Peripheral T follicular helper cell function during HIV Infection

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T follicular Helper Cells (Tfh)

- CD4 T helper cell subset
- Characterized by PD-1, CD40L, ICOS and CXCR5
  - IL-21 secretion
- Reside in lymph node germinal centers
- Peripheral Tfh (pTfh)
- Activate B cells
  - Differentiate into plasmablasts

Vinuesa et. al (2005)
B Cell Differentiation

Antigen encounter

2\textsuperscript{nd} Antigen encounter

Activated mature B cell → Short-lived plasmablast

Antibody Secretion

Moir, S. and A.S. Fauci (2009)
HIV

- Human immunodeficiency virus
- CD4+ and CD8+ T cells exhaustion
  - Decrease effector function
  - Increase inhibitory receptor expression
- B cell dysregulation
  - Hypergammaglobulinemia
- pTfh role in HIV-1 infection
Purpose

• Overall Goal: Determine the relationship between pTfh help and the maintenance of B cell responses in chronic HIV-1 infection

• Goal of Project: Develop a sensitive assay to measure ability of T helper cells to induce the differentiation of B cells into antibody-secreting plasmablasts

• Methods:
  – 5 HIV- controls and HIV+ individuals
  – Readout of T cell help = plasmablast frequency and antibody production
**Co-Culture Conditions**

Peripheral blood mononuclear cells → Magnetic Beads

- CD19+ B cells
- CD4+ T cells

CD4+ T cells and CD19+ B cells → Incubate for 7 days

Stimulants

Collect supernatant

Flow Cytometry

*Plasmablasts*

ELISA

*IgG Antibody*
Stimulants

CD4 T cells and CD19+ B cells

**Positive Control:** sCD40L+ IL-21 (B cell stim)

**Antigen-Specific:** Tetanus toxoid (T cell stim)

**HIV-1 Antigen:** SF162 gp140 trimer (T cell stim)
Gating Scheme for Plasmablasts

Lymphocytes

Size

Granularity

CD3

Viable CD3- Cells

Viability

Viable CD3+

Viable CD3-

CD27 vs CD38

CD20

CD19 vs CD20

CD19

non B cell

CD19+CD20

CD19+CD20-

non B cell

CD27

Plasmablast

Unstimulated

0.13%

Stimulated

20.0%

CD27 PE-A

CD38 PE-Cy7-A

CD38 PE-Cy7-A
Plasmablast Frequency

![Graph showing the change in percentage of PLBs out of total B cells in HIV- and HIV+ individuals under sCD40L + IL-21 stimulation.]

\[ \text{sCD40L + IL-21} \]

\[
\begin{align*}
\% \text{ Change of PBs out of total B cells} \\
\text{stimulated - unstimulated}
\end{align*}
\]

- HIV-: [Data points and bars indicating change]
- HIV+: [Data points and bars indicating change]

\( p = 0.155 \)

*: paired t-test between response to stimulation compared to unstim, \( p < 0.05 \)

**NS:** not significant

![Graph showing the change in percentage of PLBs out of total B cells in HIV- and HIV+ individuals under Tetanus toxoid stimulation.]

\[ \text{Tetanus toxoid} \]

- HIV-: [Data points and bars indicating change]
- HIV+: [Data points and bars indicating change]

\( P = 0.421 \)

![Graph showing the change in percentage of PLBs out of total B cells in HIV- and HIV+ individuals under SF162 gp140 trimer stimulation.]

\[ \text{SF162 gp140 trimer} \]

- HIV-: [Data points and bars indicating change]
- HIV+: [Data points and bars indicating change]

\( p = 0.047 \)

**NS:** not significant
*: paired t-test between response to stimulation compared to unstim, p<0.05
NS: not significant
Summary

1. Developed two assays to measure T cell help to B cells by potent stimulation
2. Detected recall responses to Tetanus toxoid in HIV- and HIV+ subjects
3. Detected HIV-specific responses in HIV+ subjects that were not present in HIV- subjects
CD4+ T cells

Incubate for 7 days with sCD40L and IL-21

Flow Cytometry

Cell Sorter

pTfh (CXCR5+)

Non-pTfh (CXCR5-)

CXCR5+ and CD19+ B cells

Flow Cytometry

Plasmablasts

ELISA

IgG Antibody
CXCR5 +/- and CD19 B cell Co-Culture

HIV-: 30030 (white bars)
HIV+: 10060 (black bars)

• Positive control elicited high B cell responses for CXCR5 +/- Co-cultures
Conclusion

• Optimized a B cell flow cytometry panel and ELISA
  – Detect B cell responses to potent stimulation (sCD40L+IL-21)
  – Detect recall responses to Tetanus toxoid for HIV- and HIV+ individuals
  – Detect HIV-1 specific responses for HIV+ individuals that were not present in HIV- individuals

• pTfh cell sorting assay is optimized for future experiments
Future Directions

• Increase sample size of HIV- and HIV+
  – Including HIV+ individuals with varying viral loads and CD4 counts
• Measuring pTfh function using wider panel of HIV-1 antigens
• Investigate specific interactions between pTfh and B cells in hopes of identifying molecules/pathways that may be targetable in a therapy or vaccine
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