

Immunopathology and Infectious Disease

Methamphetamine Enhances HIV Infection of Macrophages

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Epidemiological studies have demonstrated that the use of methamphetamine (meth), a sympathomimetic stimulant, is particularly common among patients infected with HIV. However, there is a lack of direct evidence that meth promotes HIV infection of target cells. This study examined whether meth is able to enhance HIV infection of macrophages, the primary target site for the virus. Meth treatment resulted in a significant and dose-dependent increase of HIV reverse transcriptase activity in human blood monocyte-derived macrophages. Dopamine D1 receptor antagonists (SCH23390 and SKF83566) blocked this meth-mediated increase in the HIV infectivity of macrophages. Investigation of the underlying mechanisms of meth action showed that meth up-regulated the expression of the HIV entry co-receptor CCR5 on macrophages. Additionally, meth inhibited the expression of endogenous interferon- α and signal transducer and activator of transcription-1 in macrophages. These findings provide direct *in vitro* evidence to support the possibility that meth may function as a cofactor in the immunopathogenesis of HIV infection and may lead to the future development of innate immunity-based intervention for meth users with HIV infection. (*Am J Pathol* 2008, 172:1617–1624; DOI: 10.2353/ajpath.2008.070971)

drugs, with more than 35 million users worldwide. In the United States, approximately 1.5 million individuals regularly use/abuse meth.^{1,2} An estimated 11 million Americans at the age of 12 and older reported trying meth at least once during their lifetime. Meth use and HIV type 1 infection frequently coexist because of the association of meth use with engagement of high-risk behaviors.^{3–6} The risk for HIV infection attributable to meth use continues to increase.^{7–9} Several studies have shown that there is a high prevalence of HIV infection among meth users^{10–12} and that among men who sell sex to men, those who use meth have a higher HIV risk than nonusers.¹³ Active meth users displayed higher levels of HIV loads than nonusers,¹⁴ which may be attributable to increased viral replication, as was shown in an animal study.¹⁵ However, the direct effects of meth on HIV infection and HIV disease progression are still poorly understood.¹⁶ In particular, the deleterious effect of meth on the host's immune response and its role in the immunopathogenesis of HIV infection remain to be elucidated. Therefore, study of the interactions between meth and HIV has become a greater research priority.¹⁷

The microenvironment in which the interactions between HIV and target cells take place has a crucial role in modulating HIV infectivity. Besides CD4⁺ T lymphocytes, cells from the mononuclear phagocyte system are the primary targets for HIV infection. Monocytes and macrophages as the primary sites of HIV replication are among the first cells infected by HIV and later function as reservoirs for the virus.^{18,19} Although abuse of drug such as opioids have been implicated in modulation of functions of monocytes/macrophages²⁰ and microglia,²¹ there is limited information about the impact of meth on functions of monocytes/macrophages. Meth inhibited polyinosinic: polycytidylic acid-induced antiviral activity in murine peritoneal macrophages.²² Meth also modulated the patterns of gene expression in monocyte-derived immature and

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Methamphetamine (meth) and related amphetamine compounds are among the most commonly used illicit

mature dendritic cell.^{23,24} Although these findings suggest that meth is immunosuppressive, there is a lack of direct evidence at cellular and molecular levels to demonstrate that meth has the ability to enhance HIV infection of macrophages, the primary target for the virus. In the present study, we investigated the impact of meth on HIV infection of human blood monocyte-derived macrophages and explored the mechanisms underlying the meth action on HIV infection.

Materials and Methods

Monocyte Isolation and Culture

Peripheral blood samples from healthy adult donors were provided by the University of Pennsylvania Center for AIDS Research, which has Institutional Review Board review and approval for the sample collection. These blood samples were screened for all normal viral blood-borne pathogens and certified to be pathogen free. Monocytes were purified according to a previously described technique.²⁵ In brief, heparinized blood was separated by centrifugation over lymphocyte separation medium (Organon Teknika, Durham, NC) at 400 to 500 × *g* for 45 minutes. The mononuclear cell layer was collected and incubated with Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) in a 2% gelatin-coated flask for 45 minutes at 37°C, followed by removal of the non-adherent cells with Dulbecco's modified Eagle's medium. Adherent monocytes were detached with 10 mmol/L EDTA. After the initial purification, greater than 97% of the cells were monocytes, as determined by nonspecific esterase staining and flow cytometry analysis using monoclonal antibody against CD14, the marker specific for monocytes and macrophages. Isolated monocytes were plated in 24- or 48-well culture plates at a density of 5 or 2.5 × 10⁵ cells/well in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Whereas monocytes refer to freshly isolated (within 24 hours) monocytes, macrophages refer to 7-day-cultured monocytes *in vitro*. Monocyte and macrophage viability was monitored by trypan blue exclusion and maintenance of cell adherence.

Reagents

Methamphetamine and the dopamine D1 receptor (D1R) antagonists (SCH23390 and SKF83566) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Fluorescein isothiocyanate-conjugated antibodies against CD14, CD4, and CCR5 and the control IgGs (IgG1, IgG2a, and IgG2b) were obtained from PharMingen (San Diego, CA). Enzyme-linked immunosorbent assay kit for interferon- α (IFN- α) protein was purchased from PBL Biomedical Laboratories (Piscataway, NJ). Rabbit polyclonal antibodies against actin and signal transducer and activator of transcription1 (STAT1) were obtained from Sigma-Aldrich. Rabbit polyclonal antibody against Dopamine D1 receptor was purchased from Calbiochem (La Jolla, CA). Fluorescein isothiocyanate-conjugated goat anti-rabbit IgG

antibody was purchased from Southern Biotechnology Associates, Inc. (Birmingham, AL).

Meth and/or D1R Antagonist Treatment

Seven-day-cultured macrophages (2.5 × 10⁵ cells/well) were treated with different concentrations (1, 10, 100, and 250 μ mol/L) of meth for 3, 6, and 24 hours. These concentrations of meth are comparable with the levels found in the blood, urine, or tissue samples of meth-using subjects.^{23,26–29} To investigate whether D1 receptor antagonists block meth-induced up-regulation of HIV infection, 10 μ mol/L D1 receptor antagonist (SCH23390 or SKF83566) was added to the macrophages cultures 1 hour before meth treatment for 24 hours. The cell cultures were re-fed with fresh media containing meth and/or SCH23390 or SKF83566 every 4 days. There were no cytotoxic effects of meth, SCH23390, and SKF83566 treatment on macrophages as demonstrated by trypan blue dye staining (data not shown).

Infection of Macrophages with HIV Bal Strain

The macrophage-tropic R5 strain (Bal) was obtained from the AIDS Research and Reference Reagent Program (NIH, Bethesda, MD). Macrophages were infected with equal amounts of cell-free HIV Bal (p24 20 ng/10⁶ cells) for 2 hours at 37°C after 24 hours of treatment with or without meth. The cells were then washed three times with Dulbecco's modified Eagle's medium to remove unabsorbed virus, and fresh media containing meth and/or SCH23390 or SKF83566 were added to the cell cultures. The final wash was tested for HIV reverse transcriptase (RT) activity and shown to be free of residual inocula. Untreated cells served as a control. Culture supernatants were collected for HIV RT activity assay at days 4, 8, and 12 after infection.

HIV RT Assay

HIV RT activity was determined based on the technique of Guo et al²⁰ and Ho et al³⁰ with modifications. In brief, 10 μ l of culture supernatants from macrophages infected with or without HIV was added to a cocktail containing poly(A), oligo(dT) (Amersham Biosciences, Inc., Piscataway, NJ), MgCl₂, and [³²P]dTTP (Amersham Biosciences, Inc.) and incubated for 20 hours at 37°C. Then, 30 μ l of the cocktail was spotted onto DE81 paper, dried, and washed five times with 2× saline-sodium citrate buffer and once with 95% ethanol. The filter paper was then air-dried. Radioactivity was counted in a liquid scintillation counter (PerkinElmer Life Sciences, Boston, MA).

Flow Cytometry

To determine whether meth affects the expression of CD14, CD4, and CCR5 receptors on macrophages, the cells were incubated with or without 100 μ mol/L meth for 24 hours and then removed from the culture plate and

resuspended in 100 μ l of PBS. After incubation with 20 μ l of fluorescein isothiocyanate-conjugated antibodies against CD14, CD4, and CCR5 for 45 minutes at 4°C, the cells were washed twice with PBS and fixed with 1% paraformaldehyde in PBS. Fluorescein isothiocyanate-conjugated control IgG was used as a control antibody. Fluorescence-positive cells were analyzed on an EPICS-elite flow cytometer (Beckman Coulter, Inc., Hialeah, FL).

Real-Time RT-PCR

Total RNA was extracted from macrophages using Tri-Reagent (Molecular Research Center, Cincinnati, OH) as previously described.³¹ Total cellular RNA (1 μ g) was subjected to reverse transcription using the reverse transcription system from Promega (Madison, WI). The real time RT-PCR for the quantification of D1R, IFN- α , and glyceraldehyde-3-phosphate dehydrogenase mRNA was performed with the iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA) as previously described.³² The amplified products were visualized and analyzed using the software MyiQ provided with the thermocycler (iCycler iQ real time PCR detection system; Bio-Rad Laboratories). The levels of glyceraldehyde-3-phosphate dehydrogenase mRNA were used as an endogenous reference to normalize the quantities of targets mRNA. The special oligonucleotide primers used in this study were listed as follows: D1R, 5'-AAACCCACAAGCCCCTCTGA-3' (sense) and 5'-GATGAATTAGCCCACCCAAAC-3' (antisense)³³; IFN- α , 5'-TTTCTCCTGCCTGAAGGACAG-3' (sense) and 5'-GCTCATGATTTCTGCTCTGACA-3' (antisense); and glyceraldehyde-3-phosphate dehydrogenase, 5'-GGTGGTCTCCTCTGACTTCAACA-3' (sense) and 5'-GTTGCTGTAGCCAAATTCGTTGT-3' (anti-sense). The oligonucleotide primers were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA).

Immunofluorescence Assay

The macrophages were cultured on glass coverslips at a density of 0.25×10^6 /well in 24-well plates. The macrophages were washed with $1 \times$ cold PBS (with Ca^{2+} and Mg^{2+}) twice. Cells were fixed at 4°C in 4% paraformaldehyde-4% sucrose in PBS for 20 minutes and then permeated in cold methanol (100%) for additional 10 minutes followed by 0.2% Triton X-100 for additional 10 minutes. Cells were blocked in Block Solution (Pierce, Rockford, IL) for 1 hour at room temperature. To examine expression of D1R, rabbit polyclonal antibody (1:500) against D1R was used as the primary antibody. After washing five times with $1 \times$ PBS, the cells were incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG antibody (green, 1:100) for 1 hour. After washing five times with $1 \times$ PBS, the cells were mounted on glass coverslips in mounting media (Biomedex, Foster City, CA) and viewed with a fluorescence microscopy (Zeiss, Jena, Germany). Hoechst 33342 was used for nuclei morphology.

Western Blot

Total cell lysates of macrophages were prepared using a radioimmune precipitation assay buffer (Promega) with 1% protease inhibitor cocktail (Sigma-Aldrich). Protein concentrations were determined by the protein assay kit (Bio-Rad Laboratories). Western blot assay was carried out as previously described.³¹ STAT1 and actin proteins were detected using rabbit anti-STAT1 (1:1000; Sigma-Aldrich) and anti-actin (1:3000; Sigma-Aldrich) polyclonal antibodies, respectively. Peroxidase-conjugated goat anti-rabbit antibody (1:10,000; Jackson ImmunoResearch Laboratories, West Grove, PA) was used as the second antibody. The bound antibodies were recognized by using SuperSignal West Pico Chemiluminescent Substrate Kit (Pierce) according to the manufacturer's instruction. Prestained molecular markers (Bio-Rad Laboratories) were used to determine molecular weight of immunoreactive bands.

Enzyme-Linked Immunosorbent Assay

Total cell lysates from the cultured macrophages were prepared using a radioimmune precipitation assay buffer (Promega). Enzyme-linked immunosorbent assay for IFN- α was performed according to the protocol provided by the manufacturer (PBL Biomedical Laboratories).

Statistical Analysis

Student's *t*-test was used to evaluate the significance of difference between groups, and multiple comparisons were performed by regression analysis and one-way analysis of variance. *P* values of less than 0.05 were considered significant. All data are presented as mean \pm SD. Statistical analyses were performed with SPSS 11.5 for Windows. Statistical significance was defined as *P* < 0.05.

Results

Meth Enhances HIV Infection of Macrophages

We first determined the effect of meth on HIV infection of macrophages. As shown in Figure 1, meth treatment resulted in increase of HIV RT activity. This meth-mediated increase of HIV RT activities is statistically significant. In addition, the increase of HIV RT activity was dose- and time-dependent (Figure 1, A and B). The highest enhancement of HIV by meth was observed with a dose of 250 μ mol/L (Figure 1A) at day 8 after infection (Figure 1B).

D1R Is Involved in Meth-Mediated Up-Regulation of HIV Infection

D1R has been implicated in the pathological effect of meth on the target cells. Thus, it is of importance to determine whether macrophages express D1R that is

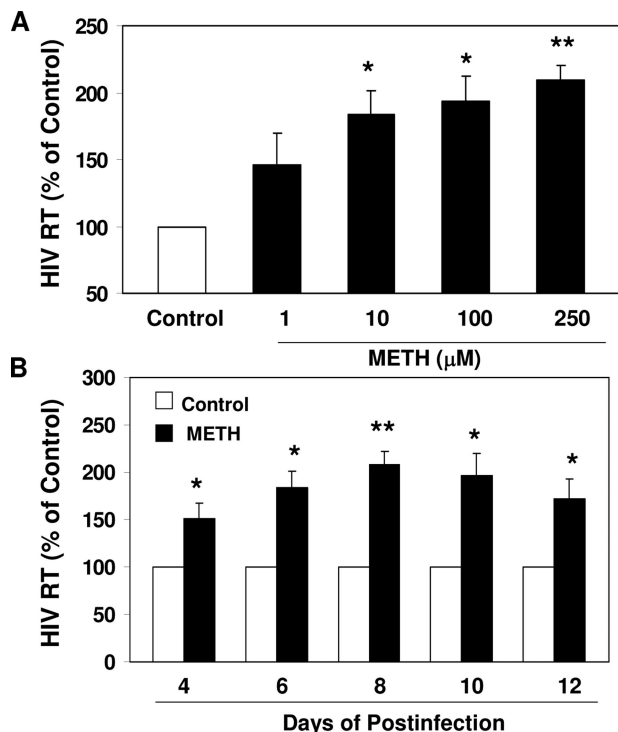


Figure 1. Dose-dependent (A) and time-course (B) effect of meth on HIV Bal replication in macrophages. **A:** Seven-day-cultured macrophages were incubated with or without meth at indicated concentrations for 24 hours and then incubated with HIV Bal strain for 2 hours in the presence or absence of meth. Day-8 culture supernatants were collected for HIV RT assay. **B:** Seven-day-cultured macrophages were incubated with or without 100 μmol/L meth for 24 hours before infection with HIV Bal strain for 2 hours and then cultured for 12 days. HIV RT activity was determined in cultured supernatants at indicated time points after infection. Data are expressed as HIV RT activity in meth-treated cells (percentage of control) compared with those in untreated cells, which are defined as 100%. The results represent the mean ± SD of three independent experiments using macrophages from three different donors. Statistical analysis was performed by one-way analysis of variance (A) or Student's *t*-test (B), and significance is shown with **P* < 0.05 and ***P* < 0.01 (meth versus control).

involved in the meth action on HIV replication. We performed a conventional RT-PCR assay using the primary pair specific for D1R detection. Ethidium bromide staining of RT-PCR-amplified products from macrophages showed a visible 471-bp band that is identical to that from human neuronal cells (NT2-N) (Figure 2A). We also observed that there is expression of D1R in macrophages at protein level (Figure 2B). We then examined whether the enhancing effect of meth on the HIV Bal infection was mediated through the D1R. The D1R antagonists (SCH23390 and SKF83566) completely abrogated the enhancing effect of meth on HIV RT activity (Figure 3), whereas the antagonists alone had little effect on HIV infection of macrophages (Figure 3).

Meth Induces HIV Entry Receptor CCR5 Expression

Because CCR5 receptor is a primary co-receptor for HIV M-tropic strain entry into macrophages,³⁴ we examined whether meth has the ability to modulate the expression of CCR5 receptor. Meth treatment (100 μmol/L) signifi-

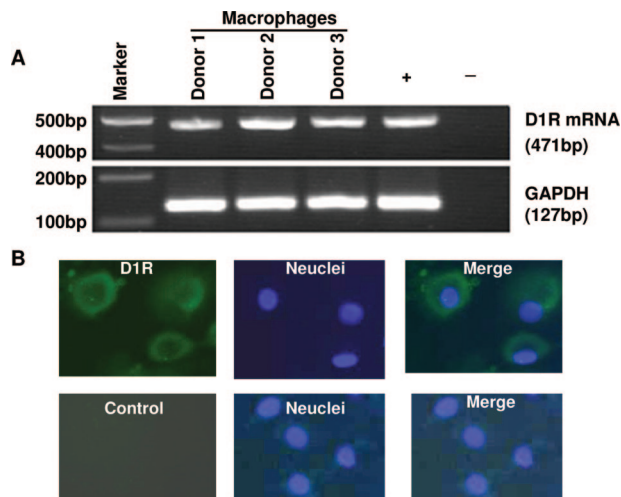


Figure 2. Dopamine 1 receptor (D1R) expression in macrophages. **A:** Total mRNA was extracted from 7-day-cultured macrophages and subjected to the real time RT-PCR for D1R. Sizes are estimated from DNA ladder (100-bp fragments) co-electrophoresed as markers. **Lane 1,** markers; **lanes 2 to 4,** macrophages from three different donors, respectively; **lane 5,** human neuronal cells (NT2-N) as a positive control (+); **lane 6,** negative control (-); the same cells as used in the positive control but processed without reverse transcriptase. **B:** The macrophages plated on coverslips were fixed, permeabilized, and stained with (top panel) or without (bottom panel) antibody to D1R (green) and with nuclear staining dye (blue). The specimens were examined by a fluorescence microscopy with magnification of ×200. One representative result of three independent experiments is shown.

cantly up-regulated CCR5 expression on macrophages as determined by flow cytometry (Figure 4). This meth action on CCR5 expression, however, was not mediated by D1R, because the D1R antagonist (SCH23390 and SKF 83566) did not block the meth effect (data not shown). To determine the specificity of the effect of meth on CCR5 expression, we also examined whether macrophage marker (CD14) and CD4 receptor are affected by meth. Meth, when added to macrophage cultures, had

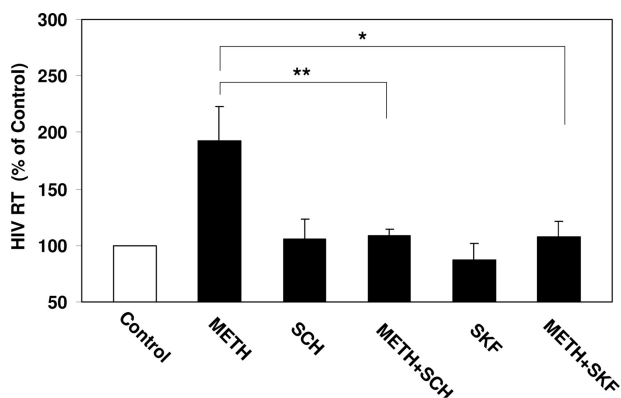


Figure 3. Effect of the D1R antagonists on meth-mediated up-regulation of HIV Bal infection. Seven-day-cultured macrophages were incubated with or without the D1R antagonist, 10 μmol/L SCH23390, or 10 μmol/L SKF83566 for 1 hour before treatment with or without 100 μmol/L meth for 24 hours and then infected with HIV Bal strain for 2 hours in the presence or absence of meth and/or SCH23390 or SKF83566. Culture supernatants were collected at day 8 after infection for HIV RT assay. Data are expressed as HIV RT activity in meth-treated cells (percentage of control) to those in untreated cells, which is defined as 100%. The results represent the mean ± SD of three independent experiments. Statistical analysis was performed using one-way analysis of variance, and significance is shown with **P* < 0.05 and ***P* < 0.01 (METH + SCH or METH + SKF versus METH).

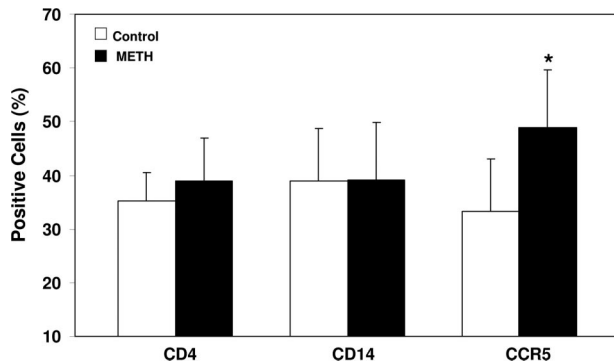


Figure 4. Effect of meth on the expression of CD4, CD14, and CCR5 receptors. Seven-day-cultured macrophages were treated with or without 100 $\mu\text{mol/L}$ meth for 24 hours. Expression of CD4, CD14, and CCR5 on macrophages was determined by flow cytometry assay. The data shown are the percentage of positive cells for the indicated receptors and represent the mean \pm SD of three independent experiments. Statistical analysis was performed using Student's *t*-test, and significance is shown with * $P < 0.05$ (meth versus control).

little effect on the expression of CD14 and CD4 receptors (Figure 4).

Meth Suppresses Endogenous IFN- α and STAT1 Expression

To look for potential mediators involved in meth-mediated enhancement of HIV replication, we examined the impact of meth on IFN- α expression by macrophages. IFN- α is a potent antiviral cytokine that impedes HIV infection of macrophages.^{35–37} We hypothesized that meth inhibits endogenous IFN- α expression in macrophages, which could be a mechanism involved in meth-mediated up-regulation of HIV. Meth treatment of macrophages resulted in a significant decrease of endogenous IFN- α at both mRNA and protein levels (Figure 5, A and B, respectively). This meth-mediated down-regulation of IFN- α in macrophage was completely blocked by the D1R antagonists (SCH23390 and SKF83566) (Figure 6). To further determine whether meth, through suppressing IFN signaling pathway, enhances HIV replication, we investigated the effect of meth on the expression of STAT1, the major component of the IFN signaling cascade.^{38,39} Macrophages treated with meth expressed lower levels of STAT1 proteins than untreated macrophages (Figure 7).

Discussion

Epidemiological studies have demonstrated that meth use is particularly common among HIV-infected patients.^{10,40} However, very little is known about the deleterious effect of meth on the host's immune response and the role of meth in the immunopathogenesis of HIV infection. It has been proposed that the modulatory effects of meth on the immune functions related to response to HIV infection may increase the susceptibility of an individual to initial HIV infection and promote the development of HIV infection toward AIDS.¹⁴ One of the ways to address the complex interactions between meth use and HIV is to use a permissive cell system such as macrophages.

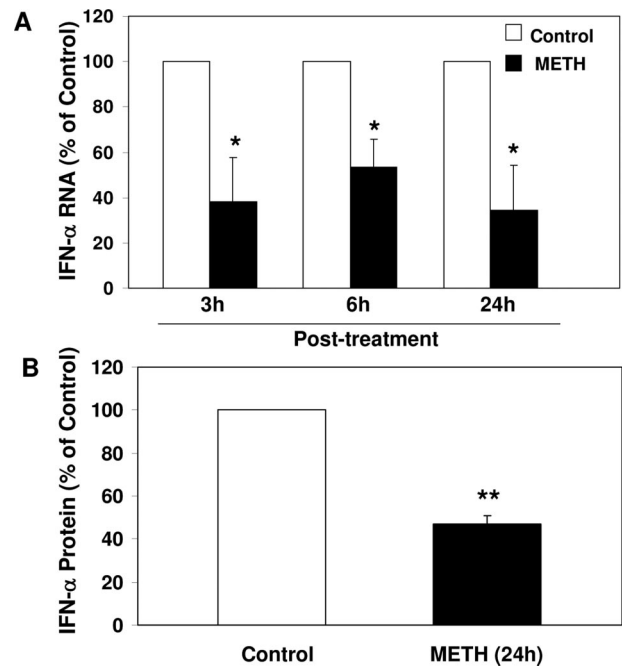


Figure 5. Effect of meth on endogenous IFN- α mRNA (A) and protein (B) expression in macrophages. **A:** Seven-day-cultured macrophages were treated with or without 100 $\mu\text{mol/L}$ meth for the indicated time points, and then cellular RNA were subjected to the real-time RT-PCR for IFN- α mRNA. **B:** Cell lysates from seven-day-cultured macrophages treated with or without 100 $\mu\text{mol/L}$ METH for 24 hours were subjected to enzyme-linked immunosorbent assay for IFN- α protein. Data are expressed as IFN- α mRNA (A) or protein (B) levels in meth-treated cells (percentage of control) to those in untreated cells, which are defined as 100%. The results represent the mean \pm SD of three independent experiments. Statistical analysis was performed by Student's *t*-test and significance is shown with * $P < 0.05$ and ** $P < 0.01$ (meth versus control).

Macrophages play a central role in the immunopathogenesis of HIV disease. Monocytes/macrophages are involved in HIV infection during all stages of disease in which they serve as major target cells, reservoirs, vehicle

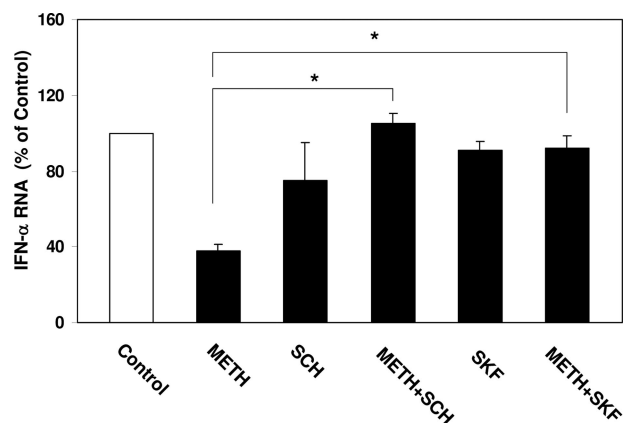


Figure 6. Effect of the D1R antagonists on meth-mediated down-regulation of IFN- α . Seven-day-cultured macrophages were incubated with or without the D1R antagonist, 10 $\mu\text{mol/L}$ SCH23390, or 10 $\mu\text{mol/L}$ SKF83566 for 1 hour before treatment with or without 100 $\mu\text{mol/L}$ meth for 3 hours. Cellular RNA was subjected to the real-time RT-PCR for IFN- α mRNA. Data are expressed as IFN- α mRNA levels in meth-treated cells (percentage of control) to those in untreated cells, which are defined as 100%. The results represent the mean \pm SD of three independent experiments. Statistical analysis was performed using one-way analysis of variance, and significance is shown with * $P < 0.05$ and ** $P < 0.01$ (METH + SCH or METH + SKF versus METH).

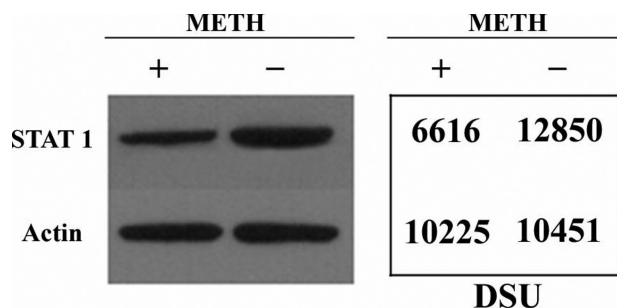


Figure 7. Effect of meth on STAT1 expression. Seven-day-cultured macrophages were incubated with or without 100 μ mol/L meth for 24 hours. Equal amount of proteins extracted from the cells was subjected to the Western blot assay using rabbit antibodies against STAT1 and actin. The numbers in the **right panel** are the signal intensities expressed as densitometry scanning units (DSU) of protein bands of Western blot shown in the **left panel**. One representative experiment is shown.

to other tissues, and transmitters of the virus to CD4⁺ T cell. Thus, it is necessary to determine whether meth has the ability to enhance HIV infection of macrophages. Our findings that meth increased HIV replication in macrophages (Figure 1) provide direct *in vitro* evidence to support the clinical study¹⁴ showing that plasma virus loads were higher in meth users. In addition, our study is in agreement with the investigation showing that meth exposure can accelerate feline immunodeficiency virus replication.¹⁵

Direct action of meth on cellular functions may require its interaction with the dopamine receptors on the target cells. Because D1R has long been implicated in mediating the persistent dopaminergic deficits caused by meth,^{41,42} we postulated that the D1R expressed on macrophages is involved in immunoregulating macrophage functions. We showed that human blood monocyte-derived macrophages expressed D1R (Figure 2). Most importantly, we demonstrated that D1R expressed on macrophages is functional, because the D1R antagonists blocked the action of meth on HIV infection of macrophages (Figure 3). This finding is supported by a recent report⁴³ showing that meth-mediated up-regulation of DC-SIGN on monocyte-derived dendritic cells was reversed by the D1R antagonist SCH23390. Because both dendritic cells and macrophages are antigen-presenting cells, it is possible that D1R is involved in the meth action in the both cell systems. We also examined the hypothesis that meth has the ability to stimulate D1R expression by macrophages. It has been reported that D1R protein is elevated in nucleus accumbens of human, chronic methamphetamine users.⁴⁴

However, our experiments examining the impact of meth on D1R failed to reveal the evidence that meth stimulates D1R expression (data not shown), suggesting that up-regulation of D1R is not a mechanism responsible for the meth action on HIV. In addition to dopamine receptors, dopamine was also shown to be involved in the regulation of HIV gene expression in both neuronal cells and cells of the immune system.⁴⁵ Dopamine activates HIV expression in chronically infected T cells.⁴⁶ Thus, it is possible that meth, through the induction of endogenous dopamine in macrophages, enhanced HIV

replication. We, however, were unable to detect the expression of endogenous dopamine by primary macrophages. This finding is in disagreement with the report that a macrophage cell line produced endogenous dopamine.⁴⁷ This discrepancy could be due to the difference in the cell types used. In our study, we used primary monocyte-derived macrophages. Nevertheless, the *in vivo* interpretations of these observations remain to be determined.

In the present study, we have demonstrated two potential mechanisms by which meth may enhance HIV replication. We first showed that meth enhanced HIV infection and replication by inducing the expression of CCR5 receptor on macrophages. This finding supports the report²³ that meth up-regulates CCR5 receptor expression in monocyte-derived mature dendritic cells. Our data also are in agreement with the observations of Gavrilin et al¹⁵ showing that meth influences the first step of virus (feline immunodeficiency virus) interaction during cell-to-cell transmission of virus. The effect of meth on CCR5, however, is not mediated by D1R, because the D1R antagonists failed to block the meth action (data not shown), suggesting that meth modulates CCR5 expression by a different mechanism. As a key HIV entry co-receptor, CCR5 plays an important role in macrophage tropic or nonsyncytium-inducing HIV strain infection of macrophages.^{34,48,49} Thus, our finding that meth up-regulated CCR5 expression (Figure 4) provides a plausible mechanism involved in meth-mediated enhancement of HIV infection of macrophages.

In addition, we investigated the impact of meth on the expression on endogenous IFN- α , a potent antiviral cytokine, in macrophages. The role of meth in modulating cytokine expression has been examined by others. For example, meth exposure significantly inhibits Th1 cytokine (interleukin-2 and IFN- γ) production in splenocytes.^{50,51} Meth is also able to significantly increase the expression of tumor necrosis factor- α and interleukin-6,⁵¹ the cytokines that have potential to enhance HIV replication.⁵² However, it is unclear whether meth can modulate IFN- α expression. Our study for the first time demonstrates that meth has the ability to suppress the expression of intracellular IFN- α in macrophages (Figure 5). This meth action on IFN- α is mediated by D1R, because the D1R antagonists completely blocked the effect of meth on IFN- α expression by macrophages (Figure 6). IFN- α is a key element of the innate defense mechanism against viral infections. It has been demonstrated that IFN- α is a potent inhibitor of HIV infection of CCR5⁺CD4⁺ macrophages.^{36,37} Thus, the suppression of intracellular IFN- α expression by meth would provide a favorable microenvironment for HIV replication in macrophages. To further determine whether meth, through suppressing the IFN signaling pathway, inhibits IFN- α expression in macrophages, we examined the hypothesis that meth inhibits the expression of STAT1, a crucial factor in mediating IFN-dependent biological responses, including the activation of the antiviral state and the induction of type I IFN expression.⁵³⁻⁵⁵ The finding that meth suppressed STAT1 expression (Figure 7) supports our hypothesis and provides a mechanism for the meth-mediated down-

regulation of endogenous IFN- α in macrophages. These data also support the studies by others^{22,23} showing that meth is immunosuppressive.

Taken together, meth, through the enhancement of CCR5 expression and the suppression of intracellular IFN- α /STAT1 expression, promotes HIV infection of macrophage. These findings provide direct evidence at cellular and molecular levels to support the concept that meth has a cofactor role in enhancing HIV infection and replication. However, further clinical studies are required to validate our *in vitro* observations and to delineate the mechanisms of meth-mediated enhancement of HIV infection *in vivo*. These studies will be critical not only for our basic understanding of meth-mediated HIV immunopathogenesis but also for the design and development of innate immunity-based intervention and treatment strategies for meth users with HIV infection.

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