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Review

Opiates, immune system, acquired immunodeficiency syndrome, and nonhuman primate model

Richard J Noel Jr,[1] Vanessa Rivera-Amill,[1] Shilpa Buch,[2] and Anil Kumar[3]

[1]Ponce School of Medicine, Ponce, Puerto Rico; [2]Kansas University Medical Center, Kansas City, Kansas, USA; and [3]Division of Pharmacology, School of Pharmacy, University of Missouri, Kansas City, Missouri, USA

Both human immunodeficiency virus (HIV) and illicit drug addiction remain major health problems not only in the United States but all over globe. The effect of drug addiction on HIV/AIDS (acquired immunodeficiency syndrome) has been somewhat underexplored. However, in United States more than one fourth of HIV-positive individuals are injection drug users. Opiates are known to negatively affect the immune system, and therefore may have deleterious effects on progression of disease among HIV-infected individuals. This review discusses the effects of opiates on immune system as well as its effect on HIV replication and AIDS progression. In addition, the effects of opiates on disease progression in non-human primate model of AIDS is presented with at least one possible reason for rapid disease progression in multi-virus the challenge model. *Journal of NeuroVirology* (2008) **14,** 279–285.

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Drug abuse and its related consequences are a major health problem in many parts of the world including United States. The rising number of injection drug users who become infected with human immunodeficiency virus (HIV) lead to the increasing interest as to whether drugs of abuse affect the pathogenesis of HIV viral infection. In the United States, from 2001 through 2005, the total number of new cases of HIV/AIDS (acquired immunodeficiency syndrome) decreased slightly; however, AIDS prevalence (i.e., the number of persons living with HIV/AIDS) has increased steadily since 2001. At the end of 2005, an estimated 421,873 persons in the 50 states and the District of Columbia were living with HIV/AIDS, of which 95,385 are injection drug users (CDC, 2007). The prevalence of multidrug abuse, including heroin, cocaine, and methamphetamine represents in the United States, a major difficulty in human studies to directly link a specific agent with observed clinical health parameters. This is due in part to the irregular use of drugs, use of multiple drugs at different times, and unrelated infections. In this article we focus on the consequences of opiates in the immune system and relate these to HIV infection and AIDS disease progression in the macaque model of AIDS.

Opiates have broad effects on the immune system

Numerous studies have revealed that opiates regulate various aspects of immune system function (Stefano *et al*, 1996; Friedman *et al*, 2006b). These compounds act as immunomodulators that modify the immune response to mitogens, antigens, and antibodies that cross-link the T-cell receptor (Sharp, 2003). In addition, immune cells have opiate receptors contributing to a direct action on these cells. A study by Gaveriaux *et al* demonstrated that chronic morphine administration induces lymphoid organ atrophy, diminishes the ratio of cells that are both $CD4^+$ and $CD8^+$ positive

Address correspondence to Anil Kumar, PhD, Division of Pharmacology, School of Pharmacy, University of Missouri, Kansas City, Health Science Building, 2464 Charlotte Street, Kansas City, MO 64108-2718, USA. E-mail: kumaran@umkc.edu

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cells in the thymus, and reduces natural killer activity in wild-type mice. None of these effects was observed in mu-opioid receptor (MOR)-deficient mice after morphine treatment (Gaveriaux-Ruff *et al*, 1998). Opiate-induced effects can also be mediated indirectly via the central nervous system (CNS). These regions include the anterior hypothalamus, arcuate nucleus/ventromedial hypothalamus, medial thalamus, medial amygdala, dorsal hippocampus, and the periacquiductal gray matter. The hypothalamic-pituitary-adrenal axis and the sympathetic nervous system are particularly important in immune system modulation (Matta *et al*, 1995; Vallejo *et al*, 2004).

Heroin abusers exhibit reduced immune system responses and increased incidence of infections (Vallejo *et al*, 2004; Ocasio *et al*, 2004). MacFarlane *et al* showed that morphine markedly potentiates *Salmonella* infection at the gastrointestinal portal of entry and enhances subsequent dissemination of *Salmonella* organisms in mice (MacFarlane *et al*, 2000). The opioid antagonist naltrexone significantly blocked *Salmonella* colonization in Peyer's patches and reduced *Salmonella* burden in other organs. The study by Ocasio *et al* revealed that morphine treatment leads to a potentiated lipopolysaccharide (LPS)-induced inflammation and accelerated progression to septic shock in rats. Animals that were treated with morphine and LPS developed hypothermia, decreased mean arterial pressure (MAP), increased plasma thrombin–antithrombin III (TAT) complex, and approximately 67% of animals exhibited progressive intramicrovascular coagulation (Ocasio *et al*, 2004). Wang and colleagues investigated the effect of morphine on $CD8^+$ T cell–mediated, noncytotoxic, anti-HIV activity in latently infected human immune cells and found that these activities were inhibited by morphine. This effect could be abrogated by treatment with naltrexone, providing additional evidence to support the notion that opioids play a role in impairing the anti-HIV function of the immune system (Wang *et al*, 2005; Friedman *et al*, 2006). Singhal *et al* evaluated the molecular mechanism of opiate-induced T-cell apoptosis. Both morphine and DAGO ([D-Ala_2,*N*-Me-Phe_4, Gly_5-ol]enkephalin) enhanced T-cell apoptosis by the c-Jun NH_2-terminal kinase pathway (Singhal *et al*, 2001; Friedman *et al*, 2006). Chronic administration of relatively low doses of morphine in a cohort of rhesus monkeys over a 2-year period have been shown to affect immunocompetence, suppressing peripheral blood mononuclear cell (PBMC) natural killer (NK) cell activity, decreasing the percentage of $CD4^+$ circulating lymphocytes (Carr and France, 1993a, 1993b). Killiam *et al* reported that opioid-treated monkeys were more vulnerable to opportunistic infections, and showed depressed weight gain and a reduction in the variety of in-cage behaviors exhibited by the monkeys (Killam *et al*, 1996).

Opiates, HIV/AIDS, and nonhuman primate model

The impact of opiates on HIV and AIDS has been difficult to define

Although the effects of morphine on the immune system are reasonably well described, the interaction between the virus and the immune system in the presence of morphine, in either HIV or simian immunodeficiency virus (SIV) infection, is a mixture of both widely accepted and controversial effects, particularly on the overall impact of opioids on progression of disease. Clearly, *in vitro* viral replication is enhanced by the presence of morphine (Li *et al*, 2002, 2003; Chuang *et al*, 1993; Peterson *et al*, 1990, 1993, 1994). This enhancement results from morphine-induced changes in immune cell gene expression in the host, including perturbations in β-chemokines and CCR5 coreceptor (Guo *et al*, 2002). Pharmacological antagonism of morphine effectively reverses the enhancement of viral replication, indicating a receptor-mediated effect (Li *et al*, 2002; Ho *et al*, 2003). The adverse effect of substance of abuse has also been shown in a HIV-infected cohort where cessation of drug abuse resulted in slower disease progression (Ronald *et al*, 1994). However, epidemiological studies have driven the controversy surrounding the importance of morphine abuse in accelerating HIV infection and progression (Bouwman *et al*, 1998; Bell *et al*, 1998, 2006).

Macaque models of morphine and SIV infection have stimulated controversy surrounding the role of morphine in AIDS

Animal studies of HIV infection have provided some evidence that morphine may potentiate the severity and rate of disease progression of HIV-related disease. In a study by Suzuki *et al*, morphine-dependence resulted in an exacerbation of the SIV infection in rhesus macaques. Two macaques were chronically injected with morphine and two were injected with saline. All four animals were then infected with SIVmac239. Although the sample size was too small to draw any meaningful conclusions, the results showed that viremia could be controlled in saline-treated but not in the morphine-treated animals. In the morphine-treated animals, virus could be detected from PBMC cultures without the inclusion of immortalized T cells, whereas, virus titers from the PBMC of saline-treated animals were undetectable without cocultivation. Furthermore, increased viremia was documented only once at 18 months after infection (Suzuki *et al*, 2002; Chuang *et al*, 2005).

Despite the evidence that opioids may adversely affect the immune system, and increase the rate of HIV/AIDS disease progression, there is suggestive evidence from both clinical observations and basic studies that opiate exposure may protect the host

from progression of HIV-1 infections (Donahoe and Vlahov, 1998; Kapadia *et al*, 2005). In a study by Donahoe *et al*, six opiate-dependent rhesus monkeys were infected with the sooty mangabey strain of SIV (SIVsmm9). They found no evidence that opiates exacerbated the course of infection and the development of simian AIDS. In fact, chronic administration of morphine led to decelerated disease progression, with increased expression of the virus appearing during the periods of withdrawal. Some AIDS-like symptoms that normally develop in SIV-infected monkeys were expressed to lower extent in the opiate-dependent animals. Most significantly, no opiate-dependent animals died in the first 2 years of the study from AIDS-related symptoms (Donahoe *et al*, 1993). Based on this limited study, it could be argued that opiates had a protective effect; however, the absence of concurrent control animals prevented clear interpretations, as historical controls were used for comparison. Furthermore, the SIVsmm9 does not cause a loss of circulating $CD4^+$ T cells until late-stage infection. Infection with HIV type 1 (HIV-1) is characterized in the acute phase by a marked decrease in the numbers of circulating $CD4^+$ T cells, followed by a steady decline until a point is reached where the levels of viremia rises sharply and AIDS develops.

Marcario *et al* adapted a model of SIVmac infection in rhesus macaques (SIVmacR71/17E). Infection with this R5 virus parallels what happens in human infection with HIV, except on a faster time scale (Marcario *et al*, 2008). In contrast to the previously mentioned models, this group found no effect of morphine on the clinical outcome of the infection during the 33-week observation period, specifically on viremia, cerebrospinal fluid (CSF) viral titers, or survival. However, they did find histopathological differences in the brains of two morphine-dependent infected animals with clinical disease. Morphine-treated animals developed lesions in the white matter of the brain, whereas untreated infected animals developed gray matter encephalitis.

Differences in the virus stocks used in these studies and in drug-dosing regimens may account for differences in outcomes. Nevertheless, the existence of three lines of study illustrates the complexity of the effects of opioids on the immune system.

The morphine-addicted SIV/SHIV model provides an experimental parallel to HIV/AIDS

We developed a unique model of morphine and viral infection/AIDS to address some of the aforementioned shortcomings (Kumar *et al*, 2004). A cohort of six rhesus macaques were introduced and made addicted to morphine over a period of 2 weeks, followed by 4 months of chronic morphine dosing prior to infection (Figure 1). In parallel, three additional macaques were identically infected but without introduction of morphine. In order to simulate the immunological parameters of HIV infection in humans, we employed a three-virus combination, delivered by intramuscular injection, to introduce infection. The virus combination, SIV/17E-Fr, $SHIV_{89.6}P$, and $SHIV_{KU}$, were selected due to the induction of more uniform disease, rapid and precipitous $CD4^+$ decline, and rapid progression to AIDS/death (Kumar *et al*, 2004). The model produced very rapid disease and death in 50% of the morphine-addicted macaques. The progression was characterized by maintenance of high plasma and CSF viral loads, loss of $CD4^+$ T cells with failure to recover, and the near total absence of an adaptive immune response (Kumar *et al*, 2004, 2006). Differential polymerase chain reaction (PCR) reactions demonstrated that the SIV/17E-Fr had the widest tissue distribution and earliest migration into the CNS (Kumar *et al*, 2006), particularly among the morphine-addicted macaques where it was detected about 20 weeks earlier than the non-morphine controls (week 8 versus 28 post infection). The SHIV/SIV morphine macaque model produced neuropathogenesis in two thirds of rapid progressors (Kumar *et al*, 2006). Subsequent follow-up for the first year of infection resulted in progression and emergence of neuroAIDS in a third morphine-dependent macaque at week 51, bringing the total to 50% of all morphine-addicted animals, whereas none of the nonmorphine controls demonstrated neuropathology. Thus in this model, neuropathology was augmented by morphine abuse as a disease cofactor.

Viral evolution and morphine-dependent rapid progression

The death of half of the morphine cohort by 20 weeks post infection led to the question of how morphine altered the viral-host dynamics to drive rapid progression. The rapid progressors exhibited very high viral loads and no immune response. Based on the importance of both viral replication and the immune response to driving viral evolution, we used this model to investigate the possible role in viral sequence evolution on driving viral pathogenesis, particularly in the 50% subset of morphine-dependent macaques that showed dramatically accelerated progression as well as a high proportion of neuroAIDS. Studies of the SIV/17E-Fr evolution at three loci, *tat, vpr*, and *env* in both plasma and CSF, predominantly showed inverse correlation with disease; however, we also found an interesting exception to this pattern for the V4 region of envelope, as well as a lack of progression-evolution correlation for a fourth locus, *nef.* Each of these outcomes offers a paradigm for the involvement of viral evolution during morphine-accelerated pathogenesis.

tat and vpr had similar patterns in both plasma and CSF

Phylogenetic analyses of individual cloned sequences of the SIV/17E-Fr of each animal showed a remarkably consistent pattern of inverse correlation

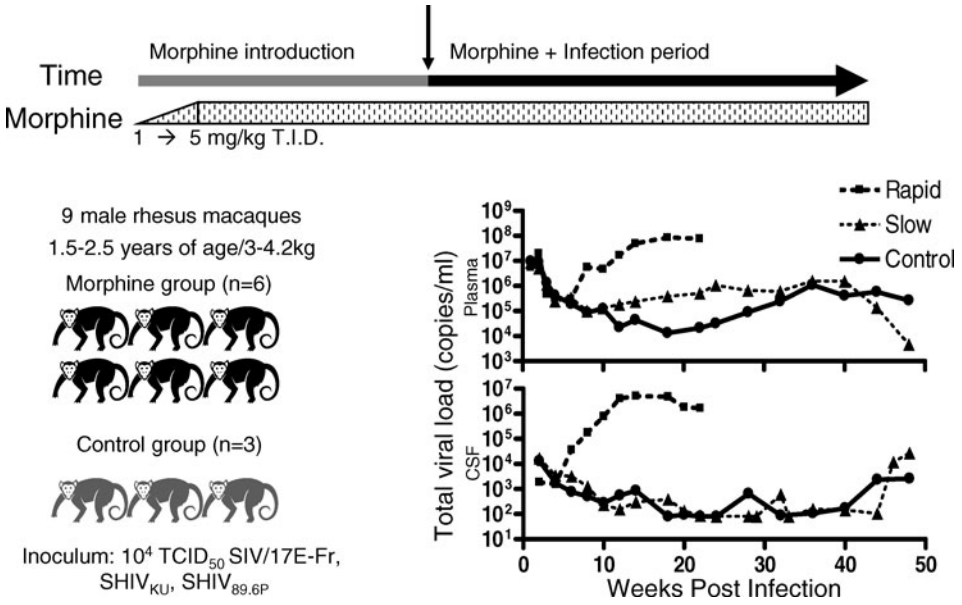

Figure 1 Schematic representation of the establishment of morphine-dependence and infection of animals. Nine 1.5- to 2.5-year-old male rhesus macaques (*Macaca mulatta*) were obtained from the Caribbean Research Primate Center and maintained in the AAALAC-approved Animal Facility at the University of Puerto Rico Medical School. The experimental protocol was approved by the Institutional Animal Care and Use Committee. The body weight of these animals ranged between 3 and 4.2 kg. The animals tested negative for tuberculosis, simian T-cell leukemia virus type-1, and simian retrovirus. The monkeys were divided into two groups (morphine-dependent and control) of six and three, respectively. Morphine dependence was established by injecting increasing doses of morphine (1 to 5 mg/kg of body weight over a 2-week period) every 8 h by the intramuscular route. Morphine injections were maintained throughout the study. The control animals were given the same amount of normal saline at the same time. After 20 weeks of the initiation of morphine/saline injections, all animals were injected intravenously (indicated by vertical arrow on timeline) with a 2-ml inoculum containing 10^4 50% tissue culture infectious doses each of $SHIV_{KU-1B}$, $SHIV_{89.6}P$, and SIV/17E-Fr. *Graph:* Plasma (*top*) and cerebrospinal fluid (CSF; *bottom*) viral load in morphine-dependent rapid progressors (■), slow progressors (▲), and control macaques (●). Sequential plasma and CSF samples were collected, and viral load was determined by real-time RT-PCR. The results are presented as RNA copy/ml plasma or CSF.

with progression. *tat* and *vpr* evolution in plasma were clearly lower in morphine-addicted rapid progressors compared to nonmorphine controls, showing between 90% (Noel and Kumar, 2006) and 60% (Noel and Kumar, 2007) lower diversity than control macaques for *tat* and *vpr*, respectively. Notably, the pattern of evolution among the slow-progressor, morphine-addicted macaques was quite similar to that of the controls. As may be expected, the evolution showed some relationship to immune status, as the rapid progressors failed to mount any detectable immune response, whereas the morphine slow progressors and controls each showed virus-specific T cells by 4 weeks and binding antibodies by 8 weeks post infection (Kumar *et al*, 2006). In the CSF for both genes, rapidly progressing, morphine-addicted monkeys had roughly half the overall sequence diversity as the control animals (Noel *et al*, 2006a; Noel and Kumar, 2007). Interestingly, 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 slow progressors, all of which showed viral-specific immune responses. In contrast, viral quasispecies compartmentalization between the plasma and CSF virus was evident in the clustering patterns of sequence clones from all animals with detectable immune responses, but notably absent in the three rapid progressors (Noel *et al*, 2006a; Noel and Kumar, 2007). This lack of independent clustering of plasma and CSF sequences was not present for the other genes in our analyses to date. An additional interesting observation came from analysis of the nature of the mutations in the CSF *tat* sequences that indicate morphine may have some impact on viral point mutation. There was a clear trend of both increased transitions (Ts) and decreased transversions (Tv) in morphine-treated monkeys compared to controls to yield a Ts:Tv ratio of 13.7 in morphine-dependent animals compared to 3.2 in controls. Examination of only the rapid progressor subset of morphine cohort yield an even greater ratio of 24.0 (Noel *et al*, 2006). There is some precedent for morphine-mediated defects in cellular DNA repair (Madden *et al*, 2002), which raises the potential that morphine may directly affect the viral evolution through more than alteration of the host immune response and stimulation of viral replication.

Viral envelope evolution showed regional differences in morphine and control macaques
The viral envelope was analyzed in two parts covering V1/V2 (Tirado *et al*, 2004) or V3–V5 (Rivera-Amill *et al*, 2007) and showed two clear patterns. The V1/V2 analysis focused exclusively on plasma, showing that viral evolution was clearly less, with only 50% as much diversity, in the rapid progressors (Tirado and Kumar, 2006), in a manner similar to *tat* and *vpr*. However, a separate amplicon from the same plasma samples covering the V3–V5 region showed a conflicting pattern of evolution with respect to morphine addiction and disease rate, making it impossible to draw a generalized picture of envelope evolution under morphine-dependent rapid progression. A more narrow analysis of individual variable regions led to a clear result. V4, in contrast to all other regions identified, showed a direct correlation between evolution and morphine-dependent rapid progression (Rivera-Amill *et al*, 2007). In SIV, V4 has been implicated in determining coreceptor use and that may account for the unique result for this variable region in these rapid progressors (Cho *et al*, 1998; Hu *et al*, 2000; Smyth *et al*, 1998; Rivera-Amill *et al*, 2007). The V3–V5 analysis was also performed for CSF, where the plasma pattern was reproduced. As with *tat* and *vpr*, the balance of synonymous to nonsynonymous nucleotide changes did not suggest immune pressure in any of the monkeys, but in contrast to the *tat* and *vpr* phylogenetic trees, all animals showed signs of separate clustering, indicating distinct compartmentalization of the plasma and CSF viral variants. Although there was some evidence of compartment mixing in the trees, it did not appear to be related to either morphine treatment nor disease progression rate (Rivera-Amill *et al*, 2007).

nef evolution was influenced by viral recombination and was not related to morphine or progression rate
A similar study of *nef* evolution in the plasma showed no effect of morphine abuse on gene evolution. All animals, regardless of morphine status, showed roughly 1.25% diversity (range 1.17% to 1.47%) during the first 20 weeks of study. Thus although disease progression was accelerated in half of the morphine cohort, there was no observable effect on evolution of SIV *nef*. Furthermore, there was no nonsynonymous substitution pattern that emerged in the morphine-addicted macaques or the rapid progressors to indicate the involvement of *nef* on rapid progression. Undoubtedly, *nef* is critical to disease progression (Deacon *et al*, 1995; Kestler *et al*, 1991; Mackay *et al*, 2002), and the lack of an observed effect in this model, when *tat*, *vpr*, and envelope had shown associations with progression and morphine abuse was unexpected. There were some confounders, largely in the nature of the viral inoculum that was a SHIV/SIV mixture. Contrary to *tat*, *vpr*, and envelope, which were all amplified with forward and reverse primers specific only for SIV/17E-Fr, the reverse primer for *nef* was common to all viral forms in the initial infection. We found recombinant virus at a relatively equal occurrence in both rapid and slow progressors and in morphine-dependent and morphine-free macaques. Our studies with *tat* suggested that morphine may alter viral point mutation (Noel *et al*, 2006a); morphine has been shown to possibly affect the human genome as well (Madden *et al*, 2002). In contrast, the results obtained from *nef* sequencing hint that morphine has little or no effect on viral recombination, which also provides a strong driving force for evolution (Noel *et al*, 2006b).

Conclusions

This model demonstrates the multiple effects that morphine may have on both the host and the virus and raises possible relationships between the rapid pathogenesis in the morphine cohort and the role of viral evolution. Virus evolution is driven by replication, immune selection, viral recombination, and cellular and tissue compartmentalization effects. The model clearly shows that morphine dependence impairs the immune response, increases viral replication and raises set-point in both plasma and CSF, and promotes rapid progression and death in 50% of infected animals. Neuropathology appears more severe, with higher CSF viral loads and neuroAIDS in two thirds of rapid progressors (Kumar *et al*, 2004, 2006). Interestingly, analysis of mutation type in *tat* CSF sequences showed the possible influence of morphine on point mutation preference (Noel *et al*, 2006a), even though our studies with *nef* in plasma suggested that recombination rates are independent of morphine (Noel *et al*, 2006b). Thus it appears that morphine affects the viral-host dynamics at multiple levels that may drive evolution (immune pressure, immune cell coreceptor expression, viral replication rate, point mutation), but is still selective in that it does not appear to influence viral recombination, which is also critical for evolution and disease pathogenesis.

Drug abuse is a critical element of the HIV/AIDS epidemic, yet from epidemiological studies in humans, it remains difficult to determine the role of drugs of abuse in altering immune parameters and viral-host interactions. These data are critically relevant to clinical management of HIV, as drug abuse in infected populations will impact on treatment and vaccine approaches. There are very interesting scientific questions that remain as well. For example, morphine may directly alter the mutation rate or preference directly at the cellular level. Morphine may affect viral compartmentalization and some of these influences may differ for the various viral genes. These are important questions that will help us to better understand the relationships between the virus and host cells in the various risk groups in the human population.

References

Bell JE, Arango JC, Anthony IC (2006). Neurobiology of multiple insults: HIV-1-associated brain disorders in those who use illicit drugs. *J Neuroimmune Pharmacol* **1:** 182–191.

Bell JE, Brettle RP, Chiswick A, and Simmonds P (1998). HIV encephalitis, proviral load and dementia in drug users and homosexuals with AIDS. *Effect of neocortical involvement. Brain* **121:** 2043–2052.

Bouwman FH, Skolasky RL, Hes D, Selnes OA, Glass JD, Nance-Sproson TE, Royal W, Dal Pan GJ, and McArthur JC (1998). Variable progression of HIV-associated dementia. *Neurology* **50:** 1814–1820.

Carr DJ, France CP (1993a). Immune alterations in chronic morphine-treated rhesus monkeys. *Adv Exp Med Biol* **335:** 35–39.

Carr DJ, France CP (1993b). Immune alterations in morphine-treated rhesus monkeys. *J. Pharmacol. Exp. Ther* **267:** 9–15.

Centers for Disease Control and Prevention (CDC) (2007). *HIV/AIDS Surveillance Report, 2005*, vol. 17, revised edition. Atlanta: US Department of Health and Human Services, Centers for Disease Control and Prevention; 2007.

Cho MW, Lee MK, Carney MC, Berson JF, Doms RW, and Martin MA (1998). Identification of determinants on a dualtropic human immunodeficiency virus type 1 envelope glycoprotein that confer usage of CXCR4. *J Virol* **72:** 2509–2515.

Chuang LF, Killam KF Jr, and Chuang RY (1993). Increased replication of simian immunodeficiency virus in CEM x174 cells by morphine sulfate. *Biochem. Biophys. Res Commun.* **195:** 1165–1173.

Chuang RY, Suzuki S, Chuang TK, Miyagi T, Chuang LF, and Doi RH (2005). Opioids and the progression of simian AIDS. *Front Biosci* **10:** 1666–1677.

Deacon NJ, Tsykin A, Solomon A, Smith K, Ludford-Menting M, Hooker DJ, McPhee DA, Greenway AL, Ellett A, Chatfield C, Lawson VA, Crowe S, Maerz A, Sonza S, Learmont J, Sullivan JS, Cunningham A, Dwyer D, Dowton D, and Mills J (1995). Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. *Science* **270:** 988–991.

Donahoe RM, Byrd LD, McClure HM, Fultz P, Brantley M, Marsteller F, Ansari AA, Wenzel D, Aceto M (1993). Consequences of opiate-dependency in a monkey model of AIDS. *Adv Exp Med Biol* **335:** 21–28.

Donahoe RM, Vlahov D (1998). Opiates as potential cofactors in progression of HIV-1 infections to AIDS. *J Neuroimmunol* **83:** 77–87.

Friedman H, Pross S, Klein TW (2006). Addictive drugs and their relationship with infectious diseases. *FEMS Immunol Med MicroBiol* **47:** 330–342.

Gaveriaux-Ruff C, Matthes H.W, Peluso J, Kieffer BL (1998). Abolition of morphine-immunosuppression in mice lacking the mu-opioid receptor gene. *Proc. Natl. Acad. Sci. U. S. A* **95:** 6326–6330.

Guo CJ , Li Y, Tian S, Wang X, Douglas SD, and Ho WZ, (2002). Morphine enhances HIV infection of human blood mononuclear phagocytes through modulation of beta-chemokines and CCR5 receptor. *J Investig Med* **50:** 435–442.

Ho WZ, Guo CJ, Yuan CS, Douglas SD, Moss J (2003). Methylnaltrexone Antagonizes opioid-mediated enhancement of HIV Infection of human blood mononuclear phagocytes. *J Pharmacol Exp Ther* **307:** 1158–1162.

Hu QX, Barry AP, Wang ZX, Connolly SM, Peiper SC, Greenberg ML (2000). Evolution of the human immunodeficiency virus type 1 envelope during infection reveals molecular corollaries of specificity for coreceptor utilization and AIDS pathogenesis. *J. Virol.* **74,** 11858–11872.

Kapadia F, Vlahov D, Donahoe RM, 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.

Kestler HW III, Ringler DJ, Mori K, Panicali DL, Sehgal PK, Daniel MD, and Desrosiers RC (1991). Importance of the nef gene for maintenance of high virus loads and for development of AIDS.*Cell.* **65:** 51–662.

Killam KF, Chuang LF, Chuang RY (1996). Opioid dependency and the progression of simian AIDS: opioid dependency and behavioral observations. *Adv Exp Med Biol* **402:** 43–51.

Kumar R, Orsoni S, Norman L, Verma AS, Tirado G, Giavedoni LD, Staprans S, Miller GM, Buch SJ, 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:** 192–206.

Kumar R, Torres C, Yamamura Y, Rodriguez I, Martinez M, Staprans S, Donahoe RM, Kraiselburd E, Stephens EB, and Kumar A (2004). 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.

Li Y, Merrill JD, Mooney K, Song L, Wang X, Guo CJ, Savani RC, Metzger DS, Douglas SD, Ho WZ (2003). Morphine enhances HIV infection of neonatal macrophages.*Pediatr. Res* **54:** 282–288.

Li Y, Wang X, Tian S, Guo CJ, Douglas SD, Ho WZ (2002). Methadone enhances human immunodeficiency virus infection of human immune cells. *J Infect Dis* **185:** 118–122.

MacFarlane AS, Peng X, Meissler JJ Jr, Rogers TJ, Geller EB, Adler MW, and Eisenstein TK (2000). Morphine increases susceptibility to oral *Salmonella typhimurium* infection. *J Infect Dis* **181:** 1350–1358.

Mackay GA, Niu Y, Liu ZQ, Mukherjee S, Li Z, Adany I, Buch S, Zhuge W, McClure HM, Narayan O, Smith MS (2002). Presence of Intact vpu and nef genes in nonpathogenic SHIV is essential for acquisition of pathogenicity of this virus by serial passage in macaques. *Virology.* **295:** 133–146.

Madden JJ, Wang Y, Lankford-Turner P, Donahoe RM (2002). Does reduced DNA repair capacity play a role in HIV infection and progression in the lymphocytes of opiate addicts? J Acquir. *Immune. Defic. Syndr.* **31(Suppl 2):** S78–S83, S78–S83.

Marcario JK, Riazi M, Adany I, Kenjale H, Fleming K, Marquis J, Nemon O, Mayo MS, Yankee T, Narayan O, Cheney PD (2008). Effect of morphine on the neuropathogenesis of SIVmac infection in Indian Rhesus Macaques. *J. Neuroimmune. Pharmacol.* **3:** 12–25.

Matta S, Saphier D, Lysle D, and Sharp B (1995). The brain-immune axis: role of opiates and other substances of abuse, the hypothalamic-pituitary-adrenal axis and behavior.*Adv Exp Med Biol* **373:** 1–9.

Noel RJ Jr, 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 RJ Jr, Kumar A (2007). SIV Vpr evolution is inversely related to disease progression in a morphine-dependent rhesus macaque model of AIDS.*Virology.* **359:** 397–404.

Noel RJ Jr, Marrero-Otero Z, Kumar R, Chompre-Gonzalez GS, Verma AS, 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 RJ Jr, 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. Retroviruses* **22:** 817–823.

Ocasio FM, Jiang Y, House SD, Chang SL (2004). Chronic morphine accelerates the progression of lipopolysaccharide-induced sepsis to septic shock. *J Neuroimmunol* **149:** 90–100.

Peterson PK, Gekker G, Hu S, Anderson WR, Kravitz F, Portoghese PS, Balfour HH Jr, Chao CC (1994). Morphine amplifies HIV-1 expression in chronically infected promonocytes cocultured with human brain cells. *J. Neuroimmunol.* **50:** 167–175.

Peterson PK, Gekker G, Schut R, Hu S, Balfour HH Jr, and Chao CC (1993). Enhancement of HIV-1 replication by opiates and cocaine: the cytokine connection. *Adv Exp Med Biol* **335:181–8:** 181–188.

Peterson PK, Sharp BM, Gekker G, Portoghese PS, Sannerud K, Balfour HH Jr (1990). Morphine promotes the growth of HIV-1 in human peripheral blood mononuclear cell cocultures *AIDS* **4:** 869–873.

Rivera-Amill V, Noel RJ Jr, Orsini S, Tirado G, Garcia JM, Buch S, Kumar A (2007). Variable region 4 of SIV envelope correlates with rapid disease progression in morphine-exposed macaques infected with SIV/SHIV. *Virology* **358:** 373–383.

Ronald PJ, Robertson JR, Elton RA (1994). Continued drug use and other cofactors for progression to AIDS among injecting drug users. *AIDS* **8:** 339–343.

Sharp BM (2003). Opioid receptor expression and intracellular signaling by cells involved in host defense and immunity. *Adv Exp Med Biol* **521:** 98–105.

Singhal P, Kapasi A, Reddy K, Franki N (2001). Opiates promote T cell apoptosis through JNK and caspase pathway. *Adv Exp Med Biol* **493:** 127–135.

Smyth RJ, Yi Y, Singh A, Collman RG (1998). Determinants of entry cofactor utilization and tropism in a dualtropic human immunodeficiency virus type 1 primary isolate. *J Virol* **72:** 4478–4484.

Stefano GB, Scharrer B, Smith EM, Hughes TK Jr, Magazine HI, Bilfinger TV, Hartman AR, Fricchione GL, Liu Y, Makman MH (1996). Opioid and opiate immunoregulatory processes. *Crit Rev Immunol* **16:** 109–144.

Suzuki S, Chuang AJ, Chuang LF, Doi RH, Chuang RY (2002). Morphine promotes simian acquired immunodeficiency syndrome virus replication in monkey peripheral mononuclear cells: induction of CC chemokine receptor 5 expression for virus entry. *J Infect Dis* **185:** 1826–1829.

Tirado G, Jove G, Kumar R, Noel RJ, Reyes E, Sepulveda G, Yamamura Y, Kumar A (2004). Compartmentalization of drug resistance-associated mutations in a treatment-naive HIV-infected female. *AIDS Res Hum. Retroviruses* **20:** 684–686.

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

Vallejo R, Leon-Casasola O, Benyamin R (2004). Opioid therapy and immunosuppression: a review. *Am J Ther* **11:** 354–365.

Wang X, Tan N, Douglas SD, Zhang T, Wang YJ, Ho WZ (2005). Morphine inhibits CD8+ T cell-mediated, non-cytolytic, anti-HIV activity in latently infected immune cells. *J Leukoc Biol* **78:** 772–776.