Responses to quadrivalent influenza vaccine reveal the landscape of CD32 expression on circulating T-follicular helper cells in men living with HIV infection.

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Background

Inactivated influenza vaccine induces a specialised subset of CD4+CXCR5+ circulating T-follicular helper cells (cTFH) that provide help to B-cells1. Investigations of potential biomarkers for HIV integration have identified rare CD4+ T-cells highly expressing the FC γ receptor CD32, but with unknown function2. We hypothesised that CD32 is upregulated on cTFH in response to influenza vaccine.

Method

1. Recruitment

Healthcare controls n = 14
Men living with HIV infection suppressed on ART n = 16

2. Study Design

2017/18 Northern Hemorrhagic Influenza session

Day 0
QIV administered post bleed

Day 7
Day 28

3. Sample processing

PBMCs isolated stored at -180°C

4. Experiment Design

16-parameter staining for T and B-cell subsets

5. Analysis with unsupervised algorithms

T-SNE FlowJo v10.4.2

Results 1. CXCR5+CD4+ T-cell subsets with variable expression of the Fc gamma receptor CD32

Figure 1. Phenotypic characterisation of one participant’s CXCR5 expressing CD4+ T-cell subsets using t-SNE. Panels show heat maps of relative marker expression. Scale above each panel, indicates low and high expression.

Results 2. Functional hierarchy of CXCR5+CD4+ subsets

Figure 2. Phenotypic characterisation of CXCR5/CXCR5+CD4+ T-cell subsets using spanning-tree progression analysis of density-normalised events (SPADE), collated data from all participants.

Node colour indicates relative expression of CXCR5 from low (green) to high (red). Node size indicates relative population frequency.

A branched hierarchy of clustered nodes of CD4+ T-cells corresponding to P1, P2 and P3. A central memory CXCR5-high node gave rise to a CXCR5+CD32-high node that was unaffected by QIV and two vaccine-inducible activated ICOS+PD-1+CD38+CXCR5+CXCR5+CD32-high nodes.

Table 1. Relative expression of proteins on CD4+ T-cells

<table>
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<tr>
<th>Cluster Number</th>
<th>CD4</th>
<th>CD45RA</th>
<th>CD38</th>
<th>ICOS</th>
<th>CD32</th>
<th>PD-1</th>
<th>CD127</th>
<th>CD127</th>
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<tr>
<td>P3</td>
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Conclusions

Circulating CXCR5+CD4+ T-cells fall into three major related populations indicating a novel role for Fcγ receptor CD32 on T-cells in response;

- a rare subset of CD4+CXCR5-highCD32-high T-cells unresponsive to vaccination
- cTFH subset responding to QIV with increased frequency of CD32 post immunisation
- cTFH like subset unresponsive to vaccination

These populations were unaffected by HIV status which supports the findings that CD32 positivity on T-cells is unrelated to HIV integration.