MAPPING THE HETEROGENEITY OF CCR5+ CD4 T CELLS BY HIGH DIMENSIONAL FLOW CYTOMETRY

Zaunders J[1], Munier ML[2], Mach M[2], Bascombe F[3], Carey D[2], Howe A[2], Xu Y[2], Milner B[4], Obeid S[4] , Mills C[4], Ambati C[4] and Kelleher A[1,2]

[1]Centre for Applied Medical Research, St Vincent's Hospital, [2]Kirby Institute, UNSW Sydney, [3]Translational Research Centre and [4]Medical Imaging, St Vincent's Hospital.

Background: The subset of CD4 T cells that express the cell surface chemokine receptor, CCR5, are the most important target of HIV-1 infection. However, the functions, phenotypes and anatomical locations of CCR5+ CD4 T cells are poorly understood.

Methods: 20-parameter flow cytometry using the 5-laser BD Symphony has been undertaken to better define CCR5+ CD4 T cells in peripheral blood in healthy adults. CCR5 staining, in combination with 19 other fluorochrome-labelled monoclonal antibodies (mAb), was optimized for the Symphony. Ultrasound-guided lymph node fine needle biopsies of axillary nodes were performed on healthy adult volunteers prior to and following Fluvax.

Results: Lymphocytes were gated on forward and side scatter, and CD3+ CD4+ T cells were gated on CD45RO+ memory cells. CCR5+ memory CD4 T cells were then analysed for expression of Treg markers (CD25hiCD127lo), chemokine receptors (CXCR5, CXCR3, CCR4, CCR6), c-type lectins (CD62L, CD161), integrins (□4, ß7), activation markers (CD38, HLA-DR), and differentiation markers (CD27, CD28, CD73). Altogether, >150 different functional and trafficking phenotypes of CCR5+ CD4 T cells were seen, including Tregs, non-Tregs, gut-homing, non-gut homing, skin-homing, Th1, Th17, activated, resting, cytotoxic and non-cytotoxic cells. Also, activated CCR5+ CD4 T cells were greatly expanded in draining axillary lymph nodes at day 5 following Fluvax.

Discussion: These results reveal for the first time the extreme heterogeneity of CCR5+ CD4 T cells in blood and in lymphoid tissue, with significant implications for rational approaches to prophylaxis for HIV-1 infection and for purging of the HIV-1 reservoir in those already infected.