MACHINE LEARNING REVEALS T-CELL ACTIVATION PATHWAYS INDUCED BY INFLUENZA VACCINE

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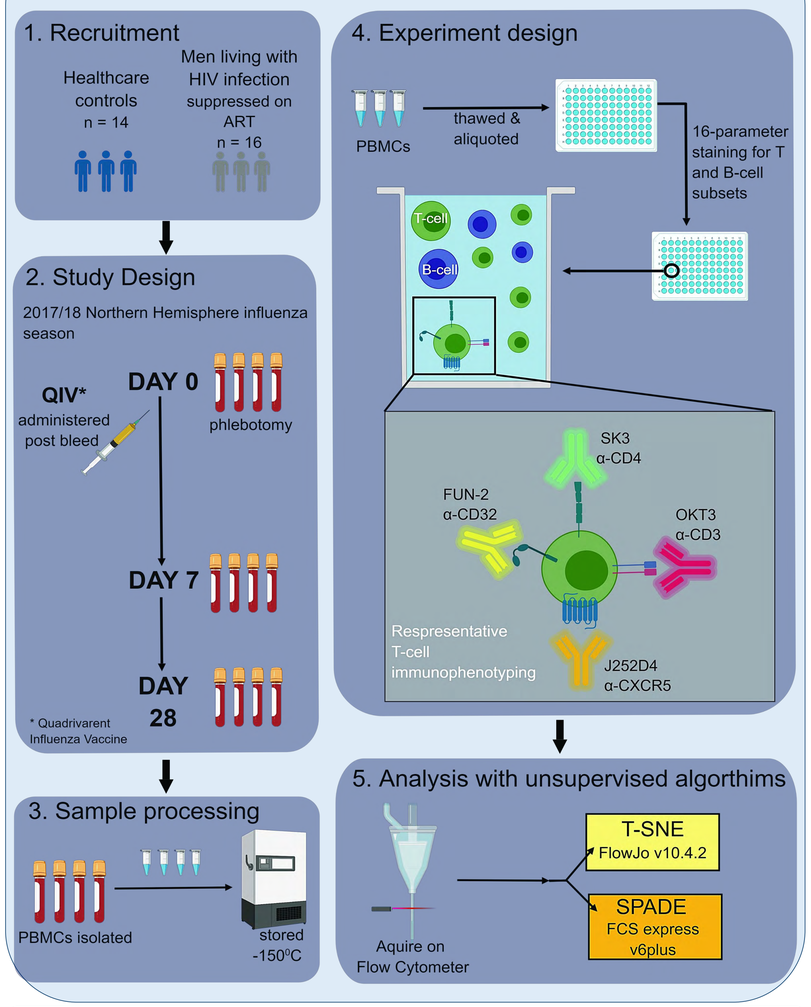
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Background

Seasonal influenza vaccine responses are dependent on a specialised subset of CD4⁺ T-cells; CXCR5⁺ circulating T-follicular helper cells (cTFH)[1]. The function of cTFH may be compromised by HIV infection. Investigations of potential biomarkers for HIV integration have identified rare CD4⁺ T-cells highly expressing the Fc gamma receptor (FcγR) CD32 with unknown function[2].

We hypothesised that CD32 is upregulated on cTFH in response to inactivated influenza vaccine and used unsupervised computer algorithms to explore the cellular relationships arising.

Methods



Results 1. CD4⁺CXCR5⁺ T-cell Subsets With Variable Expression of the Fc Gamma Receptor (FcγR) CD32

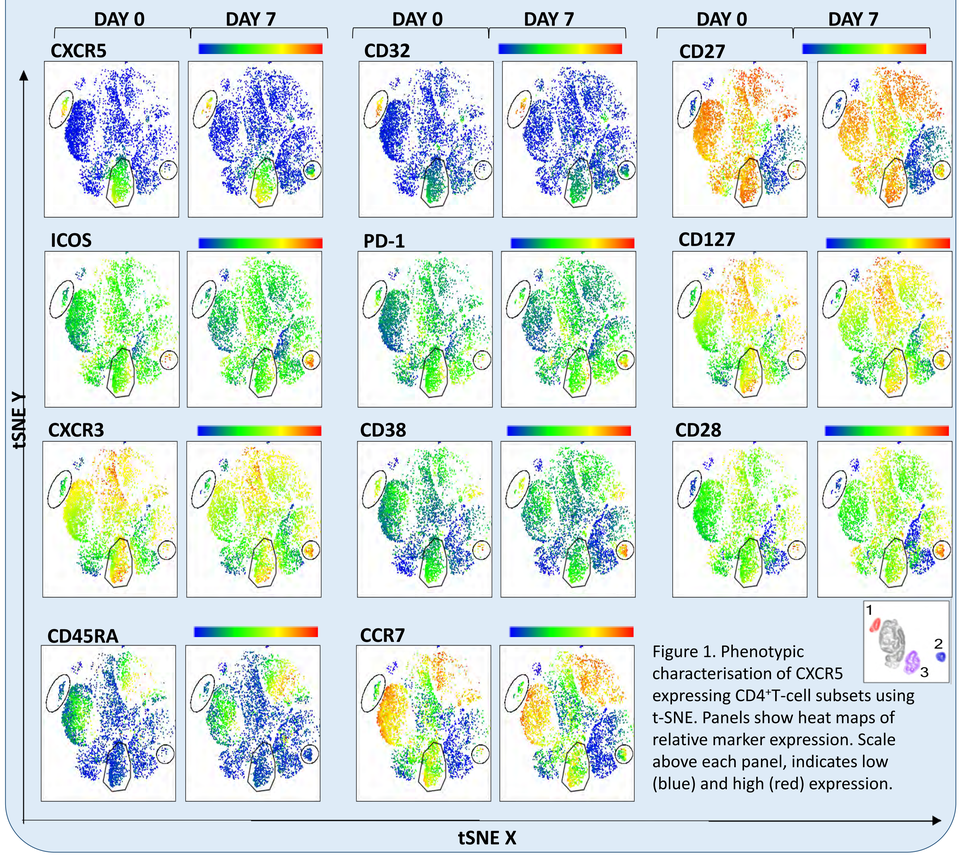


Figure 1. Phenotypic characterisation of CXCR5 expressing CD4⁺T-cell subsets using t-SNE. Panels show heat maps of relative marker expression. Scale above each panel, indicates low (blue) and high (red) expression.

Results 2. Increased Frequency of CD32⁺cTFH Post QIV

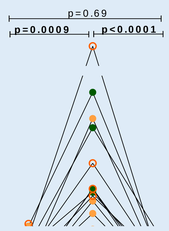


Figure 2. CXCR5⁺PD-1⁺CD32⁺CD4⁺ T-cell frequency pre and post QIV. Data analysed using 2D gating and Friedman's test.

Results 3. Diversity in CD4⁺CXCR5⁺ T-cell Responses

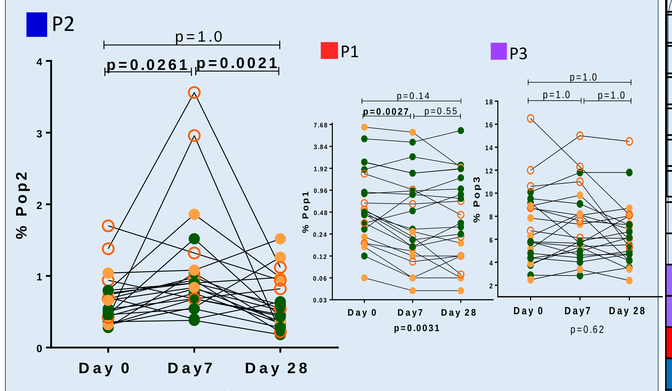


Figure 3. Frequency of CD4⁺CXCR5⁺ T-cell t-SNE subsets pre and post QIV. P1 (CXCR5^{hi}CD32^{hi}) and P3 (CXCR5^{mid}CD32$^{lo/mid}$) frequency was constant but P2 (CXCR5$^{lo/mid}$ CD32$^{lo/mid}$) was more frequent at Day 7 post QIV (p=0.0261); p values calculated with Friedman's Test.

Results 4. Functional Hierarchy of CD4⁺CXCR5⁺ T-cell Subsets

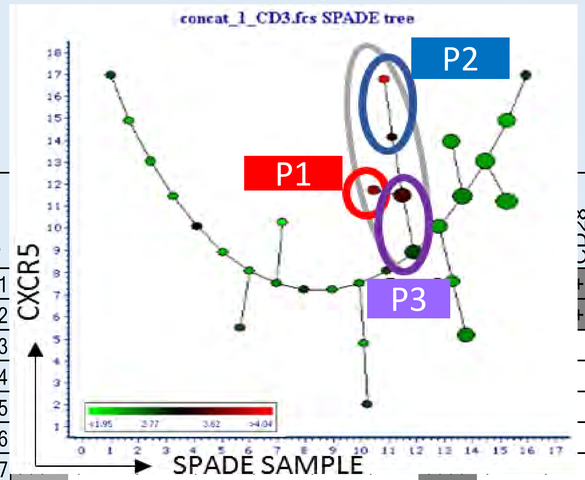


Figure 4 showing all partici... Node size ... gave rise ... vaccine-in...

Table 1: Relative Expression of CD4⁺CXCR5⁺ T-cell Subsets

Cluster Number	CD4	CXCR3	CXCR5	ICOS	PD-1	CD38	CD32	CD45RA	CCR7	CD28	CD27	CD127
1											+++	+++
2											+++	+++
3												+
4												+
5												+
6												+
7												+
8	+++	+	+	+	+	+	+	++++	+	+	+	+
9	++	+++	+	+	+	+	+	+	+	+	+	++
10	++		+	+	+	+	+	+	+	+	+	+
11	++	+++	+	++	++	+	++	+	+	+	+++	++
12	++	+++	+	++	+	+	+	+	+	++++	++++	++
13	++		+	+	+	+	+	+	+	++++	++++	++
14	++	+++	+	++	+	+	+	+++	++++	+++	++++	++
15	++	+++	+	++	+	+++	+	+++	+++	++	+++	+++
16	++	+++	+	++	++	+	+	+	+	++++	+++	++
P3	+	++	+	+	+	+	+	+	+	++++	+++	+++
P1	+	+	+	+	+	+	+	+	+	+	+	+
P2	+	+	+	+	+	+	+	++++	+	+	+	+
22	+	+	+++	+	+	+	+++	++++	+	+	+	+
23	+++	+	+	+	+	+	+	++++	+	+	+	+
24	+++	+	+	+	+	+	+	++++	+	+	+	+
25	++	+++	+	+	+	+	+	+	+	+	+	++
26	++		+	+	+	+	+	+	+	+	+	+
27	++	+++	+	++	++	+	++	+	+	+	+++	++
28	++	+++	+	++	+	+	+	+	+	++++	++++	++
29	++	+++	+	++	+	+	+	+	+	++++	++++	++
30	++	++++	+	++	+	+	+	++++	++++	+++	++++	++
31	++	++++	+	++	+	+++	+	++++	++++	+++	+++	+++
32	++	+++	+	++	++	+	+	+	+++	++++	+++	++
17	++++	++++	+++	++	++	+	++	+	++	+++	+++	++
18	++++	++++	+++	++	+	+++	++	+	++++	+++	++++	++
19	++++	+	++++	+	+	++++	++++	+	++++	+	+	+
20	++++	++++	++++	++++	+++	+++	++	+	++++	++++	++++	++++
21	++++	++++	++++	++++	++++	++++	++++	++	++++	++++	+++	++++
22	++++	++++	+	++	+	+	+	+	+	+++	+++	+++
33	++	+++	+	++	+	+	+	+	+	++++	+++	+
34	++	+++	+	++	+	+	+	+	+	+	+	+

References. 1.Ueno H. Human Circulating T Follicular Helper Cell Subsets in Health and Disease. J Clin Immunol 2016; 36 Suppl 1: 34-9. 2. Martin GE, Pace M, Thornhill JP, et al. CD32-Expressing CD4 T Cells Are Phenotypically Diverse and Can Contain Proviral HIV DNA. Front Immunol 2018; 9: 928.

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