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Impact of Methamphetamine Use on Immunologic and Virologic Markers during Suppressive ART

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BACKGROUND

► Methamphetamine is a widely used recreational drug in North America, and especially among HIV-infected men who have sex with men (MSM)[1].

► In HIV-infected subjects, methamphetamine (meth)-users are at increased risk for multiple infectious diseases and have more pronounced neuronal injury, cognitive impairment and accelerated progression to AIDS, compared with HIV-infected individuals who do not use meth.[2,3]

► It is unclear if the associations between methamphetamine use and HIV disease progression are uniquely a consequence of reduced ART compliance and higher risk behavior associated with meth consumption.

► *In vitro* studies have shown methamphetamine is associated with increased cellular HIV transcription, immune activation and up-regulation of surface HIV co-receptor CCR5 expression with consequent enhanced HIV infection[4-7].

► However, little is known about the *in vivo* effects of methamphetamine on T lymphocytes and how it may lead to compromises in regulation of immune function.

OBJECTIVE

To evaluate the effect of methamphetamine (and other recreational drugs) on virologic and immunological markers in HIV-suppressed individuals

METHODS

► Design and subjects: Baseline samples from 50 HIV-infected MSM on long-term ART with plasma HIV RNA <50 copies/ml and who were enrolled in a one year prospective clinical trial (Poster #943) to reduce sexually transmitted infections were studied. Adherence to ART, methamphetamine and other drug use (other club drugs, marijuana, cocaine, heroine and alcohol) were assessed monthly with a self-administered internet-based survey along with other risk behaviors. Meth-users (and/or other drug-users) reported consumption of methamphetamine (and/or other drugs) at least in one survey over the period of the study.

► Immune Activation Markers: CD8 and CD4 T-cell subsets and the expression of activation markers in frozen PBMCs samples were analyzed by flow cytometry using the following antibody combinations:

Activation: HLA-DR-FITC, CD45RA-PE, CD14-ECD, CD38-PE-Cy7, CD27-APC, CD3-APC-CH7, CD4-PerCP-Cy5.5 and CD8-V450, Aqua L/D

Proliferation: Ki67-FITC, CD45RA-PE,CCR7-PE-Cy7, CD27-APC, CD3-APC-CH7, CD4-PerC-Cy5.5 and CD8-V450, Aqua L/D

CCR5: CCR5-FITC, CD45RA-PE, CD14-ECD, CCR7-PE-Cy7, CD27-APC, CD3-APC-CH7, CD4-PerC-Cy5.5 and CD8-V450, Aqua L/D

► Episomal and Total HIV cDNA forms: Total HIV DNA (pol), 2-long terminal repeat (2-LTR), and cellular HIV RNA (unspliced gag and multiply-spliced encoding for tat/rev) were measured in PBMCs by droplet digital PCR[8].

► Semen: Levels of HIV RNA, CMV and EBV DNA were measured in seminal plasma by RT-PCR[9].

► Statistical Analysis: Outcome variables were assessed for normal distribution and compared by two sided p value by either Student t-test (for normal data) or Mann-Whitney U test (for non-normal data) comparing self reported drug use to no drug use.

RESULTS

Table 1. Patient characteristics

All subjects (n=50)			
Age (years), mean±95% CI	46±3	**Time on ART** (years), mean±95% CI	4.0±0.9
Sex (male), n (%)	50 (100)	**VL <50 copies/mL**, n (%)	50 (100)
Race, n (%)		**Antiretroviral treatment**[1], n(%)	
White	21 (42)	PI-based	30 (60)
Hispanic	17 (4)	NNRTI-based	17 (34)
Black	10 (20)	Raltegravir included in regimen	7 (14)
Other	2 (4)	**Drug use**, n (%)	
CD4 T-cell counts (cell/µl), mean±95% CI	637±48	Marijuana	20 (40)
CD4 Percentage, mean±95% CI	32.2±2.1	Methamphetamine	16 (32)
CD8 T-cell counts (cell/µl), mean±95% CI	845±115	Other Club Drugs[2]	13 (26)
CD8 Percentage, mean±95% CI	40.1±2.9	Alcohol[3]	12 (24)
Ratio CD4/CD8, mean±95% CI	0.9±0.1	Cocaine	11 (22)

[1] ART regimen are not mutually exclusive, [2]Other club drugs: Ecstasy, GHB, Amyl nitrates, ketamine, [3]5 or more alcoholic drinks on a single occasion > 5 times/month

Table 2. Effect of drugs on proliferation, immune activation and HIV reservoir

P-values (Mann-Mann-Whitney) obtained by comparing users of a particular drug (left column) and non-users are indicated. Upward arrowheads (↑) indicate significantly higher levels in drug-users while downward arrowheads (↓) significantly lower levels in drug-users

			CD8 T cells				CD4 T cells				
	number of drug-users[1]	number of meth-users[1]	Ki67+	CD45RA-CD38+	HLA-DR+CD38+	CCR5+	Ki67+	CD45RA-CD38+	HLA-DR+CD38+	CCR5+	Log_{10} HIV DNA
Marijuana	20	8	0.169	0.778	0.090 ↓	0.969	0.154	0.236	0.899	0.469	0.684
Methamphetamine	16	16	0.002 ↑	0.836	0.544	0.837	0.005 ↑	0.005 ↑	0.119	0.174	0.09 ↑
Other Club Drug [2]	13	8	0.138	0.090 ↑	0.758	0.301	0.157	0.010 ↑	0.448	0.617	0.757
Alcohol[3]	12	5	0.637	0.248	0.545	0.472	0.567	0.956	0.228	0.663	0.724
Cocaine	11	8	0.038 ↑	0.651	0.264	0.192	0.295	0.643	0.827	0.632	0.243

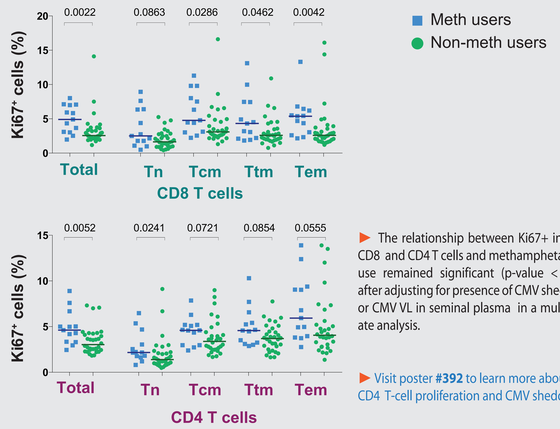
[1]Drug-users reported consumption of the specified drug at least in one survey over the period of the study. [2]Other club drugs: Ecstasy, GHB, Amyl nitrates, ketamine. [3] 5 or more alcoholic drinks on a single occasion > 5 times/month

Table 3. Characteristics of Meth and non-meth users

	Meth[1] n=16	Non-Meth n=34	*P*-value[5]
Age (years), mean (95% CI)	43 (39-48)	47 (44-51)	*0.121*
Time on ART (years), median [IQR]	4.4 [2.2-5.3]	3.4 [1.7-5.5]	*0.510*
CD4 T-cell counts (cell/µl), mean (95% CI)	640 (519-759)	636 (585-686)	*0.956*
CD8 T-cell counts (cell/µl), mean (95% CI)	985 (670-1301)	782 (660-903)	*0.129*
Ratio CD4/CD8, mean (95% CI)	0.75 (0.59-0.90)	0.97 (0.8-1.13)	***0.09***
Antiretroviral treatment, n (%)			
PI-based	13 (87)	17 (50)	***0.02***
NNRTI-based	4 (27)	16 (47)	***0.22***
Raltegravir containing regimen	1 (7)	6 (18)	*0.406*
Virus Shedding in semen, n (%)			
HIV	3 (19)	1 (3)	***0.09***
CMV	12 (75)	9 (26)	***0.0019***
EBV	4 (25)	8 (24)	*1.00*
Any other STD[4] , n (%)	2 (12.5)	3 (8.8)	*0.64*
Drug use[1], n (%)			
% of Positive surveys for Methamphetamine, median [IQR]	40 [21-79]	NA	
Marijuana[1]	11 (69)	9 (26)	***0.0063***
Cocaine[1]	8 (50)	3 (9)	***0.002***
Other Club Drug [1,2]	8 (50)	5 (15)	***0.014***
Alcohol[1,3]	5 (31)	7 (21)	*0.496*

[1]Drug-users reported consumption of the specified drug at least in one survey over the period of the study, [2]Other club drugs: Ecstasy, GHB, Amyl nitrates, ketamine, [3]5 or more alcoholic drinks on a single occasion > 5 times/month, [4]5 or more alcoholic drinks on a single occasion > 5 times/month, [5]Including Gonorrhea, Chlamydia, Syphilis, trichomonas, mycoplasma, [6]2-tail t-test

Effect of Meth-use on proliferation



► The relationship between Ki67+ in total CD8 and CD4 T cells and methamphetamine use remained significant (p-value < 0.05) after adjusting for presence of CMV shedding or CMV VL in seminal plasma in a multivariate analysis.

► Visit poster **#392** to learn more about CD4 T-cell proliferation and CMV shedding

Figure 1. Comparison of the Proliferation frequencies (Ki67+) of total and different CD8 and CD4 T-cell subsets between meth users and non-meth users
Individual data and median values are plotted.

Effect of Meth-use on immune activation

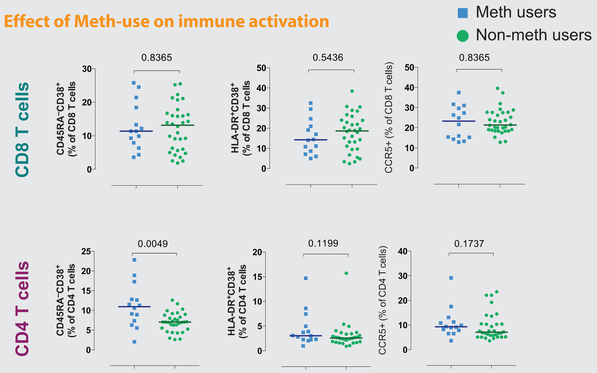


Figure 2.Comparison of the immune activation in CD8 and CD4 T-cell comparments.
Individual data and median values are plotted.

Effect of Meth-use on HIV viral reservoir

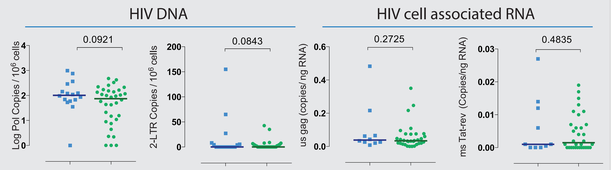


Figure 3. Comparison of the total HIV DNA, 2-LTR circles and cell associated HIV RNA (unspliced gag and multispliced tat-rev).
Individual data and median values are plotted.

CONCLUSIONS

► Among all drugs self-reported, Methamphetamine use had the most significant impact on both CD4 and CD8 T-cell proliferation (and activation). No effect was observed in modulating CCR5 expression levels.

► The increased levels of proliferarion in all T-cells subsets suggested a general and unspecific effect of meth in these cells *in vivo*.

► Despite long term ART and fully suppressed HIV RNA in blood plasma, there was a trend for meth users to have higher levels of proviral HIV reservoir and more detectable HIV RNA in seminal plasma.

► Both these findings could explain increased HIV transmission and worse HIV disease progression in subjects reporting meth use.

► Meth-users had also significantly more episodes of seminal CMV shedding compared to non-meth users, suggesting that CMV could play a role in meth-related complications and should be considered in future studies.

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