Differentiation and function of follicular helper CD4 T cells ($T_{FH}$)

Shane Crotty
the immunobiology of B cell and CD4 T cell responses to vaccines

- **Naive B cell**
- **antigen**
- **activated B cells**
- **plasmablast/short-lived plasma cell**
- **germinal center**
- **short-lived plasma cell**
- **long-lived plasma cell**
- **memory B cell**
the immunobiology of B cell and CD4 T cell responses to vaccines
How do you get T cell help to B cells?

The diagram illustrates the interaction between T cells and B cells in the context of the immune response. It shows the following key elements:

- **T cell zone**: Site where T cells are located.
- **MZ follicle**: Marginal zone follicle where B cells reside.
- **Germinal center (GC)**: Area where B cells undergo affinity maturation and germinal center formation.
- **Plasmablast/plasma cells**: Cells that produce antibodies.
- **Memory B cells**: Long-lived B cells that can respond quickly to future infections.
- **Exit to blood and bone marrow**: Pathway for B cells to leave the lymph node.

The diagram also highlights the roles of different T cell subsets:

- **Th1**: T helper 1 cells, which are involved in the immune response against intracellular pathogens.
- **Th2**: T helper 2 cells, which are involved in the immune response against extracellular pathogens.
- **Th17**: T helper 17 cells, which are involved in chronic inflammation.
- **Treg**: Regulatory T cells, which suppress immune responses.

The diagram is modified from Crotty AE 2011.
GC B cell state
GC B memory B cell
Tfh
GC B plasma cell
mutation
cell division
GC B apoptosis
Bcl6 is a central regulator of Tfh differentiation and T cell help to B cells

5000 SMARTA + 10⁵ LCMVarm → day 7-8
analyze T and B cells

virus-specific CD4 T cells

Control

Bcl6⁺

32%

85%

Science, 2009
Bcl6 is a central regulator of Tfh differentiation and T cell help to B cells

5000 SMARTA + 10^5 LCMVarm → day 7-8 analyze T and B cells

virus-specific CD4 T cells

Control

Bcl6+

GFP+

Bcl6+

germinal center B cells

D45

NP-Ova IgG (x10^3)

no cells GFP Bcl6

Science, 2009
Bcl6 and Tfh differentiation are antagonized by Blimp-1
Bcl6 and Tfh differentiation are antagonized by Blimp-1
SAP is required in CD4 T cells for GCs and long term humoral immunity

**Figure 1**

**Figure 2**

**Table**

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>LCMV-specific IgG ASC per 10^6 cells</th>
<th>LCMV-specific memory B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>10^2</td>
</tr>
<tr>
<td>25</td>
<td>100</td>
<td>10^3</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
<td>10^4</td>
</tr>
<tr>
<td>75</td>
<td>100</td>
<td>10^5</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>10^5</td>
</tr>
<tr>
<td>125</td>
<td>100</td>
<td>10^5</td>
</tr>
<tr>
<td>150</td>
<td>100</td>
<td>10^5</td>
</tr>
</tbody>
</table>

**Legend**

- Black = WT
- Red = SAP KO

Nature, 2003

JI 2007
Tfh cells are required for germinal centers, and therefore the bulk of B cell memory: memory B cells and long-lived plasma cells.

Tfh cells are limiting for this process.
**Figure 3**

- **SM CD4 T cell %**
  - 0.0
  - 0.4
  - 0.8
  - 1
  - 3
  - 4
  - 5

- **WT**
  - CD25+/-- anti-IL-2
  - IL-2

- **Bcl6**
  - **CD25**
  - CXCR5

- **Memory B cells**
  - **plasmablast/plasma cell**

- **Wildtype**
  - **plasmablast/plasma cell**
  - **plasmablast/plasma cell**

- **Germininal center**
  - **FDC**
  - **GC Tfh**

- **Tfh sites of Infection/Inflammation**

- **DC**
  - **Nai**
  - **Bcl6**
  - **PD-1 MFI**

- **Th1**
  - **Th2**
  - **Th17**
  - **Treg**

- **Bone marrow**
  - **Blood and exit to**

- **Immunity 2011**
  - **JEM 2012**
  - **JL 2013**
Tfh cell fate commitment

- **Fig 3A**
  - IL2R
  - Blimp1

- **Fig 3B**
  - 41.7
  - 46.5

- **Fig 3C**
  - D3 LCMV-infected mice
  - Infected-matched mice
  - Day 3 p.i.
  - Day 8 p.i.
  - Transfers
  - FACS analysis

- **Fig 3D**
  - IL-2Rα
  - IL-2Rα

- **Fig 3E**
  - CD45.1+ SM
  - IL2Rα

- **Fig 3F**
  - Bcl6
  - Blimp1

- **Fig 3G**
  - IFN-γ

Choi et al., JI 2013
Tfh cell fate commitment

Choi et al., JI 2013
Human Tfh cells
Human Tfh and GC Tfh cells

Tonsil or LN

Gated on CD4+ CD45RO+

Figure 1

**Fold change in MFI**

Kroenke et al., 2012
Why do some people generate broadly neutralizing antibodies against HIV?

1. Tfh cells are required for germinal centers, and therefore the bulk of B cell memory: memory B cells and long-lived plasma cells.

2. Tfh cells are limiting for this process.

3. Generation of broadly neutralizing Abs against HIV/SIV requires extensive somatic mutation, and therefore requires exquisitely optimized germinal center responses.

How can a vaccine elicit broadly neutralizing antibodies against HIV?
Why do some people generate broadly neutralizing antibodies against HIV?

IAVI Protocol C cohort: 800+ HIV+ African individuals tracked longitudinally and examined for HIV neutralizing antibodies

- Top = Neutralization of 5-6 representative HIV strains
- Low = Neutralization of < 2 HIV strains
Are blood CXCR5\(^+\) cells predictive of HIV neutralizing antibody responses?

\[
\begin{array}{c}
\text{total CXCR5}^+ \% \\
\hline
0.029 \quad \text{ns}
\end{array}
\]

\[
\begin{array}{c}
\% \text{CXCR5}^+ \text{ of CD4}^+ \\
\hline
0 \quad 5 \quad 10 \quad 15 \quad 20 \quad 25
\end{array}
\]

\[
\begin{array}{c}
\text{HIV}^- \quad \text{Top} \quad \text{Low} \quad \text{HIV}^+
\end{array}
\]
Are blood recently activated Tfh cells predictive of HIV neutralizing antibody responses?

ICOS+ PD1\textsuperscript{hi} CXCR5+ recently activated Tfh cells
Which subset of blood CXCR5$^+$ cells is most related to $T_{FH}$ cells from lymphoid tissue?

Immunity, Sept 2013
Which subset of blood CXCR5$^+$ cells is most related to T$_{FH}$ cells from lymphoid tissue?
Is PD-1 expression stable on blood CXCR5+ cells?

Day3  Day5  Day10  Day15  Day20

PD-1+

PD-1-

CXCR5

PD1+

PD1-

PD-1+

PD-1-
Which subset of blood CXCR5$^+$ cells is most related to $T_{FH}$ cells from lymphoid tissue?

Top 100 genes differentially regulated in comparison to blood CXCR5$^-$ cells

Blood CD4$^+$CD45RO$^+$

*CXCR5$^-$

*PD1$^+$CXCR3$^+$

*PD1$^+$CXCR3$^-$

*PD1$^-$CXCR3$^-$

*blood CXCR5$^+$

Tonsil CD4$^+$CD45RO$^+$

CXCR5$^-$

GC Tfh
Blood CXCR5+ PD1+ CD4 T cell secreted molecules

**CXCR5+**

<table>
<thead>
<tr>
<th>PD1+</th>
<th>PD1-</th>
<th>PD1+</th>
<th>PD1-</th>
<th>CXCR5-</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCR3-</td>
<td>0.5</td>
<td>0.3</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>CXCR3-</td>
<td>38</td>
<td>21</td>
<td>42</td>
<td>24</td>
</tr>
</tbody>
</table>

**Unstim**

**PMA/Iono**

% of IL-21+ cells

% of IL-4+ cells

% of IL-IFN+ cells

% of IL-2+ cells
Blood CXCR5+ CD4 T cell help to B cells

CXCR5+

PD1+ CXCR3-
PD1- CXCR3-
PD1+ CXCR3+
PD1- CXCR3+
CXCR5-

IgG

B cells

IgM

Plasmablasts

PD1+ CXCR3-
P D1- CXCR3-
P D1+ CXCR3+
P D1- CXCR3+
CXCR5-
P D1+ CXCR3-
P D1- CXCR3-
P D1+ CXCR3+
P D1- CXCR3+
CXCR5-
Are CXCR5$^+$ PD1$^+$ cells memory T$_{FH}$ cells?
Why do some people generate broadly neutralizing antibodies against HIV?

IAVI Protocol C cohort: 800+ HIV+ African individuals tracked longitudinally and examined for HIV neutralizing antibodies.

* Top = Neutralization of 5-6 representative HIV strains
* Low = Neutralization of < 2 HIV strains
Are blood CXCR5⁺ PD1⁺ cells predictive of HIV neutralizing antibody responses?

earliest available time point

0.0037

C

time of bnAb development

0.0010
PD1<sup>+</sup>CXCR3<sup>-</sup>CXCR5<sup>+</sup> CD4 T cells are highly functional memory Tfh cells, and they are a biomarker associated with the capacity to make broadly neutralizing antibodies against HIV.
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Pew Scholars
GC B cell state
- GC B memory B cell
- Tfh
- GC B plasma cell
- mutation
- cell division
- GC B apoptosis

T B
T B
plasmablast/plasma cell

germinal center

exit to blood and bone marrow

Th1 sites of infection/inflammation
- Th2
- Th17
- Treg
- Tfh

GC Tfh

plasma cells

MZ follicle

FDC

B B
B B
B B
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B B
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B B
B B
B B
B B
B B
B B

T T

T T

B T

Th1

Tfh

Tfh

Tfh

Tfh

DC

T cell zone

T B