Transcriptional and Surface Receptor Signatures of Macrophages Enriched for Latent HIV

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Background:
HIV reservoirs persist in macrophages in people with HIV despite antiretroviral therapy, representing a barrier to cure. Difficulty in identifying and studying latent HIV in macrophages *in vitro* has limited understanding of mechanisms governing latency. We aimed to define transcriptional profiles associated with latent infection across distinct macrophage types and identify surface markers defining latently infected macrophages.

Methods:
An established *in vitro* HIV latency model was utilised, incorporating primary human monocyte-derived macrophages (MDM) and alveolar-like MDMs (AlvMDM) infected with a HIV-GFP reporter virus. Cells were FACS-sorted based on GFP expression and subjected to single-cell RNA sequencing. Latently infected macrophages were bioinformatically defined as GFP-/HIV RNA+ and compared to bystander (GFP-/HIV RNA-) cells for differential gene expression and pathway enrichment analyses. GFP- cells were sorted based on expression of selected surface receptors, and total HIV DNA quantified by qPCR.

Results:
Despite detectable HIV RNA expression, non-productively infected macrophages exhibited moderate transcriptional changes relative to bystander cells, with more pronounced alterations in AlvMDM than MDM (133 vs 35 differentially expressed genes, respectively) with only one overlapping gene (*FBP1*). Differentially expressed genes were associated with apoptosis (MDM - *GIMAP8*, *IFI27,* AlvMDM - *BEX2, MDM2)*, cell cycle regulation (AlvMDM - *CDKN1A)*, and immune response (MDM - *CD40, AlvMDM - TNFRSF9, CCL22)*, while pathway-level analyses indicated broader perturbations in transcription, proteasome activity, and mitochondrial function. Latently infected MDM and AlvMDM exhibited increased expression of genes encoding surface receptors, including *MERTK* and *TNFRSF9*, respectively. Notably, purification of AlvMDM with high surface *TNFRSF9* expression enriched for cells with higher HIV DNA content ($p<0.01$).

Conclusion:
Latent HIV infection in macrophages is associated with subtle transcriptional changes which differ by macrophage subtype. Differential expression of surface

receptors may facilitate identification and targeting of latent macrophage reservoirs in tissues, with important implications for HIV elimination strategies.

Disclosure of Interest Statement:
None to declare.