Follicular SIV-specific CD8 T cells Contribute to Enhanced Control

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Study Objectives

• Integrated DNA is the major molecular form of HIV in CD4 T cells of ART treated patients and PD-1+ CD4 T cells in blood harbor highest levels of integrated viral DNA. Chomont et al. Nat Med (2009)

• However, little information is available on the fate of PD-1+ CD4 T cells following HIV or SIV infection especially in lymphoid and mucosal tissue, preferential sites of virus replication.

• Here, we determined the fate of PD-1+ CD4 T cells in blood, lymph nodes and rectal tissue following a pathogenic SIV infection in rhesus macaques in the setting of uncontrolled and vaccine-mediated controlled infection.
PD-1$^{\text{hi}}$ CD4 T cells are enriched in sites of preferential SIV replication in SIV naïve RM.

Gated on Memory CD4
PD-1^{hi} CD4 T cells are phenotypically Tfh cells
PD-1\textsuperscript{hi} CD4 T cells are mostly localized in the Rectal Follicle of SIV Naïve RM
What Happens to These PD-1^{hi} Cells During Chronic SIV Infection?
Cohort of SIV infected Non-Controller and Vaccinated Controller Rhesus Macaques

Non-Controllers

- Mostly unvaccinated
- Some received DNA/MVA 239 vaccine

Controllers

- All received DNA/MVA 239 vaccine
PD-1$^{hi}$ CD4 T Cells Accumulate in Lymphoid Sites of Non-Controllers but NOT in Controllers
Lower frequency of PD-1$^{hi}$ CD4 T cells is associated with decreased plasma viremia.

Non-Controller RM only
PD-1$^{hi}$ Cells in Vaccine Controllers Have High In Vivo Proliferation and CCR5 Expression

### Ki-67

- Uninfected (SIV-)
- Vaccine Controllers (SIV+)
- Non-controllers (SIV+)

- Percent of Ki67+ Cells

### CCR5

- Percent of CCR5+ Cells

### CXCR5

- CXCR5+ cells (Percent of PD-1$^{hi}$ cells)
PD-1$^{\text{hi}}$ CD4 T Cells Support SIV Replication and Production (Non-Controllers)

SIV RNA levels were very low in Controllers (not shown)
PD-1^{hi} CD4 T cells accumulate in LN and rectum of non-controllers but not in controllers and support virus replication during chronic SIV infection
What Hypotheses Could Explain The Lack Of Expansion Of These PD-1^hi^ CD4 T Cells In Controllers?

- Are CD8 T Cells Present In These Sites?
- Greater Anti-viral CD8 T Cell Response?
- Altered Phenotype Of Follicular CD8 T Cells?
Higher Frequencies of SIV Specific CD8 T Cells in Lymphoid Sites of Controllers

Gag CM9+

GB+ Gag CM9+

Blood

LN

LN

Vaccine Controllers

Non-controllers

Gag CM9+

Percent of CD8 T cells

Percent of CD8 T cells

Percent of CD8 T cells

Percent of CD8 T cells

C NC

C NC

C NC

0 2 4 6 8 10

0 5 10 15 20

0 5 10 15 20

0 10 20

PD-1h Cells

(Percent of CD5+ CD4 T cells)

R = -0.74
P = 0.0005

towards an cure
people focused science driven
Higher Frequency of Functional Follicular CD8 T cells in Controllers

% CXCR5+

% CXCR5+

% GB+ CXCR5+

- Total CD8 T cells (Controller)
- SIV specific CD8 T cells (Controller)
- Total CD8 T cells (Non-controller)
- SIV specific CD8 T cells (Non-controller)
Increased Co-localization of CD8 T Cells with PD-1$^{hi}$ Cells in the Lymph Node of Controllers
In Vitro CD8 T cell Killing Assay

Brief Protocol:
• Pulse CXCR5^hi^ Tfh cells with P11c (Gag CM9) Peptide for 1 hour at 37°C
• Label CXCR5+ CD8 T cells with Cell Violet Tracer Dye
• Co-culture CXCR5+ CD8 T cells with Tfh at 2:1 ratio of effectors to targets (CD8 T cells to antigen pulsed Tfh cells)
SIV Specific CD8 T cells Isolated from the LN limit CXCR5\textsuperscript{hi} PD-1\textsuperscript{hi} CD4 T cell expansion

Frequency of Gag CM9

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<th>RM 1</th>
<th>RM 2</th>
<th>RM 3</th>
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<td>(2.9%)</td>
<td>(5.3%)</td>
<td>(0.5%)</td>
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### AXCR5\textsuperscript{hi} PD-1\textsuperscript{hi} CD4 T cells

- CXCR5\textsuperscript{hi} PD-1\textsuperscript{hi} CD4 T cells: + + +
- αCD3/CD28: - + +
- CD8 T cells: - - +

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- αCD3/CD28: - + +
- CD8 T cells: - - +
Conclusions

- PD-1hi CD4 T cells accumulate during chronic SIV infection, localize to CD8 T cell immune privileged sites such as the B cell zone of lymphoid follicles and support active virus replication.

- Vaccinated controller RM limit the aberrant expansion of PD-1hi CD4 T cells possibly through maintenance of higher frequencies of follicular SIV specific CD8 T cells capable of infiltrating B cell zone and killing infected PD-1hi CD4 T cells.

- These data implicate targeted therapies to reduce/eliminate virus-infected follicular CD4 T cells contribute significantly to enhance control of chronic SIV/HIV infections.
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