Monitoring mucosal T cell responses to vaccines (the HVTN experience)

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Overview

- Considerations for cellular analyses in mucosal samples
- Current HVTN experience examining vaccine-induced T-cell responses in the HVTN 505 mucosal sub-study and HVTN 076
- Interest in vaccine-induced changes in mucosal cells that could potentially enhance HIV infection risk (e.g., Ad5 (vector)-specific T cells, activated T cells)
Considerations

- The route of HIV infection is typically through rectal or vaginal mucosa; it can be informative to examine the effect of vaccination at mucosal sites.
- Options for mucosal tissue/cell sampling include biopsies (vaginal, cervical, rectal/GI), cervical cytobrush, semen.
- “Functional” assays typically required to measure vaccine-specific cellular responses.
- “Phenotyping” assays can enumerate changes in cell populations related to vaccination.
Biopsies and processing into single cell suspension for flow cytometry

Collection procedures for *ex vivo* assays:

- Colon: 25 biopsies by flexible sigmoidoscopy at ~40 cm
- Rectum: up to 6 biopsies taken by flexible sigmoidoscopy or by anoscopy

Biopsy processing:

- digested with collagenase and washed multiple times
- cells in suspension rested overnight for ICS studies or assayed directly for phenotypic studies

Individual biopsies can also be frozen or fixed for microscopy
Example of cell yields and viabilities from GI biopsies (HVTN 076)

Rectum

![Graph showing cell yields and viabilities from rectal biopsies.](Image)

Colon

![Graph showing cell yields and viabilities from colonic biopsies.](Image)
Accurate determination of CD3$^+$ cell percentages by Guava instrument (HVTN 076)

T cells are a relatively low proportion of total viable cells recovered from GI biopsies.
Example of CD3$^+$ T cell counts obtained from GI biopsies (HVTN 076)

A small number of rectal biopsies provides sufficient cells to examine vaccine-induced T cells specific to a few antigens. Typically, 100-200k T cells required per stimulation condition for ELISpot or intracellular cytokine staining.
Intracellular cytokine staining (ICS)

- Measures vaccine-induced T cells and adenovirus-specific T cells
- PTE$_g$ peptide pools for HIV (Env, Gag, Pol) and hexon 1 and 2 peptide pools for Ad5
  - Only selected peptide pools per mucosal sample due to limited cell yields
- IFN-γ, IL-2 and TNF-α used as primary measure
- May also include IL-17, granzyme B, CD103, CCR5
- Mucosal CD4 staining in stimulated assay poor; gate on CD3$^+$CD8$^-$ as presumed CD4$^+$ T cells
Further optimization resulted in these changes for GI biopsies:

- Percoll gradient step removed since it decreased cell yields without improving purity
- Prior to stimulation for ICS, added overnight rest in the presence of Zosyn and fungizone. This lowers background, but main reason was operational to allow biopsies to be processed mainly within normal working hours (especially important in developing countries).
ICS background (without antigen-specific stimulus) is higher for CMMC/RMMC compared with PBMC, but relatively low.

*Data for five HVTN 076 participants for multiple visits*
Sufficient cells for ICS from rectal biopsies: Example for SEB stimulation
HVTN 505 was a phase 2b trial testing efficacy in reducing HIV acquisition or controlling HIV in Ad5-seronegative, circumcised men and male-to-female transgender persons, who have sex with men.

- DNA x3 at months 0, 1 and 2
- Recombinant Ad5 at month 6
  - Each encodes Env clades A, B, C, Gag and Pol clade B; DNA additionally encodes Nef clade B
- 1250 vaccine and 1250 placebo recipients
- Vaccinations halted in April 2013 due to lack of efficacy
After trial was halted, developed a plan to obtain rectal biopsies from 15 vaccine and 15 placebo recipients within 6 months of last Ad5 vaccination.

All biopsies performed at Seattle HVTU clinic.

Made use of existing mucosal sampling protocol (PI: McElrath) to allow quick initiation.

6 biopsies per participant; 2 frozen in OCT and 4 processed fresh for phenotyping and ICS.
HVTN 505 rectal biopsies

- Frozen OCT specimens allow detection of HIV target cells *in situ* (CD4**, CCR5**, activated)

- Intracellular cytokine staining (ICS) to examine HIV- and Ad5-specific T cells;
  - IFN-γ, IL-2, TNF-α, CD40L, perforin, granzyme B, CCR5
  - For Ad5, use hexon peptide pool; previously tested use of empty Ad5 vector stimulation

- Phenotyping panel examines major lineages, activation (CD38**HLA-DR**), and expression of α4β7
To minimize the time delay from last vaccination to biopsy, there was higher priority for recruiting vaccine recipients sooner.

- Median: 28 weeks for vaccine and 45 weeks for placebo recipients.
HVTN 505 mucosal sub-study ICS results

- HIV-specific CD4\(^+\) and CD8\(^+\) T cells responses detected in blood from vaccine recipients but not in rectum

- Ad5-specific CD4\(^+\) T-cell responses detected in most participants in blood and rectum
  - Magnitude significantly higher in vaccine vs. placebo recipients in blood (p=0.024) but not rectum

- Ad5-specific CD8\(^+\) T-cell responses detected in half of participants in blood and in \(\leq 25\%\) in rectum
HVTN 505 mucosal sub-study interim results

- Gating of phenotyping data completed; data analysis in process (examples shown for lineages and homing and activation markers on CD4⁺ T cells)
- ICS does not detect HIV-specific T-cell responses in rectum
- Ad5-specific CD4⁺ and CD8⁺ T-cell responses detected in rectum, but unlike blood, the magnitude is similar for vaccine and placebo recipients
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