kondo et al. 2005; Salvi et al. 1998). These cohorts have largely centered around blood transfusion as a means of viral transfer, yet Salvi et al. reported sexual transmission (Salvi et al. 1998) while Kondo and colleagues reported the first non-B clade evidence of the role of Nef defects in long-term non-progression (Kondo et al. 2005). The newest findings regarding Nef's role in pathogenesis come from long-term studies of some of these cohorts. There are indications that these HIV-positive long-term survivors experience CD4 T cell loss even after many years of clinically absent disease (Birch et al. 2001; Churchill et al. 2004), as was also indicated in macaques infected with SIV (Hofmann-Lehmann et al. 2003). Thus, while Nef appears important for pathogenesis, progression is not abolished by defects in this viral gene. Together with the possibility for recombination to restore Nef function and the possibility for different determinants of pathogenicity in vertically infection, these outcomes cast a pervasive doubt over the use of Nef-defective virus in vaccine development.

Studies addressing the role of Nef in pathogenesis under the risk setting of drug abuse are very rare in either humans or in macaque models. A report of a single long-term nonprogressor from the injection drug risk group had characteristic defects in the *nef* gene, caused by duplication leading to premature truncation of the protein. However, the study was complicated by the existence of potential functional defects in the *vpr* gene, as well as the inability of polymerase chain reaction to amplify a fragment of *nef* at a variety of time points over to course of infection (Saksena et al. 1996). In contrast, a study of five HIV-positive intravenous drug users in Italy, all of whom were long-term survivors, demonstrated only a single individual with detectable alterations (deletions) of *nef* that would lead to loss of function (Catucci et al. 2000). Thus, the studies in drug abusing humans do not provide a clear indication of the specific role of Nef in pathogenesis. While Nef defects appear to promote nonprogression, such deficiency is not required. In addition, little information is available for the possible role of Nef in driving rapid disease progression.

Examination of *nef* evolution in our model system revealed no apparent evolutionary pattern of the SIV *nef* in the rapid progressors to indicate different selective forces or pathogenic role for Nef in this group as compared to the slow progressors or controls (Noel et al. 2006b). These findings remain compatible with a role for Nef as necessary for pathogenesis, since the overwhelming majority of *nef* sequences among all animals were apparently intact and functional. However, the results suggest that *nef* may not be specifically crucial to rapid pathogenesis, as there was no support for a mutant form of *nef* specifically enhancing the pathogenicity of the inoculum virus. An important caveat to this study was the detection of intervirus recombination between the SIV and SHIV virus; however, the presence of recombination appeared to be unaffected by disease rate and drug abuse status (Noel et al. 2006b).

Conclusions

It is clear that drug abuse continues to be an important driving force for the HIV/AIDS epidemic in the USA and throughout the world. More than 25% of AIDS patients in the

USA include a history of injection drug abuse, one that frequently involves the use of opiates. In contrast, the impact of opiates on HIV infection and progression within an individual host is less evident. Substantial evidence exists indicating that both endogenous and exogenous opioids as well as opiate abuse modulate immune function. Opiates have also been shown to facilitate HIV-1 replication in vitro. The use of rhesus macaque models of drug abuse and AIDS has shed some light on the in vivo significance of morphine addiction on disease progression. Even so, there continues to be extended debate and considerable research aimed at understanding whether (and, if so, how) opiates alter HIV-1 infection and progression to AIDS.

The earliest studies of the role of opiates on SIV progression in macaques poignantly displayed the controversial effects of morphine on disease. The use of a pathogenic form of SIV (SIVmac239) in conjunction with 5 mg/kg morphine three times per day produced an apparent negative impact on immune function including reduced neutralizing antibody titer and decreased responsiveness of T cells to in vitro stimulation compared to saline-treated macaques. This system produced rapid disease and death in a third of animals in the morphine group. A second study shortly thereafter produced a contrasting result. A lower dose of morphine given more frequently together with a relatively nonpathogenic form of SIV (SIVsmm9) appeared to provide decelerated disease compared to the control group. While both of these early studies showed evidence for immune modulation by morphine leading to variation in disease progression, neither unfortunately shed light on the possible effects of drug abuse on viral evolution. More recently, our studies clearly indicate that morphine dependence caused significantly higher replication in the blood and in the cerebral compartment. Half of the morphine addicted macaques became rapid progressors and did not develop virus-specific immune responses. Each of these animals succumbed to SIV/SHIV-induced AIDS significantly more rapidly than the control animals. It is important to note that our studies used highly pathogenic viral cocktail as well as the dosing schedule of morphine similar to the first study (5 mg/kg, three times per day). In addition, our work has clearly shown that viral evolution is altered in the setting of drug abuse, which was not evident from previous studies. Due to limits of our study design (inoculation with an already highly pathogenic virus), we cannot determine the direct impact of morphine on evolution. Morphine abuse is strongly associated with increased viral replication, which provides greater opportunity for viral mutation; however, the role of morphine in altering selection to enable evolution cannot be discerned from the sum of work to date. For example, does morphine predominantly alter immune function and affect evolution of the virus indirectly by changing immune selection through downregulation of the immune response? Alternatively, morphine may more directly alter the host target cell repertoire and constitution via alteration of coreceptor expression (Guo et al. 2002; Suzuki et al. 2002). These remain critical questions if we are to understand how morphine contributes to infection and progression to AIDS.

In addition to better understanding the impact of morphine itself on viral evolution and AIDS progression, the importance of the dose of morphine is clearly an important issue. The more frequent, smaller dosing regimen that, in conjunction with a mild form of SIV, appeared to delay disease may best reflect the consistent dosing of an HIV/AIDS patient on methadone maintenance therapy. The more frequent dosing will provide a more consistent

exposure to morphine and together with lower doses will make the peaks and valleys of the morphine concentration less sharp. On the other hand, in the setting of illicit drug abuse that is characterized by shifts in morphine availability, the higher dose at less frequent intervals may provide a more similar stress to the individual.

The impact of the pathogenic potency of the infecting virus is the second major confounding factor in the studies published to date. SIVsmm9 is of low pathogenicity and fails to induce disease in some infected animals that become asymptomatic long-term survivors.

Interestingly, even in a case where highly pathogenic forms, SIVmac239, or our virus cocktail including SIV17E, a mac239 derivative, some animals became long-term survivors. However, the occurrence of long-term survival, nearly 3 years in our work, was not different between the morphine and control groups indicating a neutral effect of morphine on slow disease when a pathogenic virus was used. In contrast, morphine-accelerated disease progression was clearly present only when virus pathogenicity was high. This may indicate that the effects of morphine on the immune system, by tending toward a weakened response, provide a stage for an aggressive virus to induce rapid progression to AIDS, while at the same time providing a poor environment for the replication of weaker viral forms. It is unclear how this may affect the overall pathogenesis in human infection, but the potential to generate an environment that permits unchecked replication of forms of higher pathogenicity (by downregulating the immune response) represents a true danger to drive rapid pathogenesis, even if it occurs only 50% of the time. It may well turn out that during acute infection, where virus capable of causing transmission tends toward lower pathogenic potential, morphine abuse may not provide accelerated disease, providing the “donor” does not transmit quasispecies capable of both transmitting and thriving in the altered immune environment. In turn, as disease progresses, continued presence of opiates will drive the more rapid emergence of highly pathogenic virus, such as the CXCR4 coreceptor-using forms associated with AIDS development. Thus, while it is impossible to directly translate the highly controlled macaque studies to the highly variable circumstances of human infection, our model together with that of Chuang and colleagues may provide the best parallel of the human epidemic as disease progresses in an infected person.

Over the years, studies have documented compartmentalization of HIV-1 genotypes in patients (Korber et al. 1994; Ritola et al. 2005; Smit et al. 2004). Using various techniques such as heteroduplex tracking assays and cloning, many of these studies reported the existence of unique sequences of *env* in brain specimens obtained at autopsy. Other investigations have also documented the existence of *tat* and *nef* sequences that were either specific to or more commonly associated with brain specimens (Blumberg et al. 1992; Boven et al. 2007; Bratanich et al. 1998; Mayne et al. 1998; Saksena et al. 1997), thus suggesting the potential for functional differences between brain-derived and peripheral *tat* and *nef* variants. Although human studies are meaningful for studying inpatient variation and compartmentalization, they are limited due to the availability of brain tissue and to the complexity of a human population infected by a highly variable virus. In addition, many of these patients have received diverse antiviral therapies which themselves are differentially permeable with regard to the blood–brain barrier. Regardless, when taking into consideration whether the patients develop HIV dementia (HIV-D), a pattern does emerge

where HIV-D sequences significantly differ from non HIVD (Power et al. 1994, 1998). HIV-D-derived sequences lead to an increased neuronal death when expressed in infectious recombinant viruses (Power et al. 1998). The limitations stated above would be even more daunting in terms of investigating evolutionary changes in HIV-1 in the CNS of IDU; this may be one explanation for the lack of studies in this area. Certainly, this lack of information only emphasizes the need for an animal model that can be used to address these questions.

There are noteworthy differences between morphine-dependent and control macaques suggesting a contribution of morphine in both the pathogenesis of SIV/SHIV infection and viral evolution. The next logical step would be to identify the mechanism behind this accelerated disease progression. Is the effect contained entirely within the host immune suppression and depends heavily on the pathogenicity of the infecting virus, is it also related to target cell coreceptor expression, or is a combination of multiple factors involved? Except for our studies, very little is known about how opiates affect tissue compartmentalization of virus, leading to accelerated disease progression, neurological impairment, and any other complications. Furthermore, there is no information available regarding the effect of opiate addiction on host genetics and conversely host genetics on the impact of opiate abuse and its possible implication in disease progression—though it is clear from all studies that immunomodulation affects viral replication and disease progression. Additional analysis in various compartments may provide a better understanding of the accelerated form of the disease in morphine-dependent macaques. In addition, it is critical to develop a better understanding of the potential role of opiate abuse in contributing to the selection of the virus in the initial infection. This will be critical to better develop a sense for how opiate abuse drive human infection and disease, which is really characterized by the presence of and simultaneous exposure to multiple viral forms. Consequently, to better understand the complex interactions between opiates, the immune system and HIV, and develop novel approaches for treatment in the opiate-abuse population, there is a critical need to fill the numerous gaps in our understanding of the cellular and molecular mechanisms by which opiate abuse may affect the outcome of HIV infection and disease progression.

Acknowledgments

This work was supported by National Institute on Drug Abuse (DA015013) and NIGMS-RCMI (RR003050).

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