Characterisation of HIV latency and reactivation in human macrophages

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INTRODUCTION

- HIV infects and establishes a reservoir in macrophages that persists in people with HIV (PWH) despite effective antiretroviral therapy (ART)[1].
- Macrophage HIV reservoirs contain replication competent virus[2] which can contribute to viral rebound upon ART cessation[3], underscoring the clinical relevance of this reservoir.
- Macrophage reservoirs reside in difficult to access tissue locations in PWH (Fig. 1) which is a barrier to HIV cure.
- *In vitro* models of latently infected primary human macrophages are useful to understand what governs latent HIV reactivation in different tissue macrophage types, which currently is poorly understood.

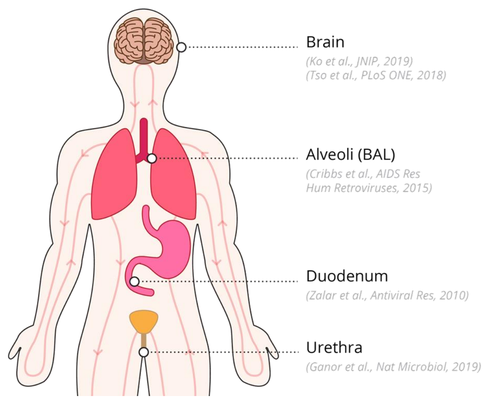

Figure 1: HIV-containing macrophages persist in various tissue sites in PWH on ART. *Image: Courtesy Hans Kek*

METHODS

- An *in vitro* model of HIV latency in primary human monocyte-derived macrophages (MDM)[4] was used to assess HIV reactivation in tissue macrophages types following stimulation (Fig. 2A&B).
- Single cell RNASeq was used to detect HIV RNA in productively and latently-infected MDM and identify transcriptomic changes associated with HIV infection state (Fig. 2C).

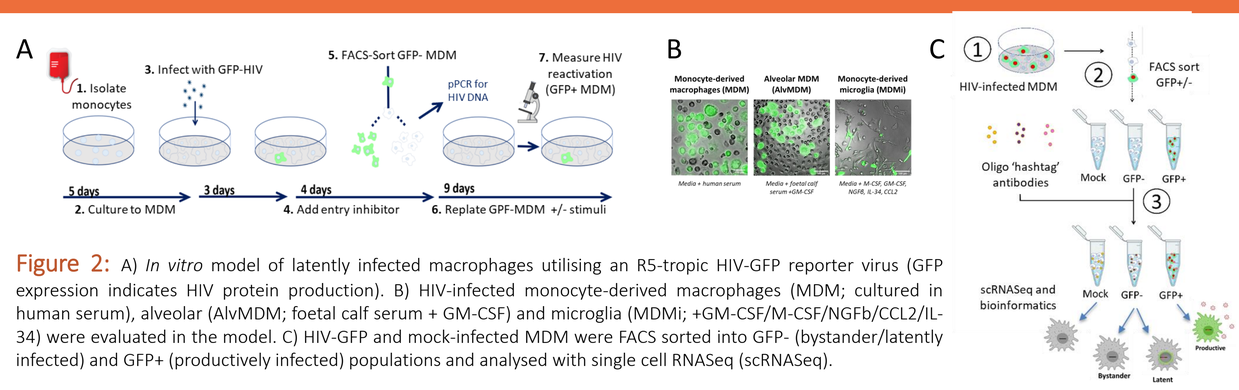

Figure 2: A) *In vitro* model of latently infected macrophages utilising an R5-tropic HIV-GFP reporter virus (GFP expression indicates HIV protein production). B) HIV-infected monocyte-derived macrophages (MDM; cultured in human serum), alveolar (AlvMDM; foetal calf serum + GM-CSF) and microglia (MDMi; +GM-CSF/M-CSF/NGFb/CCL2/IL-34) were evaluated in the model. C) HIV-GFP and mock-infected MDM were FACS sorted into GFP- (bystander/latently infected) and GFP+ (productively infected) populations and analysed with single cell RNASeq (scRNASeq).

RESULTS

1. Establishment and reactivation of latent infection is similar in MDM and AlvMDM.

- Similar level of HIV DNA in non-productively infected MDM and AlvMDM, indicating latently infected cells, whilst HIV DNA levels in MDMi were lower (Fig. 3A)
- HIV reactivation was similar in MDM and AlvMDM, but absent in MDMi (Fig. 3B&C)

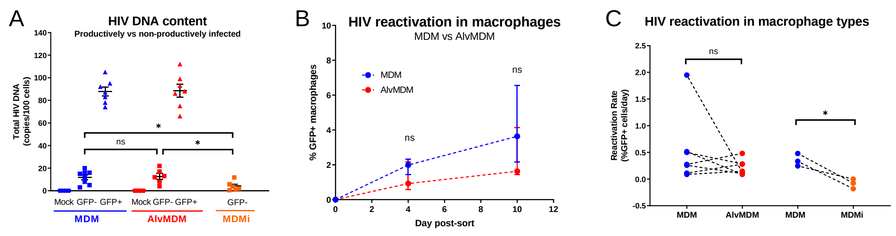

Figure 3: Productively (GFP+) and non-productively infected (GFP-) macrophages were FACS sorted and HIV DNA measured by qPCR (A). HIV reactivation in GFP- (latently infected) macrophages of different types was assessed over time (B; overall reaction; C; reactivation rate/day). *p<0.05 by paired t-test.

2. HIV reactivation from latently-infected macrophages is enhanced with M2 polarising cytokines but inhibited by TLR agonists.

- Similar effect in both MDM and AlvMDM (Fig. 4)

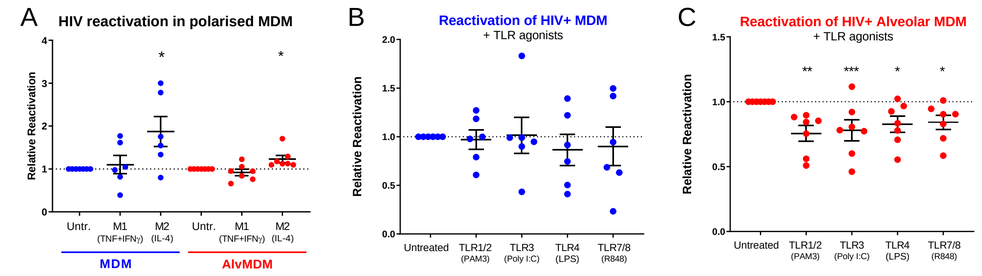

Figure 4: HIV reactivation from latently-infected MDM and AlvMDM polarised with M1 or M2 cytokines (A), or with various TLR agonists as indicated (B&C). Relative reactivation rate per day (mean +/- SEM) shown. *, **, ***p<0.05, 0.01 and 0.001 by paired t-test.

3. scRNASeq identifies latently HIV-infected macrophages.

- HIV RNA transcripts were detected in non-productively infected (GFP-) MDM (Fig. 5A), confirming latent infection in macrophages.
  - scRNASeq allowed discrimination of latently infected MDM (GFP-, HIV RNA+) from bystanders (Fig. 5B).
- Latently infected MDM were found in various subpopulations (Fig. 5C).

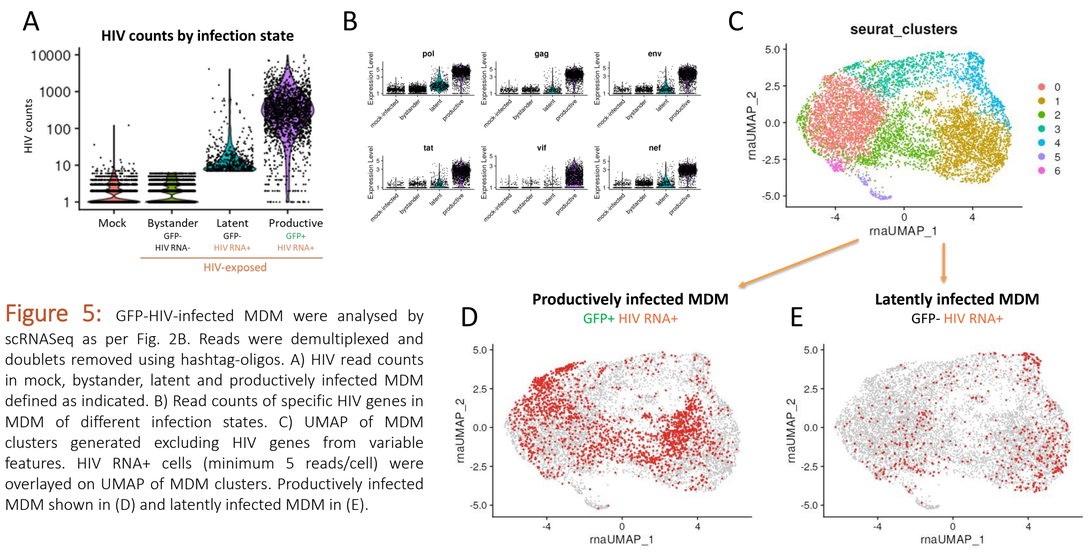

Figure 5: GFP-HIV-infected MDM were analysed by scRNASeq as per Fig. 2B. Reads were demultiplexed and doublets removed using hashtag-oligos. A) HIV read counts in mock, bystander, latent and productively infected MDM defined as indicated. B) Read counts of specific HIV genes in MDM of different infection states. C) UMAP of MDM clusters generated excluding HIV genes from variable features. HIV RNA+ cells (minimum 5 reads/cell) were overlayed on UMAP of MDM clusters. Productively infected MDM shown in (D) and latently infected MDM in (E).

4. Productively and latently HIV-infected macrophages exhibit distinct transcriptional profiles

- Productively-infected MDM exhibited altered expression of genes related to cell cycle, transcriptional control and antigen presentation (Fig. 6A).
- DEGs in latently-infected MDM were different, but also included genes related to antigen presentation and transcriptional control, as well as cytoskeleton and apoptosis (Fig. 6B).

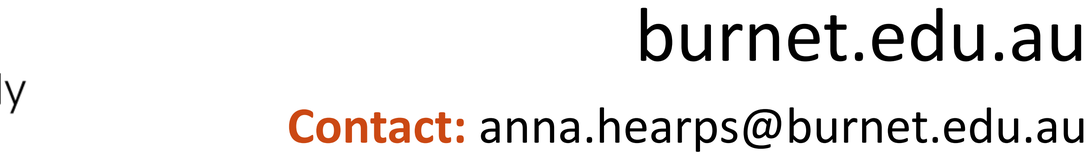

Figure 6: Volcano plot and summary table of differentially expressed genes (DEG) in productively infected (GFP+, HIV RNA+; A) and latently-infected (GFP-, HIV RNA+; B) MDM as determined by scRNASeq analysis as in Fig. 5.

CONCLUSIONS

- scRNASeq can identify latently infected macrophages.
- Latent and productive HIV infection may occur in different MDM subpopulations.
- Latent and productively-infected MDM show distinct transcriptional profiles.
- Local tissue cytokine environments and macrophage types may influence establishment and reactivation of latently in HIV-infected macrophages.

REFERENCES

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