Title:

CHARACTERISATION OF POTENTIAL HIV-TARGET MYELOID CELLS IN FORESKIN EPITHELIA

0209

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BACKGROUND

The human foreskin (FS) is an immunologically active tissue containing both lymphoid and myeloid cells. The foreskin has been shown to play an important role in HIV infection as its complete removal during medical male circumcision (MMC) has been shown to reduce the risk of HIV acquisition by up to 60%. $CD4^+CCR5^+$ Langerhan's cells (LCs) and macrophages are known to be resident in both inner and outer foreskin tissue and are potential HIV target cells. While the role of adaptive immune cells in HIV pathogenesis is extensively studied ,the role and permissiveness of myeloid cells remains elusive. It is hypothesized that epithelial tissue-resident $CD4^+CCR5^+$ cells can serve as HIV targets with the potential to support viral replication.

METHODS

- Inner and outer FS cells were migrated out of isolated epidermal tissue from adult South African men undergoing voluntary MMC.
- Epidermal sheets were obtained after dispase digestion; cells were collected after a 48-hr incubation (crawl) and tissue resident (liberase) isolated by liberase digestion of remnant tissue. Cells were immunophenotyped by flow cytometry and infected ex vivo with an HIV-1 IMC.

$CD4^+CCR5^+$ myeloid cells that migrate out of foreskin epithelia express significantly high levels of activation markers CD80/86 and HLA-DR.

Myeloid and lymphoid cells were infectable ex vivo to HIV-1 with increased p24 expression and CD4 downregulation over time.

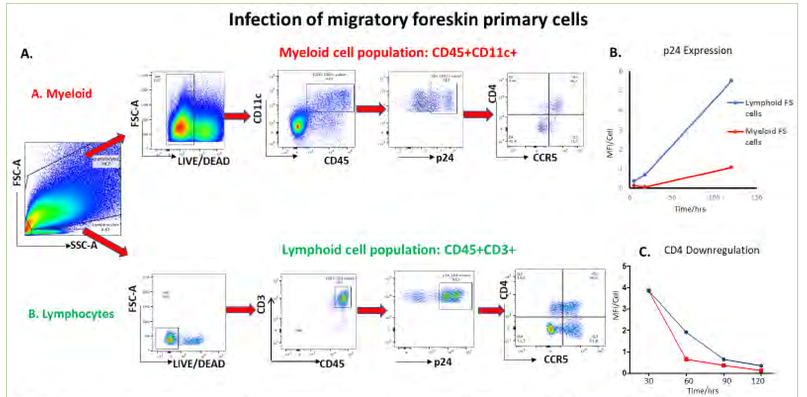

Figure 4. Multicolour flow cytometry plots for p24 expressing HIV-1 infected primary foreskin cells. **Panel A** shows **live $CD45^+CD11c^+$** HIV-1 infected non-lymphoid foreskin cells expressing **HIV-1 p24**. **Panel B** shows live **$CD45^+CD3^+$** HIV-1 infected lymphoid foreskin cells expressing **HIV-1 p24**. An NL4-3 (92TH 14-12) R5 tropic infectious molecular clone was used to generate virus used in this experiment.

RESULTS

- Migratory foreskin cells were more activated with elevated levels of CD80/86 expression (*p=0.0001*). 55% of live migratory $HLA\text{-}DR^+CD4^+CD11c^+CD14^{+/-}CD206^+$ macrophages and $CD1a^+CD207^+$ Langerhan's cells are CD80/86 high compared to 12.5% of live tissue resident macrophages. Majority of migratory Langerhan's cells were CD80/86 high (mean with SD at 95 % confidence interval). 60% of live macrophages and Langerhan's cells were CCR5 and CD206 high shown in figures 2 and 3 panel C.
- The expression of CD169 was low in both migratory and tissue resident macrophages. Langerhan's cells constituted only 2% of the isolated cells, and macrophages 0.09%.
- $CD11c^+CD45^+$ myeloid cells were infected with HIV and showed 40% p24 expression that increased with time shown in figure 4A and B.
- HIV induced CD4 downregulation in myeloid and lymphoid cell populations shown in figure 4C.

CONCLUSIONS

- Foreskin myeloid cells are $CD4^+CCR5^+$, they are prone to HIV infection due to the presence of the HIV receptor CD4 and co-receptor CCR5.
- Myeloid cells also express attachment factors of the lectin receptor family (CD206+ $CD207^+$) and to a lesser extent, the sialoadhesion molecule CD169, which have been shown to facilitate the binding of HIV to its receptors.
- The time dependant p24 amplification indicates productive infection of cells of the myeloid component in a similar manner to lymphocytes, the conventional HIV targets. Hence, myeloid cells are important key players in HIV pathogenesis that should be investigated further.

The foreskin epithelia harbours diverse myeloid and lymphoid cell subsets that are infectable to HIV-1 *ex vivo.*

ACKNOWLEDGEMENTS :

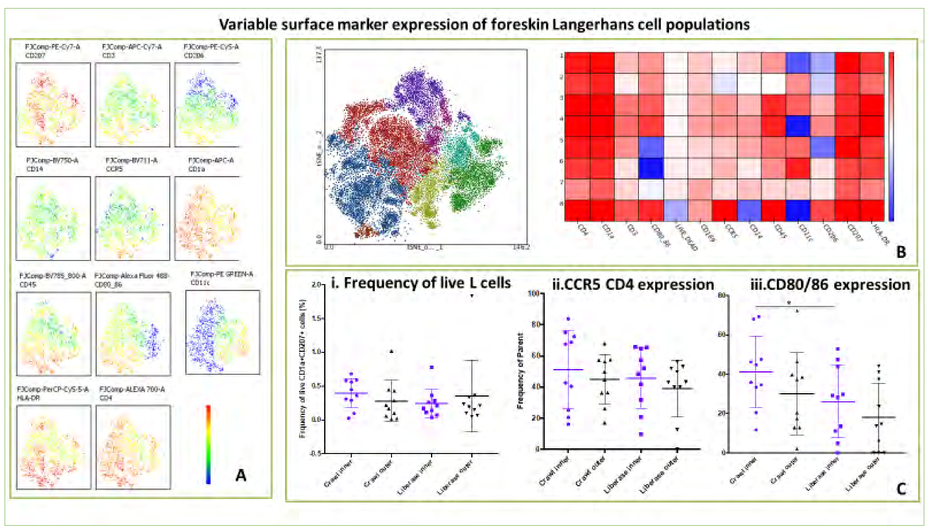

Figure 2. Variable surface marker expression of foreskin Langerhans cell populations. A. t-Stochastic Neighbor Embedding map visualization(tSNE) showing expression of specific LC surface markers in a representative individual. B. tSNE map of the LC subsets clustered by flowSOM analysis and the heatmap of the clusters showing marker expression. C. Scatter dot plots showing frequencies of **i)** live LC isolated from different methods, **ii)** CD4 CCR5 expression in LC's **iii)** expression of CD80 and 86 in LC populations.

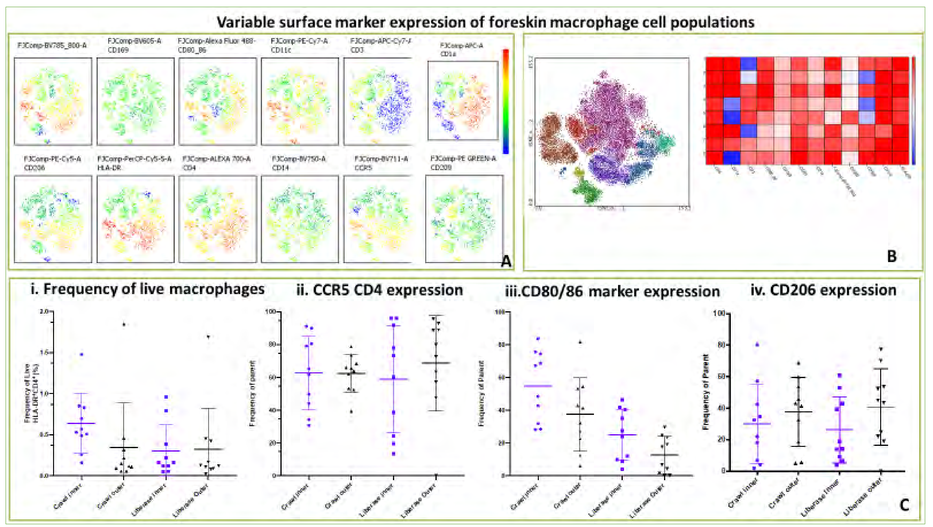

Figure 3. Variable surface marker expression of foreskin Macrophage cell populations. A. t-Stochastic Neighbor Embedding map visualization(tSNE) showing expression of specific macrophage surface markers in a representative individual. B. tSNE map of the macrophage subsets clustered by flowSOM analysis and the heatmap of the clusters showing marker expression. C. Scatter dot plots showing frequencies of **i)** live macrophages isolated from different methods, **ii)** CD4 CCR5 expression in Macrophages **iii)** expression of CD80 and 86 in macrophage populations. iv) expression of CD206 in macrophage populations.

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