DNA methylation, closed chromatin structure and nucleosomal occupancy cooperatively regulate *CXCR5* gene expression in human CD8⁺ T cells

Funsho Ogunshola[1,2], Werner Smidt[3], Anneta Naidoo[4], Trevor Khaba[2], Omolara Baiyegunhi[1,2], Thandeka Nkosi[1,2], Veron Ramsuran[3], Thumbi Ndung'u[1,2], Bruce Walker[2,5], Tulio Oliveira[3] and **Zaza Ndhlovu[1,2,5]**

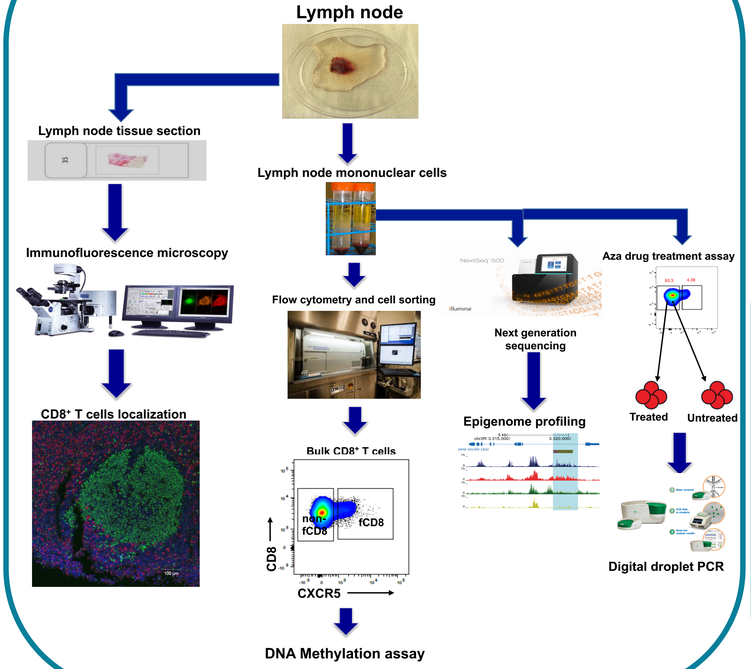
[1]Africa Health Research Institute, (AHRI), Durban, South Africa, [2]HIV Pathogenesis Programme, Doris Duke Medical Research Institute, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa. [3] Bioafrica Epidemiology, genomics and Bioinformatics, Durban, South Africa. [4]HIV Vaccines Trial Network, Cape Town, South Africa, [5]Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard University, Cambridge, USA

SANTHE
SUB-SAHARAN AFRICAN NETWORK FOR TB/HIV RESEARCH EXCELLENCE
Advancing African Science, Fighting HIV/AIDS and TB

Summary

- **Introduction**: CD8⁺ T cells located in B cell follicles play an important role in viral and tumor control. However, in human lymph nodes (LN), only a small subset of CD8⁺ T cells called follicular CD8⁺ T cells (fCD8) express CXCR5, the chemokine receptor required for cell migration into B cell follicles. We investigated why most lymph node CD8⁺ T cells (non-fCD8) do not express CXCR5, and why there is reduced CXCR5 expression in fCD8 relative to germinal centre T follicular helper cells (Tfh).
- **Materials and Methods**: Immunofluorescence microscopy (IF) staining was used to identify the location of fCD8 in the LNs. DNA methylation assay and assay for transposase accessible chromatin using sequencing (ATAC-Seq) were used to investigate the regulation of *CXCR5* gene.
- **Results**: DNA hypermethylation and closed chromatin conformation at the transcriptional start site (TSS) prevent CXCR5 expression in non-fCD8. We also found that greater nucleosomal density at the *CXCR5 TSS* is responsible for reduced CXCR5 expression in fCD8 relative to Tfh.

Experimental design



Results

CXCR5 expression is required for CD8⁺ T cells localization in the germinal center

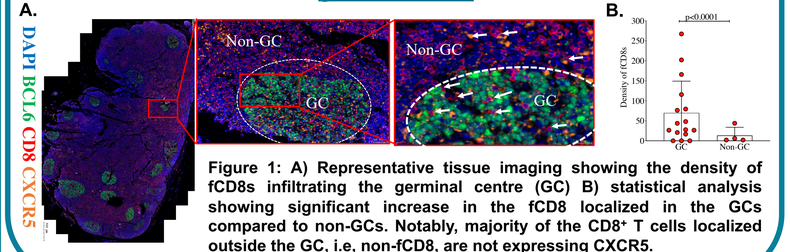


Figure 1: A) Representative tissue imaging showing the density of fCD8s infiltrating the germinal centre (GC) B) statistical analysis showing significant increase in the fCD8 localized in the GCs compared to non-GCs. Notably, majority of the CD8⁺ T cells localized outside the GC, i.e, non-fCD8, are not expressing CXCR5.

High DNA Methylation around the TSS of CXCR5 gene in non-fCD8

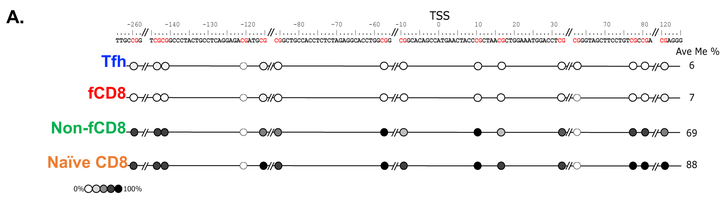


Figure 2: A) Bisulfite treatment and sequencing covering 500bp around the TSS of *CXCR5* gene revealed high DNA methylation in non-fCD8 and naïve CD8 as shown by the shaded circles B) Analysis showing % levels of DNA methylation around the TSS of the *CXCR5* gene in study subsets.

Inhibition of methyl transferase activity resulted in increased CXCR5 transcript in non-fCD8

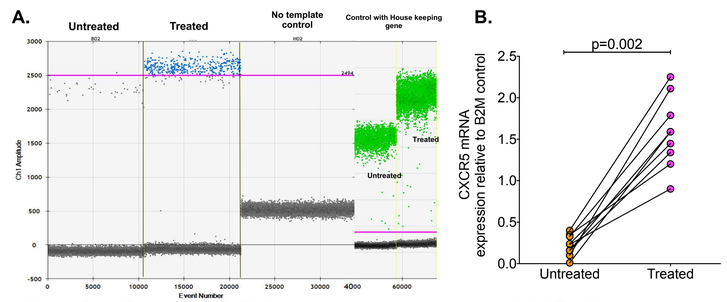


Figure 3: A) Digital Droplet PCR readout showing increase of CXCR5 mRNA transcript in purified sorted non-fCD8 after treatment with methyl transferase inhibitor for 24 hours. B) Analysis showing significant increase of CXCR5 mRNA transcript in treated compared to untreated non-fCD8.

Accessible chromatins in fCD8 is different from non-fCD8

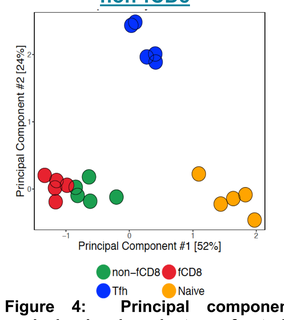


Figure 4: Principal component analysis showing clusters of study subsets. This data shows a clear delineation of cell subsets based on the epigenome landscape.

non-fCD8 maintained closed chromatin conformation at CXCR5 transcriptional start site

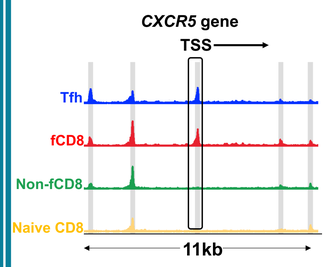


Figure 5: ATAC-Seq track of CXCR5 gene for Tfh, fCD8, non-fCD8 and Naïve CD8⁺ T cells. This data show that chromatin accessibility also contribute to the repressed state of *CXCR5* gene

Reduced turnover rate of the nucleosome at the promoter region of *CXCR5* could lead to lower levels of CXCR5 expression

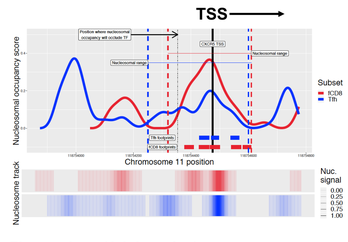


Figure 6: Height of the occupancy score showing the fraction of nucleosomal sized fragments at the chromosome 11 position. Predicted transcription factor footprints are shown as bars for the respective cell subsets. The heatmap shows the calculated nucleosomal signal from the ATAC-Seq data.

Conclusions

- ✓ **CXCR5 expression is required for CD8⁺ T cells to localize in the germinal centers of B cell follicles where HIV-1 replication persists.**
- ✓ **Hypermethylation of DNA was observed around the transcriptional start sites (TSS) of non-fCD8.**
- ✓ **Chromatin landscape of fCD8 is different from non-fCD8, particularly around the TSS.**
- ✓ **Higher nucleosomal occupancy/positioning at the TSS of fCD8 relative to Tfh.**
- ✓ **These data provide critical insights into both the underlying molecular mechanisms that repress CXCR5 in non-fCD8s and plausible mechanism responsible for the low CXCR5 expression in fCD8s, with implications for HIV cure strategy or eradication of B cell-derived tumors.**

Acknowledgements

We would like to thank the study participants, clinic as well as HPP laboratory staff. This work was supported by the SANTHE programme, an initiative funded through Welcome Trust (10775/Z/15/Z), Bill and Melinda Gates Foundation, the Mark and Lisa Schwartz Foundation, the International AIDS Vaccine Initiative (IAVI) (UKZNRSA1001), the NIAID (R37AI067073), and the Howard Hughes medical Institute.

References

- Connick, E., Mattila, T., Folkvord, J. M., Schlictemeier, R., Meditz, A. L., Ray, M. G., Mccarter, M. D., Mawhinney, S., Hage, A., White, C. & Skinner, P. J. 2007. CTL fail to accumulate at sites of HIV-1 replication in lymphoid tissue.
- Fukazawa, Y., Lum, R., Okoye, A. A., Park, H., Matsuda, K., Bae, J. Y., Hagen, S. I., Shoemaker, R., Deleage, C., Lucero, C., Morock, D., Swanson, T., Legasse, A. W., Axthelm, M. K., Hesslgesser, J., Geleziunas, R., Hirsch, V. M., Edlepsen, P. T., Piatak, M., JR., Estes, J. D., Lifson J. D. & Picker, L. J. 2015. B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers.
- Se Jin Im, Masao Hashimoto, Michael Y. Gerner, Junghwa Lee, Haydn T. Kissick, Matheus C. Burger, Qiang Shan, J. Scott Hale, Judong Lee, Tahseen H. Nasti, Arlene H. Sharpe, Gordon J. Freeman, Ronald N. Germain, Helder I. Nakaya,, Hai-Hui Xue & Rafi Ahmed. 2016. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy.
- Yew Ann Leong, Yaping Chen, Hong Sheng Ong, Di Wu, Kevin Man, Claire Deleage, Martina Minnich, Benjamin J Meckiff, Yunbo Wei, Zhaohua Hou, Dimitra Zotos, Kevin A Fenix, Anurag Atnerkar, Simon Preston, Jeffrey G Chipman, Greg J Beilman, Cody C Allison, Lei Sun, Peng Wang, Jiawei Xu, Jesse G Toe, Hao K Lu, Yong Tao, Umaimainthan Palendira, Alexander L Dent, Alan L Landay, Marc Pellegrini, Iain Comerford, Shaun R McColl, Timothy W Schacker, Heather M Long, Jacob D Estes, Meinrad Busslinger, Gabrielle T Belz, Sharon R Lewin, Axel Kallies & Di Yu. 2016. CXCR5⁺ follicular cytotoxic T cells control viral infection in B cell follicles.