Lentivirus persistence in brain: antiretroviral therapy, viral quantity and host neuroimmune interactions

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Summary

This study investigates the efficacy of ART in brain using primary human microglial cultures and brain tissues from SIV-infected nonhuman primates and a cohort of HIV-infected humans in the absence or presence of ART. We show that ART is less effective in HIV-infected microglia, and lentivirus persists in the brain despite effective ART and independent of blood viral load.

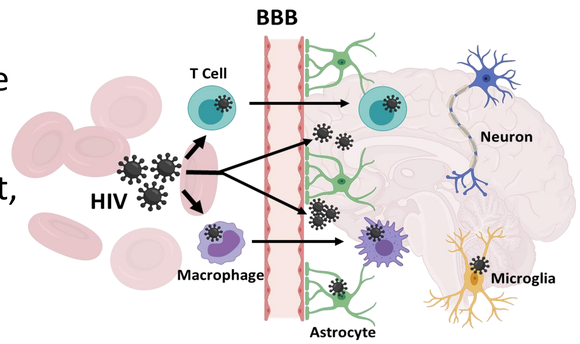
Questions

1. What is the comparative efficacy of ART drugs in suppressing HIV replication in microglia versus lymphocytes?
2. How do ART drugs, and their interruption, modulate brain viral burden in SIV-infected rhesus macaque models?
3. Does HIV-1 persist in the brain of infected patients who receive effective ART?

Introduction

Despite antiretroviral therapy (ART), HIV continues to persist in anatomical and cellular sanctuaries. HIV infects the brain and replicates in microglia (brain resident macrophages) and trafficking macrophages.

25% of HIV-infected people experience HIV associated neurocognitive impairment, despite effective ART.



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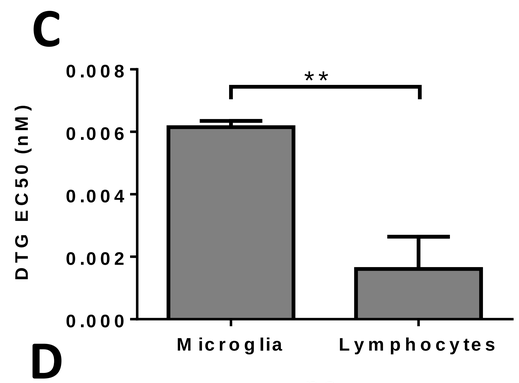
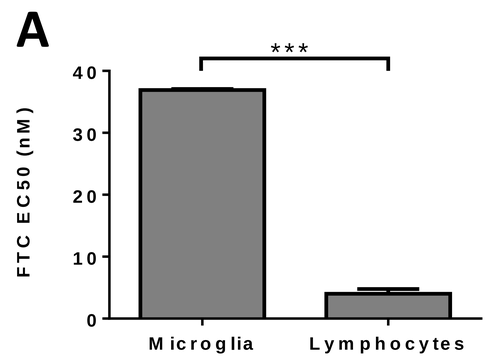
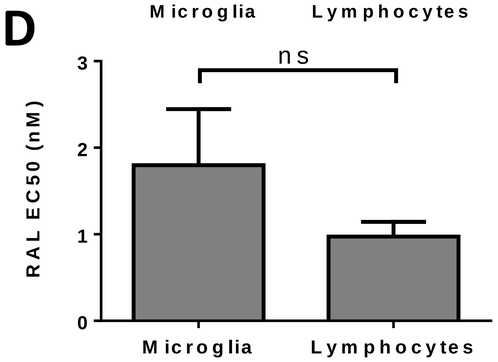
Result I: ART compounds have reduced efficacy in microglia compared to lymphocytes

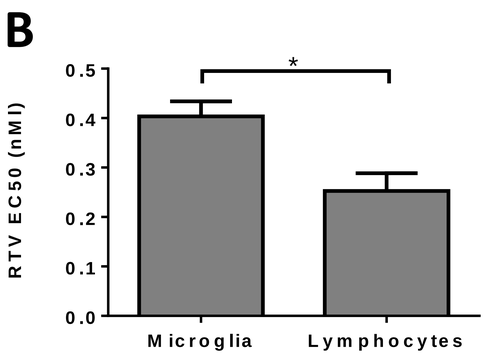
Method: to determine the EC_{50} levels of ART drugs, HIV-infected primary microglia and lymphocytes were treated with dolutegravir (DTG), ritonavir (RTV), emtricitabine (FTC), raltegravir (RAL), and tenofovir (TDF). The viral levels were measured by p24 assay.

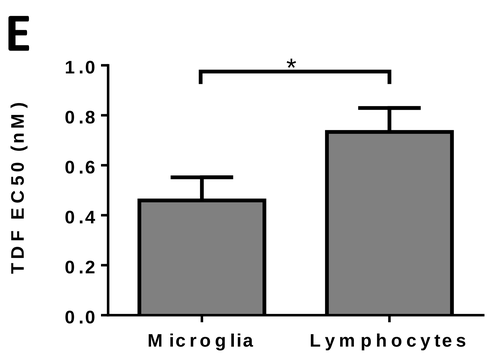
Result: FTC, DTG and RTV displayed significantly higher EC_{50} values in microglia relative to lymphocytes. In contrast, RAL showed no significant difference in antiviral efficacy in microglia versus lymphocytes while TDF inhibited viral production more efficiently in HIV-infected microglia compared to lymphocytes. This indicates reduced efficacy of most ART drugs in microglia.











Result II: ART and its interruption exerted minor effects on SIV load in brains of Chinese rhesus macaques

Chinese rhesus macaque cohort

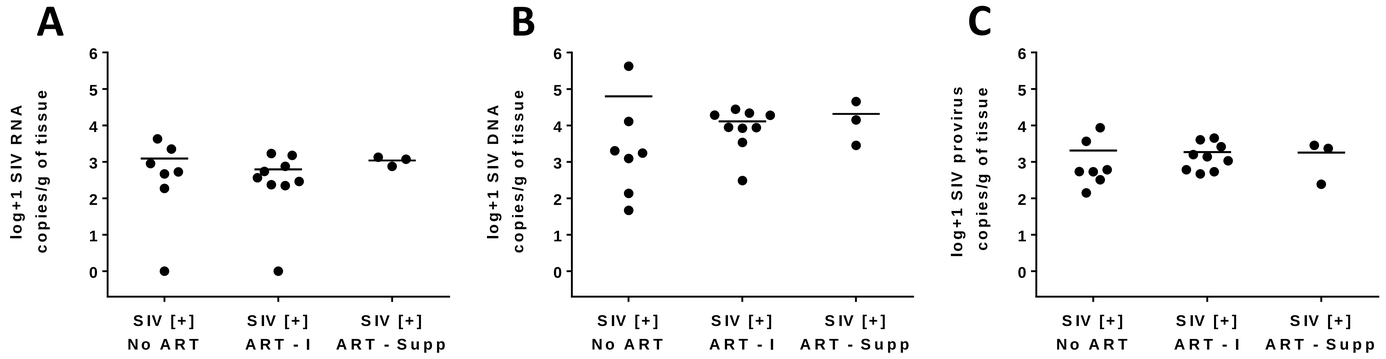
The effects of the ART (FTC, DTG, RTV, RAL, and TDF) and their interruption assessed in SIV model with three groups. ART was initiated 3 days after infection and the effects of ART, and its interruption was observed in plasma viral load.

Groups	Sex	Mean age (year) ± SD	Log Mean plasma viral copies/ml ± SD	Log Mean CSF viral copies/ml ± SD	Mean $CD3^+$ $CD4^+$ (%) ± SD
SIV [+] No ART (n=7)	**F**	**6.4 ± 1.2**	**5.62 ± 5.77**	**3.49 ± 3.76**	**30 ± 6**
SIV [+] ART-interrupted (n=8)	**F**	**5.2 ± 0.4**	**6.65 ± 7.09**	**3.57 ± 3.86**	**59 ± 10**
SIV [+] suppressed (n=3)	**F**	**5.8 ± 1.0**	**ND***	**ND***	**49 ± 6**

Detection of SIV RNA, DNA and integrated DNA in post-mortem brains of animals

Method: Total DNA and RNA were extracted from post-mortem brains of SIV-infected Rhesus macaques and viral burden was quantified by droplet digital PCR.

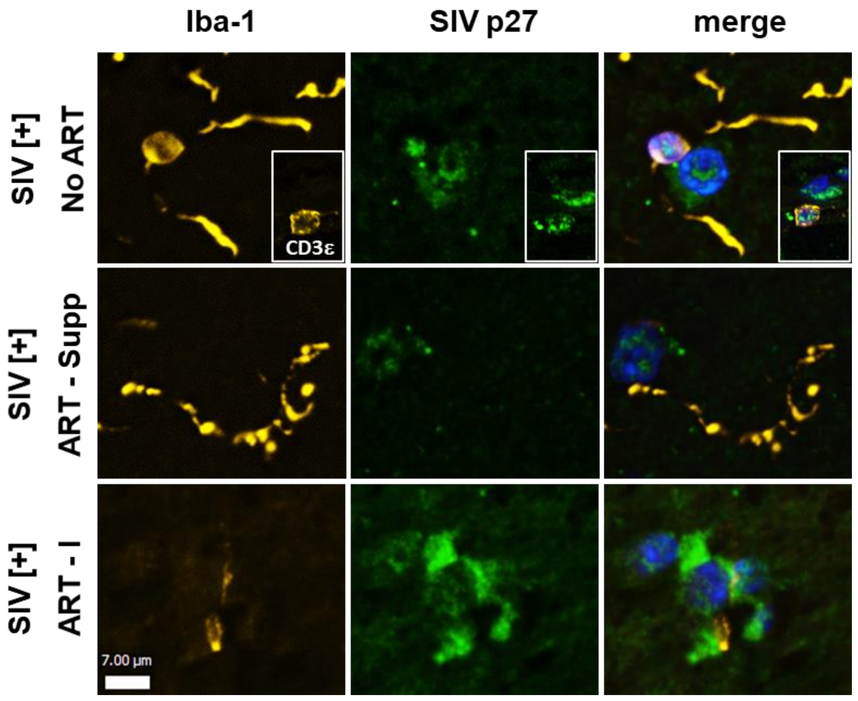
Result: SIV RNA (A), DNA (B) and integrated DNA (C) were detected in in brain tissues despite effective ART treatment with minimal effects observed during ART interruption.



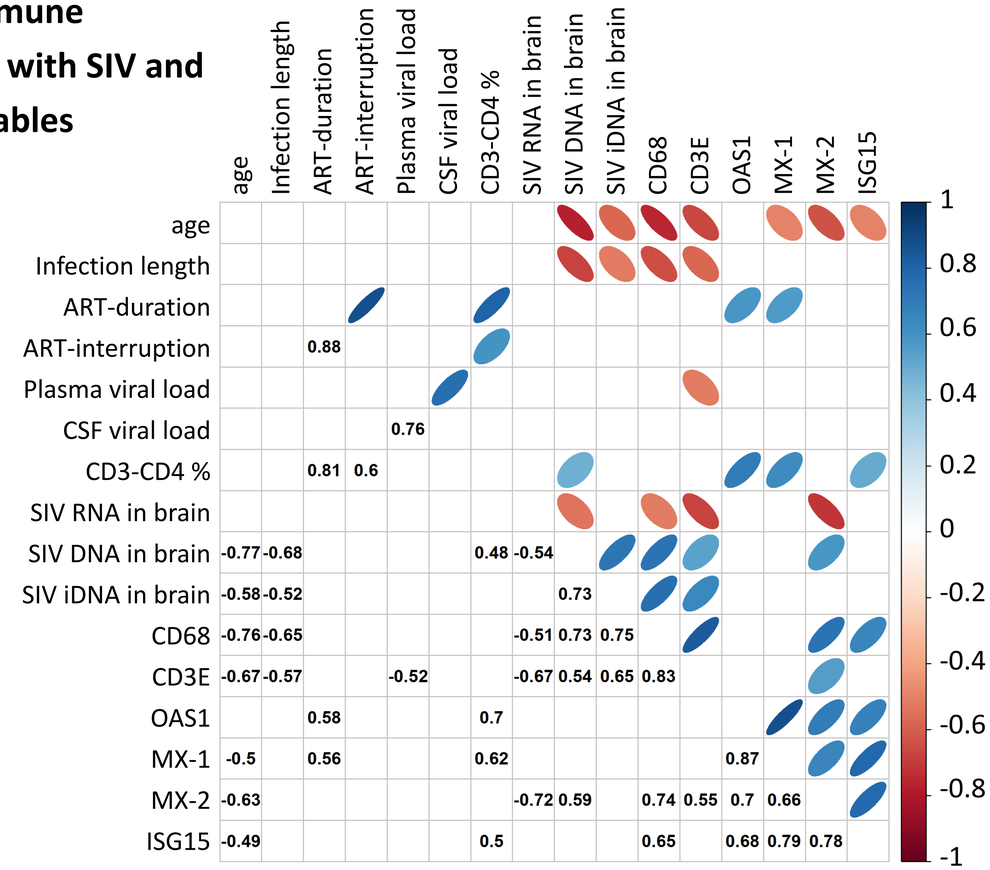
Detection of SIV p27 in microglial and T lymphocyte in brain tissue

Method:
Immunofluorescence imaging of brain tissues labeled with Iba-1 (microglial), CD3ε (T cell) and SIV p27 specific Ab.

Result:
SIV p27 was detected in parenchyma microglia, perivascular T cells and macrophages.



Neuroimmune response with SIV and host variables



Result III: HIV burden in infected individuals is unaffected by ART despite suppression of plasma viral loads

Human cohort

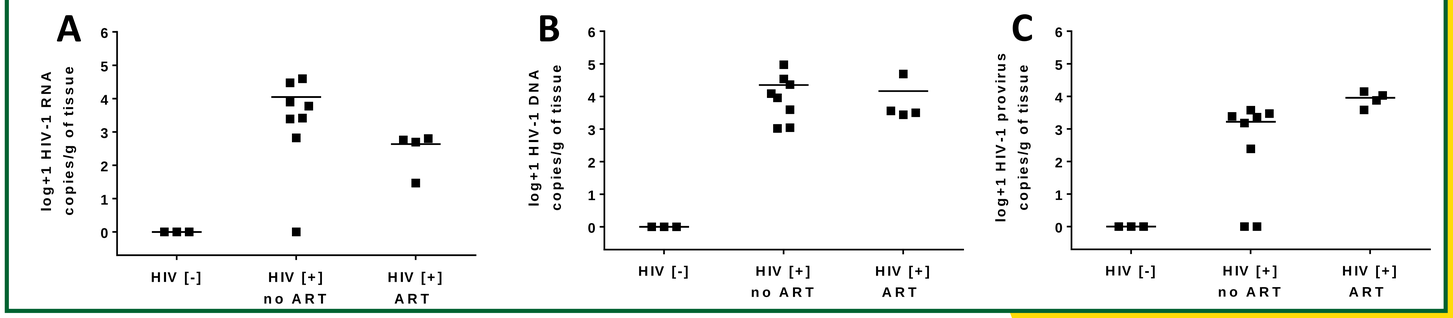
We analysed viral burden in brains from uninfected (HIV[-]), HIV-infected with uncontrolled plasma virus (HIV[+]/no ART) and controlled plasma virus, receiving effective ART for decades (HIV[+]/ART).

Groups	Sex	Mean age (year) ± SD	Log Mean plasma viral copies/ml ± SD	Average of $CD4^+$ T-cell cells/µl ± SD
HIV[-] uninfected (n=3)	3M	42.7 ± 9.6	ND*	Not available
HIV[+] no ART, unsuppressed (n=8)	6M / 2F	40 ± 6	5.63 ± 5.53	67 ± 113
HIV[+] ART, suppressed (n=4)	4M	57.8 ± 14.2	ND*	560 ± 201

Detection of HIV RNA, DNA and integrated DNA in post-mortem brains of humans

Method: Total DNA and RNA were extracted from post-mortem brains of HIV-infected and uninfected individuals. HIV-1 burden was quantified by droplet digital PCR.

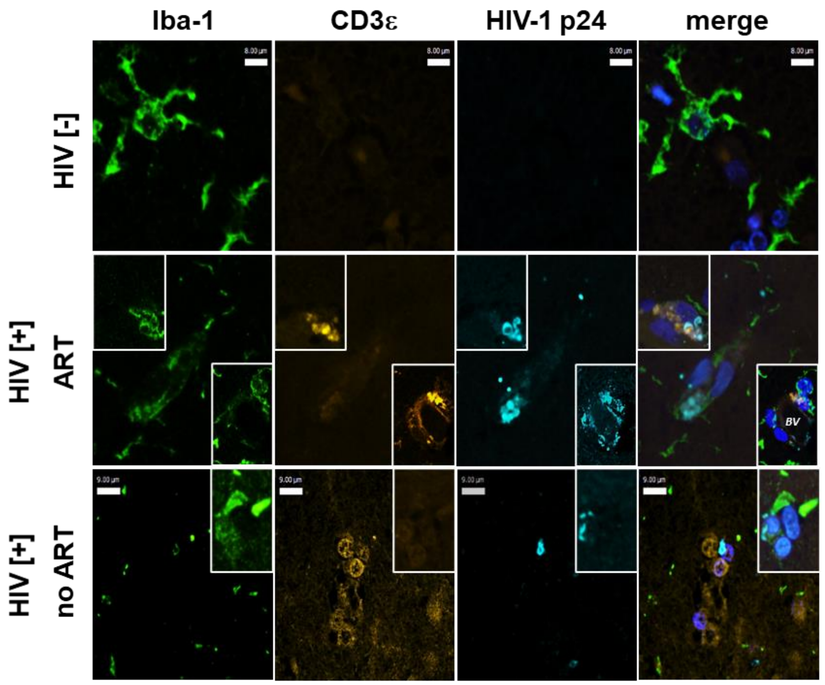
Result: HIV RNA (A), DNA (B), and integrated DNA (C), were detected in in brain tissues of suppressed HIV[+] group receiving ART treatment. HIV RNA trended toward decreased levels in HIV[+] ART but total and integrated DNA were similar to no ART group.



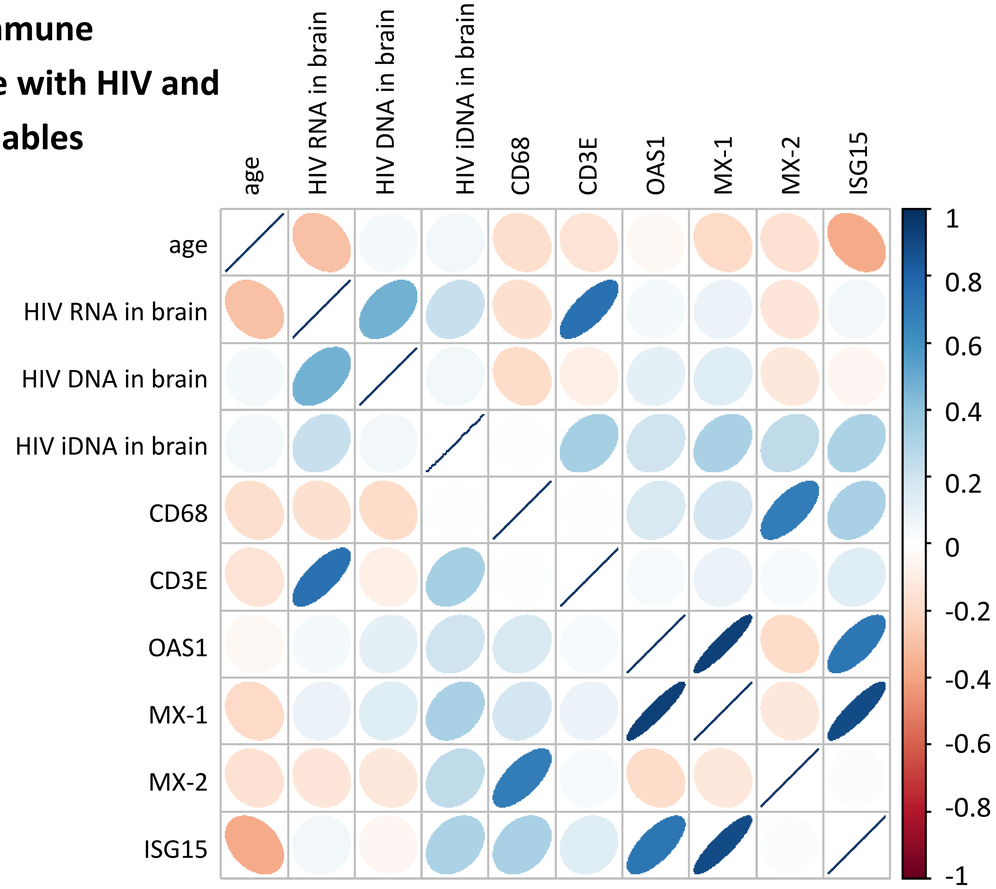
Detection of HIV p24 in microglial and T lymphocyte in brain tissue

Method:
Immunofluorescence imaging of brain tissues labeled with Iba-1 (microglial), CD3ε (T cell) and HIV p24 specific Ab.

Result:
HIV p24 was detected in parenchyma microglia, perivascular T cells and macrophages.



Neuroimmune response with HIV and host variables



Conclusions

1. ART drugs are selectively efficient depending on the HIV-infected cell type and individual drug.
2. Contemporary ART drugs exert minimal effects on brain SIV quantity in the presence or absence of ART interruption and despite control of plasma viral load.
3. Total and integrated SIV DNA quantities in brain were correlated with CSF SIV RNA quantity.
4. HIV-1 DNA, RNA and antigen persist in the brain despite effective ART.
5. There was limited correlation between SIV and HIV-1 quantities in brain and the associated neuroimmune responses.
6. These studies indicate lentivirus infections of the brain persist despite effective ART and antiviral neuroimmune responses.

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