Imaging B Cell Follicles to Investigate HIV/SIV Persistence

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Most HIV Replication Occurs In Secondary Lymphoid Tissues


Lymph Node Structure

Lymphatic Organs in Humans

Paracortex (EF)

Follicle (F)

Germinal Center (GC)
HIV Replication is Concentrated in in CD4+ T Cells in B Cell Follicles (i.e., TFH)


A CD4+ cell in F has a 31-fold (range, 6- to 155-fold) greater likelihood of being HIV RNA+ as a CD4+ cell in EF.

HIV Replication is Concentrated within Germinal Centers of Follicles

Are GC TFH More Permissive than Other CD4+ T Cells?

GFP Expression in Tonsil Subsets

GFP Expression in Sorted Tonsil Cell Subsets

GC TFH are highly permissive, but alter their phenotype during productive infection.

Why Are CTL Unable to Suppress HIV Replication in B Cell Follicles?

- Inadequate numbers of CTL
- Escape from immune recognition
- Impaired effector mechanisms
- Immune privileged site
CD8+ T Cells and Many Antiviral Proteins Are Less Abundant in B-cell Follicles

HIV-1 seropositive subjects (N=15)

<table>
<thead>
<tr>
<th>Protein</th>
<th>F Median Cells/mm² (range)</th>
<th>EF Median Cells/mm² (range)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>INF-α</td>
<td>0.4 (0.0 - 40.4)</td>
<td>2.7 (0.4 – 29.0)</td>
<td>0.0186</td>
</tr>
<tr>
<td>α-Defensins 1, 2, 3</td>
<td>2.5 (1.4 - 12.4)</td>
<td>4.9 (0.8 - 8.5)</td>
<td>0.0127</td>
</tr>
<tr>
<td>RANTES</td>
<td>282 (63 – 1122)</td>
<td>1025 (213 – 3065)</td>
<td>0.0007</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>32 (6 - 132)</td>
<td>105 (21 – 577)</td>
<td>0.0054</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>14 (0 – 299)</td>
<td>23 (9 - 244)</td>
<td>0.4251</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>1.0 (0.0 – 21.0)</td>
<td>2.7 (0.7 – 25.6)</td>
<td>0.1257</td>
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<tr>
<td>Perforin</td>
<td>4.7 (1.1 – 30.5)</td>
<td>4.1 (1.0 - 21.8)</td>
<td>0.7736</td>
</tr>
<tr>
<td>Granzyme A</td>
<td>158 (13 – 414)</td>
<td>465 (39 – 1246)</td>
<td>0.0018</td>
</tr>
<tr>
<td>CD8</td>
<td>11.8 (3.1 – 32.5)</td>
<td>56.7 (32.8 - 72.3)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Are HIV-Specific CTL deficient in B cell follicles?


Dr. Pamela Skinner
CTL Fail to Accumulate in Follicles of Untreated HIV+ Lymph Node

Connick E….Skinner P. J Immunol 2007;178:6975
SIV-Infected Rhesus Macaque Model

1. Does the SIV-infected macaque model recapitulate the distribution of virus and CTL in humans?

2. Does the same pattern occur in all secondary lymphoid tissues?
SIV RNA+ Cells Are More Frequent in F vs EF in Chronic Infection Without SAIDS

<table>
<thead>
<tr>
<th>Location</th>
<th>F:EF</th>
<th>GM</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td></td>
<td>7.1</td>
<td>4.7, 10.7</td>
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<tr>
<td>Inguinal LN</td>
<td></td>
<td>3.1</td>
<td>2.4, 4.0</td>
</tr>
<tr>
<td>Axillary LN</td>
<td></td>
<td>32.0</td>
<td>23.6, 43.4</td>
</tr>
<tr>
<td>Mes LN</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cecum</td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

SIV RNA+ Cells Remain Concentrated in F after Adjusting for Frequencies of Target Memory Cells

\[
p < 0.0001
\]

SIV-Specific Tetramer Staining Cells Are Concentrated in Extrafollicular Zones

Red = Mamu B*08/Vif RL8 tetramer
Green = CD20        Blue = CD3

Effector to Target Cell Ratios are More than 10-Fold Higher in EF vs F

Frequencies of CTL are Inversely Related to SIV RNA+ cells in EF and F

Is Virus Replication Concentrated in Follicles in the Absence of a Robust CTL Response?

- Acute SIV infection – when CTL are evolving
- SAIDS – when CTL are losing the ability to control virus
- CD8 Depletion – CD8 cells transiently eliminated

Hypothesis: Virus replication is not concentrated in F in the absence of a robust CTL response.
Frequencies of SIV RNA+ Cells in Follicles (F) and Extrafollicular Zones (EF) Vary by Disease Stage

**14 Day Acute**
- p = 0.99

**Chronic**
- p = 0.0001

**SAIDS**
- p = 0.39

**SIV RNA+ cells/mm²**

**F** vs. **EF**
- 95% CI: 0.91, 0.66, 1.26
- 3.2, 2.1, 4.9
- 1.9, 1.1, 3.4

SIV RNA Increases Primarily in the Extrafollicular Zone after CD8 Depletion


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**Graphs:**

- **y-axis:** SIV RNA copies/ml
- **x-axis:** Days post-infection
- **Legend:**
  - Pre Depletion LN
  - Post Depletion LN
  - CD8 depleting antibodies

**Legend:**

- **CM9 Tetramer+ T cells/ml blood:**
  - Follicle
  - Extrafollicular Zone

- **y-axis:** SIV RNA+ cells/mm²
- **x-axis:** Pre, Post

Few SIV-specific CTL exhibit a follicular homing phenotype

Why do CTL fail to accumulate in large numbers in F where virus is highly concentrated?

Connick E...Skinner P. J Immunol 2014; 193:5613
Follicles Are HIV Reservoirs in ART-Treated Individuals


RNAseq in Lymph Node of ART-Treated Person

(Connick E, unpublished data)

HIV RNA in situ+ (red); CD20 (white), FDC (green)
Targeting CTL to B Cell Follicles To Induce a Functional Cure

1. SIV
2. Isolate CTL and transduce CXCR5
3. Expand CXCR5+ CTL and CTL alone
4. Infuse into ART-treated macaque

Discontinue ART and assess localization of CTL and virus vis a vis untransduced CTL
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