# Neuroinflammation is associated with levels of HIV DNA in the frontal cortex of virally suppressed PWH

Sarah J Byrnes[1], Janna Jamal Eddine[1], Jingling Zhou[1], Emily Chalmers[1], Trisha Jenkins[1], Narin Osman[1], Michael Roche[1,2], Bruce J Brew[3], Jacob D Estes[1,4], **Thomas A Angelovich**[1,2,5], Melissa J Churchill[1,5,6]

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## INTRODUCTION

HIV-associated neurocognitive disorders (HAND) affect ~20% of ART-suppressed people with HIV (PWH), suggesting that HAND pathogenesis may be driven by mechanisms other than direct viral replication in the brain including chronic systemic inflammation (Fig.1). The role of viral persistence in the brain and/or systemic inflammation on neuropathology in ART-suppressed PWH is unclear.

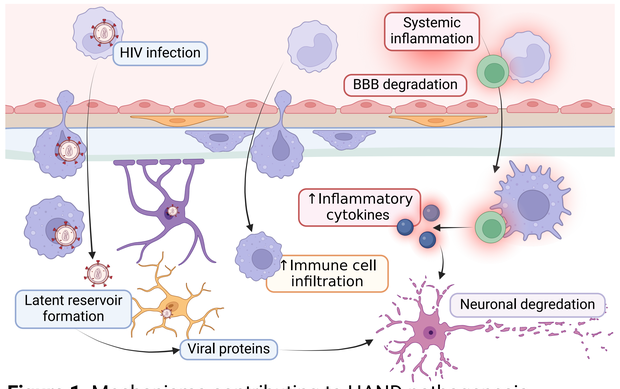



**Figure 1**. Mechanisms contributing to HAND pathogenesis

## METHODS

Multiplex IF was performed on FFPE frontal cortex tissue (Fig.2) from virally suppressed (VS; n=18; Table 1) and non-virally suppressed (nVS; n=17) PWH to measure CNS immune activation. Genomic DNA from matched frozen samples was used to quantify intact and defective HIV DNA.

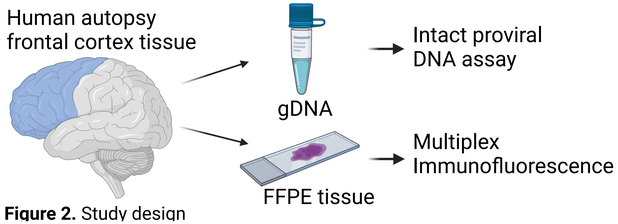



**Figure 2.** Study design

**Table 1**. Cohort

| | HIV-seronegative | Non-virally suppressed (nVS) | Virally suppressed (VS) |
|---|---|---|---|
| n | 6 | 17 | 18 |
| Age | 51 (39-61) | 47 (40-56) | 60 (53-62) |
| Sex (% Male) | 50 | 52.9 | 77.5 |
| HIV parameters | | | |
| Viral load (plasma)[a] | | 61223 (17387-367620) | UD |
| Viral load (CSF)[a] | | 361 (184-651) | UD |
| CD4 T cells (mm$^3$) | | 16 (3-101) | 163 (115-376) |
| Viral suppression (yrs) | | - | 3.8 (2.49-7.21) |

CSF: cerebrospinal fluid, UD: undetectable. [a] HIV RNA copies/ml

## RESULTS

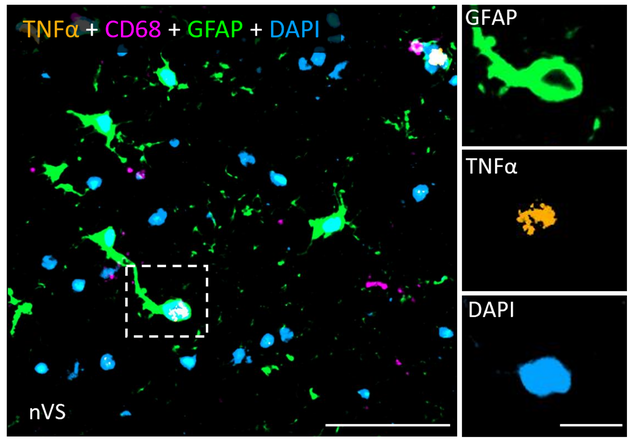



**Figure 3**. Representative image of multiplex IF. Green= GFAP/ astrocytes, Orange= TNFα, Purple= CD68/myeloid cells, Blue= DAPI/Nuclei. Image shown at x30 magnification: scale bars 50μm and 20μm (inset).

### 1. Neuroinflammation and anti-viral signalling persists despite viral suppression

Frontal cortex tissue from both non-virally suppressed and ART-suppressed PWH had an elevated frequency of Mx1+ and TNFα+ myeloid cells and astrocytes relative to HIV-seronegative individuals (Fig.4 A-D).

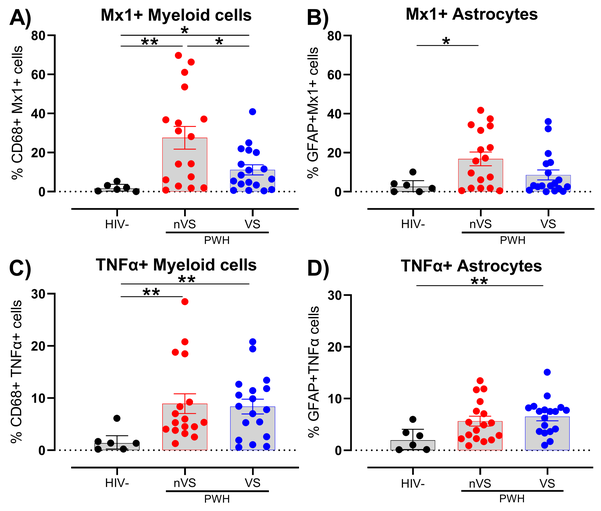



**Figure 4**. Percentage of Mx1+ (A & B) and TNFα+ (C & D), myeloid cells and astrocytes in frontal cortex tissue.

## RESULTS

### 2. Elevated astrocyte and myeloid cell immune regulation in ART-suppressed PWH

PWH had a higher frequency of myeloid cells and astrocytes expressing TGFβ1 (Fig.5 A & B) in the frontal cortex tissue relative to HIV-seronegative individuals. Similar increases were seen in both grey and white matter (Table 2).

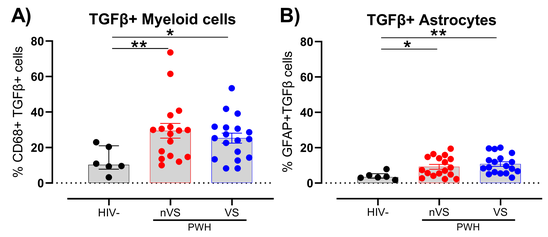



**Figure 5**. Percentage of TGFβ1 (A & B) producing myeloid cells and astrocytes in frontal cortex brain tissue.

**Table 2**: Inflammatory profiles of white and grey matter in the frontal cortex of PWH

| | | HIV- | PWH nVS | PWH VS |
|---|---|---|---|---|
| Mx1 | WM | 0.795 (0.033, 1.96) | 4.75 (0.494, 10.3) | 1.81 (0.463, 5.08) |
| | GM | 0.071 (0.046, 0.254) | 5.48 (0.974, 15.1) | 1.76 (0.127, 6.79) |
| TNFα | WM | 0.110 (0.056, 0.725) | 1.35 (0.477, 2.86) | 1.40 (0.602, 2.90) |
| | GM | 0.812 (0.059, 1.44) | 1.56 (1.11, 3.38) | 3.34 (2.03, 4.57) |
| TGFβ | WM | 1.66 (0.857, 2.64) | 4.15 (2.08, 6.75) | 3.82 (2.06, 6.35) |
| | GM | 2.58 (1.02, 3.10) | 6.72 (2.58, 7.19) | 6.94 (2.78, 9.05) |

GM: grey matter, PWH: people with HIV, nVS: non-virally suppressed, VS: virally suppressed, WM: white matter.
Percentage of total cells (Median and interquartile range shown)

### 3. Intact HIV DNA persists in the frontal cortex of ART-suppressed PWH

Intact and defective HIV DNA was detected in the frontal cortex of PWH (Fig.6). The frequency of intact DNA was not reduced in ART-suppressed PWH, supporting the presence of a stable viral reservoir in the brain.

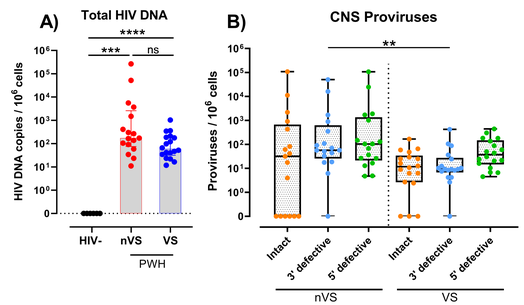



**Figure 6.** Frequency of total (A), intact, 3' defective and 5' defective (B) HIV DNA in the frontal cortex as measured by the intact proviral DNA assay.

## RESULTS

### 4. Neuroinflammation in the frontal cortex of ART-suppressed PWH is associated with total and 5' defective HIV DNA reservoir size

The level of total and 5' defective HIV proviral DNA in the frontal cortex tissue was associated with activation of anti-viral type I interferon signalling (Mx1) in ART-suppressed PWH (Fig.7 A-D). HIV proviral DNA did not correlate with TNFα or TGFβ1 expression, indicating an alternative driver of these proteins in the brain of ART-suppressed PWH.

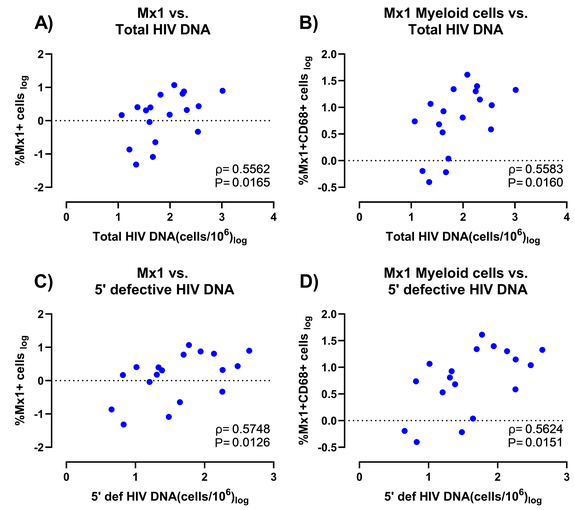



**Figure 7**. Correlative analysis of total (A & B) and 5' defective (C & D) HIV DNA with total Mx1 expression and Mx1 expression on myeloid cells in frontal cortex tissue.

## CONCLUSION

- Long-term viral suppression does not abrogate CNS viral load, neuroinflammation or immune regulation.
- Local CNS virus contributes, at least in part, to anti-viral type I interferon driven cell activation in ART-suppressed PWH.
- These findings support a role of both local and peripheral viral reservoirs in driving neuroinflammation in ART-suppressed PWH.

**Affiliations:**
1. RMIT University, Melbourne Australia. 2. Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Australia. 3. University of New South Wales and University of Notre Dame, Sydney, Australia. 4. Vaccine & Gene Therapy Institute, Oregon Health & Science University, America. 5. Burnet Institute, Melbourne, Australia. 6. Monash University, Clayton, Australia.

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