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Injection Drug Use as a “Second Hit” in the Pathogenesis of HIV-associated Pulmonary Hypertension

HIV-associated pulmonary arterial hypertension (HIV-PAH) is a serious complication that develops in approximately 1:200 of HIV-infected individuals, and an even higher prevalence of echocardiographic signs of pulmonary hypertension indicates that the disease may be more common than previously thought (1–3). Mortality is high, even in the era of antiretroviral therapy (ART) (4). Lower CD4 cell count has been associated with HIV-PAH, but successful treatment with ART has not reduced prevalence, suggesting that factors other than HIV may contribute to disease (4). Although HIV viral proteins have been suggested to play a role in the pathogenesis of HIV-PAH, not all patients with HIV develop the disease, leading to the investigation of other risk factors, or “second-hit” events (Figure 1). Intravenous drug use in HIV infection is one potential “second hit” that has been associated with HIV-PAH (5); however, the contribution of specific illicit drugs such as opioids or amphetamine derivatives, and the mechanism by which these drugs contribute to pulmonary vasculopathy in HIV-PAH, is not known.

In this issue of the *Journal*, Spikes and colleagues (pp. 1235–1243) study a nonhuman primate model to investigate the effect of morphine on simian immunodeficiency virus (SIV) infection and the development of pulmonary hypertension (6). They report that rhesus macaques pretreated with morphine for 26 weeks, followed by inoculation with macrophage-tropic SIVmac71/17E virus, and subsequent treatment with intramuscular morphine for 31 weeks after inoculation, develop significant pulmonary vascular remodeling including plexiform lesions, whereas animals either infected with SIV alone or treated with morphine alone did not. Although there is mild to moderate inflammation in all animals, the authors report increased perivascular inflammation in the SIV/morphine group. In addition, increased macrophage lung infiltration and elevated plasma levels of monocyte chemoattractant protein-1 and interleukin-8 are associated with vascular remodeling in the SIV-infected, morphine-treated macaques.

The current article’s key strength lies in its *in vitro* studies exploring potential mechanistic underpinnings for the observations made in the SIV-infected, morphine-treated macaques. In the *in vitro* studies, the synergistic effects of HIV viral proteins and morphine on proliferation, apoptosis, and oxidative stress in human pulmonary microvascular endothelial cells suggest that SIV/HIV viral proteins and morphine may initially interact to increase apoptosis, with endothelial injury subsequently promoting the proliferation of apoptosis-resistant cells. This aberrant

healing process is hypothesized to lead to angio-obliteration, potentially through increased production of reactive oxygen species and, in cases of chronic morphine and HIV trans-activator of transcription (Tat) protein exposure, increased phosphorylation and activation of vascular endothelial growth factor-2 receptor.

These studies support the concept that HIV-PAH is a “multiple-hit” phenomenon and that opiate-induced endothelial cell injury may be one such insult. Such findings are consistent with previous reports in humans describing increased prevalence of injection drug use in the epidemiology of HIV-PAH (1). Other illicit drugs such as cocaine may also increase risk of HIV-PAH. Dhillon and colleagues previously described an *in vitro* model in which combined treatment with HIV Tat protein and cocaine increases pulmonary artery endothelial cell permeability and smooth muscle cell proliferation (7).

The current findings are thought-provoking and lead to the following questions. First, what role does severity of SIV infection, as measured by CD4 T-cell levels and viral load, play in PAH pathogenesis? Second, it is interesting that all three HIV viral proteins studied had similar proapoptotic and proliferative effects on endothelial cells. Were there any HIV viral proteins such as gag, pol, or rev that did not produce this effect? Finally, the SIV and morphine-treated group developed *Pneumocystis* infection in 60% of the animals. Given prior findings by Swain and coworkers in which *Pneumocystis* exposure increased the likelihood of developing pulmonary hypertension in a rodent model, *Pneumocystis* colonization or infection could have also contributed to the development of disease (8).

This work extends previous studies demonstrating that SIV-infected macaques develop pulmonary vascular lesions consistent with HIV-PAH (9, 10). Although it has been previously postulated that HIV viral proteins play a role in the pathogenesis of HIV-PAH, the mechanism by which this happens is not known. HIV transgenic rats that express seven of the nine HIV viral proteins develop increased right ventricular pressures, right ventricular hypertrophy, and pulmonary vascular remodeling consistent with pulmonary hypertension (11). Although direct infection of endothelial cells by HIV has never been demonstrated, it is possible that soluble HIV proteins affect host target cells and lead to HIV-PAH. For example, HIV-negative regulatory factor (Nef) protein has been identified within the endothelial cells of complex plexiform lesions in Simian/human chimeric immunodeficiency virus (SHIV)-*nef*-infected rhesus macaques and HIV-infected humans (12). Similar to humans with idiopathic pulmonary arterial hypertension, aberrant Golgi trafficking has been reported in SHIV-*nef*-infected macaques (13). Macaques infected with SIV or SHIV-envelope (*env*) protein

Figure 1. Multiple hits are likely involved in the pathogenesis of HIV-associated pulmonary arterial hypertension.

also develop pathologic changes (10) as well as hemodynamic changes consistent with pulmonary hypertension (George, unpublished data). Both Env and Tat have been associated with increased oxidative stress in endothelial cells (14).

Several other potential pathways have been described in the literature. In a murine *Pneumocystis* model of immune restoration, mice that had previously been infected with (and cleared) *Pneumocystis* developed physiologic and pathologic changes consistent with pulmonary hypertension (8). Autoimmune mechanisms have also been invoked in HIV-PAH, with an increased prevalence of the disease in individuals with specific HLA-DR alleles (15). The effect of immunologic aging and premature cellular senescence in HIV and PAH is also an avenue currently under exploration (16).

The current studies by Spikes and colleagues are the first to investigate the potential pathogenic role of opioid drugs in the development of HIV-PAH. This study is a provocative initial look at the potentially synergistic interactions between morphine and the HIV viral proteins Tat, Nef, and Env in the pathogenesis of PAH. Future studies may further explore the impact of other drugs such as methamphetamines and other opioids. Identifying these risk factors as well as understanding the multiple hits in the pathogenesis of the HIV-PAH will help identify high-risk individuals and optimize treatments for this fatal disease.

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