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Effect of Methamphetamine Use on T-cell Proliferation *in vivo* and *ex vivo*

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BACKGROUND

- Methamphetamine (meth) is a widely used recreational drug among HIV-infected men who have sex with men (MSM)[1].
- In HIV-infected subjects, 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 non-meth users individuals.[2,3]
- It is unclear if these associations 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].
- Little is known about the *in vivo* effects of meth on the immune system.

OBJECTIVE

To evaluate the effect of meth use on immunological and virological parameters (*in vivo* study) and T-cell function (*ex vivo* study).

METHODS

In vivo study

- 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 (CCTG592) to reduce sexually transmitted infections were studied. Adherence to ART, meth and other drug use were assessed monthly with a self-administered internet-based survey along with other risk behaviors. Meth-users reported consumption of meth at least in one survey over the period of the study.
- Immune Markers: frozen PBMCs 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, 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
  - **PD-1:** CD57–FITC, CD45RA–PE, CD28–ECD, CD4–PerCP-Cy5.5, PD-1–PE-Cy7, CD27–APC, CD3–APC-Cy7, CD8–V450, Aqua L/D
- Semen: Levels of HIV RNA and CMV DNA were measured in seminal plasma by RT-PCR[9].
- Episomal and Total HIV cDNA forms: Total HIV DNA (pol) and 2-long terminal repeat (2-LTR) were measured in PBMCs by droplet digital PCR[8].
- Statistical Analysis: Comparisons were made between the meth users and non-meth users using Fisher exact test (for dichotomous data) or Mann Whitney U test (for continuous data). To explore the cofounding effects, we adjusted our analysis for CMV-shedding in the genital tract and use of a protease inhibitor (PI) in the ART regimen using a multivariate fixed-effects regression model when one or both of these potential confounders had a p-valued <0.1 in univariate analyses.

Ex vivo study

- Subjects: 37 HIV-infected subjects enrolled in studies at the HRNP were selected. 19 HIV-infected individuals who tested positive for meth (and negative for cocaine, opiates, barbiturates, benzodiazepines, PCP, THC, and ETOH with urine toxicology). These individuals had HIV RNA levels ranging from <50 to 230,400 copies/mL and a median CD4+ T-cell count of 402 [271-618] cells/µl. 18 HIV-infected control subjects from the same cohort who tested negative for all listed substances by urine toxicology (no history of substance abuse) were matched for HIV RNA levels and CD4+ T-cell counts.
- *Ex vivo* T-cell proliferative responses: Fresh PBMCs were cultured for 7 days in presence of phytohemagglutinin (PHA) or pathogen antigens: heat-inactivated CMV, Candida, Mycobacterium tuberculosis (MTB) protein, Toxoplasma (Toxo), HIV gag/p24/p5 and heat-inactivated (1 hour, 56ºC) supernatant of HIV infected T cells [HIVAgsupt]. Cells were pulsed with [3H]-thymidine (0.5 ACi/well) 24hours before harvest. Data were presented as counts per minute (cpm) and triplicates were averaged. St imulation index (SI) was calculated as the ratio of the mean cpm as measured for each stimulus (PHA and other antigens) to the mean cpm of unstimulated control.

RESULTS

Table 1. Patient characteristics

	Meth[1] n=16	Control n=34	*P*-value[5]
Age (years), median [IQR]	45 [36-49]	48 [40-54]	*0.11*
Time on ART (years), median [IQR]	4.4 [2.2-5.9]	3.4 [1.7-5.7]	*0.51*
Nadir CD4⁺ T cell counts (cell/µl), median [IQR]	204 [120-300]	216 [60-386]	*0.94*
CD4⁺ T-cell counts (cell/µl), median [IQR]	577 [485-835]	652 [548-714]	*0.57*
CD4⁺%, median [IQR]	31 [25-36]	34 [28-37]	*0.56*
CD8⁺ T-cell counts (cell/µl), median [IQR]	852 [571-1113]	820 [507-939]	*0.27*
CD8%, median [IQR]	42 [38-52]	38 [31-46]	***0.08***
Ratio CD4/CD8, median [IQR]	0.72 [0.49-1.0]	0.83 [0.66-1.2]	***0.13***
Antiretroviral treatment, n(%)			
PI-based	**13 (87)**	**17 (50)**	***0.02***
NNRTI-based	4 (27)	16 (47)	*0.22*
Recreational drug use[1], n (%)			
% of Positive surveys for meth, median [IQR]	40 [21-79]	NA	
Marijuana[1]	11 (69)	9 (26)	***<0.01***
Cocaine[1]	8 (50)	3 (9)	***<0.01***
Other Club Drug [1,2]	8 (50)	5 (15)	***0.01***
Any other STI[4], n (%)	2 (12.5)	3 (8.8)	*0.64*

[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, [4]Including Gonorrhea, Chlamydia, Syphilis, Trichomonas, mycoplasma, [5]Mann-Whitney U test

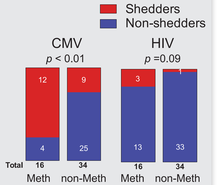


Figure 1: Seminal Virus shedding in meth and non-meth users groups.

Meth-use affects only CD4 T-cell immune activation

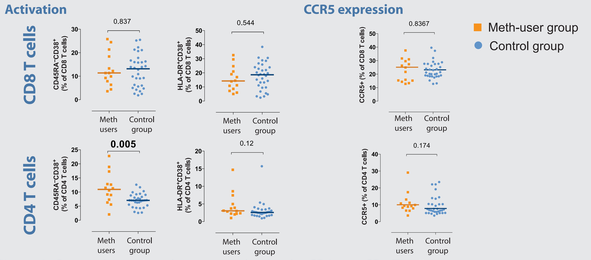


Figure 2. Comparison of the immune activation in CD8 and CD4 T-cell compartments.
Individual data and median values are plotted..Two-sided p-values (Mann Whitney U test) are indicated.

Meth-use affects both CD4 and CD8 T-cell proliferation

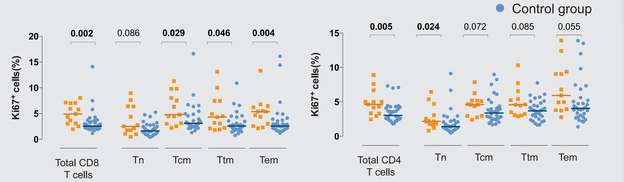
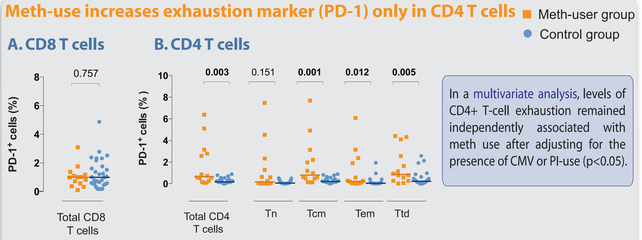


Figure 3. Comparison of the proliferation frequencies (Ki67+) of total and different CD8 and CD4 T-cell subsets between meth users and non-meth users
Individual data and median values are plotted. Two-sided p-values (Mann Whitney U test) are indicated.

Meth use remained independently associated with CD4+ and CD8+ T-cell proliferation (p<0.05 in both cases in a multivariate analysis adjusting for CMV shedding or PI-use.

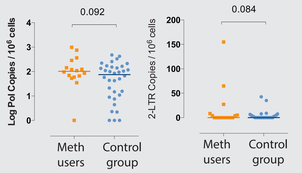
Meth-use increases exhaustion marker (PD-1) only in CD4 T cells



In a multivariate analysis, levels of CD4+ T-cell exhaustion remained independently associated with meth use after adjusting for the presence of CMV or PI-use (p<0.05).

Figure 4. Comparison of the immune exhaustion frequencies (PD-1+) of total and different CD8 (Panel A) and CD4 (Panel B) T-cell subsets between meth users and non-meth users
Individual data and median values are plotted.Two-sided p-values (Mann Whitney U test) ` are indicated.

Meth-use and HIV reservoir



After adjusting for CMV shedding or PI-use in a multivariate analysis, the increased levels of HIV DNA observed among meth users was no longer significant, suggesting that CMV shedding has a stronger effect on HIV DNA levels than a history of meth use.

Figure 5. Characterization of HIV cellular reservoir in meth users and control group.
Individual and median values are shown.Two-sided p-values (Mann Whitney U test) are indicated.

Meth-use increases spontaneous proliferation of T-cell, concomitant with a loss of antigen-specific proliferative responses

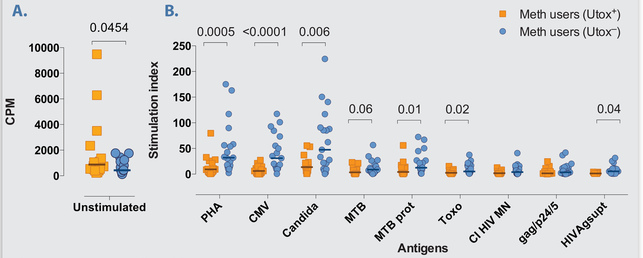


Figure 6. *Ex vivo* T-cell proliferative responses to antigen stimuli.
PBMCs from HIV infected individuals from meth users (urine toxicology positive) and non-meth users (urine toxicology negative controls) were cultured in triplicates for 7 days in absence (Panel A) or in presence of PHA and different antigen (Panel B). Individual data and median values are plotted.Two-sided p-values (Mann Whitney U test) are indicated.

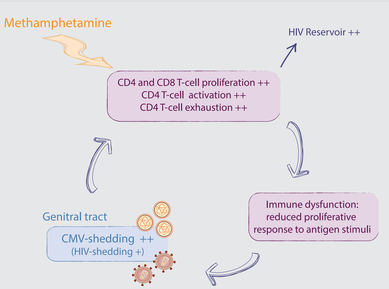


Figure 7. A model for the effect of meth use in HIV-infected ART-suppressed individuals.

CONCLUSIONS

- Meth users had significantly increased levels of CD4⁺ and CD8⁺ T-cell proliferation and, increased CD4+ T-cell activation (especially measured as the frequency of CD45RA⁻CD38⁺) and exhaustion.
- Increased proliferation in all T-cells subsets suggests a general nonspecific effect of meth use.
- Meth-users had significantly more episodes of seminal CMV-shedding suggesting that CMV-associated immune activation[10] could contribute to the overall activation.
- Despite increases in spontaneous T-cell proliferation in UTox⁺ meth users, there was a loss of multiple pathogen specific T-cell proliferative responses (i.e. CMV, HIV, Candida, MTB and Toxoplasma).
- In summary, meth use may cause sustained immune dysfunction (reduction of pathogen-specific proliferative responses), leading to a loss of control of viral replication (such as CMV shedding), which may contribute to increased immune activation, proliferation and exhaustion (Figure 6).

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