Commentary

Adding Fuel to the Fire: Methamphetamine Enhances HIV Infection

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A growing body of evidence indicates an increased risk of HIV/AIDS infection among substance abusers. A particular concern is the increasing impact of methamphetamine use and the associated vulnerability among this population to the risk of HIV-1 exposure.^{1–3} Epidemiological studies indicate that ~4.9% of Americans have tried methamphetamine at least once in their life.⁴ Furthermore, one recent survey indicates that one in three teens see only a slight or no risk in trying methamphetamine [The National Association of Counties (NACo) Methamphetamine Newsletter, October 2007; available at http://www.naco.org/Content/ContentGroups/Programs_and_Projects/Criminal_Justice/NACo'sMethamphetamineNewsletter_October2007.pdf]. Methamphetamine, a highly addictive psychostimulant, alters immune functions and increases susceptibility to infections.^{5–7} The emerging double epidemic of substance abuse and HIV-1 infection is a multifaceted problem requiring understanding the causal mechanism of how drugs of abuse could affect the host-virus dynamics accelerating progression of the infection. Among the various cellular reservoirs for HIV-1, macrophages are the early and preferred sites of virus replication.⁸ Furthermore, HIV-1-infected macrophages survive for months, actively producing and spreading the virus to neighboring cells. Macrophages remain the central source of viral replication after marked depletion of CD4⁺ cells. Additionally, macrophages are an integral part of the innate immune system, acting as the first line of host defense against pathogens and playing a pivotal role in several critical aspects of HIV-1 infection.

In this issue of *The American Journal of Pathology*, Liang and colleagues⁹ examine the ability of methamphetamine to enhance HIV-1 infection in human macrophages, by shedding new light on a highly pertinent and timely topic. This is of particular importance in determining whether methamphetamine is involved in the augmentation of HIV-1 replication and is essential for understanding its role in HIV-1 immunopathogenesis. The study provides *in vitro* evidence of methamphetamine's ability to enhance HIV-1 replication in human macrophages. However, the

interpretation of data with respect to increased HIV-1 replication in macrophages as a correlate of higher viral load in methamphetamine abusers and causality of the former on the latter has to be considered with caution. The conclusive evidence of methamphetamine's effects on increasing HIV-1 replication in human patients remains important but difficult to prove because factors such as multidrug use and antiretroviral therapy significantly affect HIV-1 progression in this population.¹⁰ The authors demonstrate up-regulation of CCR5 expression by methamphetamine on macrophages as a plausible mechanism implicated in methamphetamine-mediated augmentation of HIV-1 infectivity in macrophages. CCR5 is the chemokine receptor that HIV-1 uses as a co-receptor for macrophage infection. Future work will address the specific mechanisms by which methamphetamine influences enhanced expression of CCR5. Use of inhibitors that selectively interfere with the interaction between CCR5 and HIV-1 *in vitro* could provide further clues. Attenuation of HIV-1 infection by the antagonist of dopamine 1 receptor is a very interesting observation indicating direct immunomodulation by neurotransmitters requiring further investigation.

The authors suggest two putative mechanisms underlying the drug's effects in macrophages—enhancement of CCR5 expression and suppression of endogenous interferon- α /STAT1 expression—as the basis for methamphetamine-mediated enhancement of HIV-1 infection in macrophages. It would be interesting to determine how suppression of such proinflammatory signals contribute to HIV-1 immunopathogenesis because of methamphetamine abuse as STAT signaling deficiency may contribute to the crippling of CD4 T-cell responses to a cytokine central to the immune response by HIV-1.¹¹ Critical for control of infection is the release of soluble mediators in response to the presentation of specific antigen by antigen-presenting cells. Cytokines (eg, interleukin-2, interferon- γ , or tumor necrosis factor- α), chemokines [eg, regulated on activation, normal T cell expressed and secreted (RAN-

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TES)], and cytotoxins (eg, perforin) produced by HIV-1-specific CD8⁺ T cells are essential for elimination of virus-infected cells, thereby contributing to the control of HIV-1 replication. Studies performed in mice infected with retroviruses and exposed to methamphetamine and other dopaminergic stimulants (such as cocaine) indicate that drugs of abuse might increase viral loads via dysregulation of inflammatory cytokine production.^{12,13} Identification of molecular mechanisms regulating the immunomodulatory cytokines and chemokines induced by methamphetamine may offer additional clues of how methamphetamine abuse can affect HIV-1 infection.

Numerous studies provide evidence that methamphetamine mediates immune dysregulation^{12,14–16} and modulates expression of several genes in dendritic cells.⁶ Although methamphetamine abuse is implicated in dysregulation of immunity, the apparent causal interrelationship between methamphetamine exposure and the ability of the host to elicit protective immunity is not known. The presence of dopaminergic receptors on human lymphocytes further supports the idea that neurotransmitters or substances acting via their receptors (like methamphetamine) can affect T-cell immune reactions. HIV-1 chronic infection is associated with progressive CD4⁺ T-cell depletion and dysfunction of the immune system. T cells play critical roles in orchestrating immune responses¹⁷ because activation and proliferation of T cells are characteristic of adaptive immune responses. Similarly, the production of cytokines, such as interferon- γ , interleukin-2, and tumor necrosis factor- α are important for T cells to control viral infections. How methamphetamine disarms the adaptive immune system, rendering the host more susceptible to HIV-1 infection, is currently unknown. Identification of such underlying mechanisms will highlight new therapeutic and prophylactic methods to improve the immunity in the setting of drug abuse. One possible mechanism could be oxidative stress, leading to depletion of antioxidant stores.

Association between impaired immune responses and oxidative stress has been documented in numerous pathological conditions.^{18,19} Oxidative stress associated with methamphetamine exposure²⁰ could affect T-cell function, hampering control of HIV-1 infection. Intracellular redox disturbance affects proximal and distal T cell receptor (TCR) signaling events. T-cell development, differentiation, and proliferation are regulated by cellular interactions with the environment, and TCR plays a vital role in the interpretation of the environmental cues. One possible cause of methamphetamine-mediated immune dysfunction could be the defects in the transduction of signals after TCR stimulation induced by oxidative stress, and flaws in signaling through the TCR result in an impaired ability to mount and maintain efficient immune responses to pathogens.

Several TCR signaling molecules are affected by oxidative stress leading to impaired expression of crucial TCR-proximal signaling molecules (eg, TCR- ζ , p56lck, and LAT).²¹ In several human pathological conditions (eg, cancer, rheumatoid arthritis, AIDS, and leprosy) oxidative stress has been implicated in inhibiting TCR-dependent phosphorylation of signaling molecules required

for efficient T-cell proliferation contributing to induction of T-cell hyporesponsiveness.^{22–24} Relevant questions in this context would be whether methamphetamine-induced oxidative insults prevent proper immune activation resulting in deficient immunity and progressive infection. A paradoxical lack of effective immunity is a contributing factor in chronic infectious diseases such as HIV-1. Utilization of antioxidants as an adjunctive treatment for HIV-1 infection has been suggested previously.²⁵ Indeed, it has been shown that glutathione, a major biological antioxidant, is reduced in plasma, peripheral blood mononuclear cells, and lung tissue of HIV-1-infected patients.^{26–28} Furthermore, exposure of T cells to oxidative stress leads to the loss of transcription factor activity, defective expression of specific genes, and diminished cytokine production in response to antigen stimulation.²⁹ Thus, oxidative stress mediated by methamphetamine could be one possible mechanism behind T-cell dysfunction.

Immune dysregulation encourages the study of the interactions between methamphetamine and HIV-1 in the central nervous system. Direct neurotoxic effects of methamphetamine putatively can aggravate HIV-1-associated neuronal injury. Furthermore, the blood-brain barrier (BBB) compromise present in HIV-1 encephalitic (HIVE)³⁰ was recently demonstrated after methamphetamine administration *in vivo*.^{31,32} Brain microvascular endothelial cells, pericytes, and astrocytes form the BBB, and tight junctions connect the brain microvascular endothelial cells assuring BBB structural integrity. Formation of toxic reactive oxygen species is thought to contribute to methamphetamine-induced neurotoxicity.³³ A cationic lipophilic molecule in nature, methamphetamine can diffuse into mitochondria and be retained by these organelles.³³ Mitochondria are common targets for oxidative species, and mitochondrial dysfunction and increased energy consumption play a significant role in mediating the pro-oxidant and immunotoxic effects of methamphetamine.

Reactive oxygen species could activate myosin light chain kinase in brain endothelium leading to increased BBB permeability, tight junction and cytoskeleton modification,³⁴ and enhanced leukocyte migration across the BBB. In the context of HIVE, interactions of HIV-1-infected monocytes with brain microvascular endothelial cells activate RhoA GTPase leading to tight junction phosphorylation, increased BBB permeability, and monocyte migration across the barrier.³⁵ Furthermore, strategies to prevent activation of two GTPases, Rac1 and RhoA, in the brain microvascular endothelial cells resulted in decreased adhesion and migration of HIV-1-infected monocytes across the brain endothelium.³⁶ It is plausible that the combined effects of methamphetamine and HIV-1 central nervous system infection could be attributable to activation of transcription factors and GTPases leading to BBB dysfunction secondary to oxidative stress.

Understanding the specific mechanisms of methamphetamine abuse and HIV-1 will require utilization of relevant animal models^{37,38} that reproduce salient features of HIV-1 infection in humans and are devoid of numerous confounding factors present in human studies. These models will allow testing of approaches preventing deleterious effects of methamphetamine on immune dysfunc-

tion and neurotoxicity. An important component of such prevention strategies can be the use of antioxidants. The potential role of antioxidants is justified by studies indicating that reactive oxygen species scavengers and antioxidants attenuate the toxic effects of methamphetamine.³⁹ Furthermore, scavengers of free radicals (such as glutathione) ameliorate damage caused by oxidative stress.⁴⁰ Glutathione is essential for several immune functions, such as interleukin-2 production, interleukin-2 responses, cytotoxic T-cell activity,⁴¹ and regulation of Th1-Th2 balance.⁴² Because antioxidants improve immune responses to HIV-1, they may also help to eliminate virus-infected cells in the brain and preserve BBB function. We are just beginning to understand the multifaceted, complex effects of methamphetamine in the context of HIV-1 infection, but the limited available information suggests that this drug can facilitate the spread of the virus, cause immune dysfunction, and aggravate HIV-1-associated neurotoxicity.

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