DNA/NYVAC Vaccine Regimen Induces HIV-Specific CD4 and CD8 T-Cell responses in Intestinal Mucosa

Matthieu Perreau, PhD
Division of Immunology and Allergy, Centre Hospitalier Universitaire Vaudois
University of Lausanne
Switzerland
Background I

- Gut mucosal tissues correspond to HIV’s most frequent port of entry, the major sites for HIV spreading and CD4 T-cell depletion.

- Many efforts are made to induce protective mucosal immunity against HIV, including HIV-specific CD8 T-cell immunity.

- Replication-defective adenovirus and poxvirus-derived vectors are among the most studied T-cell based vaccine platforms against HIV-1.

- Recently, two HIV vaccine trials evaluating the efficacy of these two vectors generated different clinical outcomes.
Background II

- The phase IIb HIV vaccine trial called STEP evaluating the protective effect of MRKAd5 vaccine was prematurely stopped due to a lack of efficacy of the vaccine.
  \textit{Buchbinder et al, Lancet 2009}

- The RV-144 trial evaluating the protective effect of poxvirus-derived ALVAC-HIV in combination with a recombinant gp120 subunit vaccine prevented HIV infection (31.2% efficacy).
  \textit{Rerks-Ngarm et al, NEJM 2009}

- None of the previous studies evaluated 1) the presence of pre-existing Ad-specific or pox-specific T cells in gut mucosal tissues, which may impair HIV-vaccination efficiency, and 2) the induction of HIV-specific T cells in gut mucosal tissues.
Aims of the Study

1. To investigate the anatomical distribution in blood and gut mucosal tissues of pre-existing T cells that might crossreact with Ad-derived or pox-derived vectors
   a. following natural adenovirus infection
   b. following smallpox vaccination
Methods I

To address the first issue 25 HIV-uninfected individuals vaccinated with smallpox and potentially infected with Ads were recruited.

CFSE labeling

Mononuclear cells were isolated from Blood, rectum, and Ileum

Exposed to Ad5/NYVAC vectors For 6 days

Stained with \( \alpha \)-CD3, \( \alpha \)-CD4 and \( \alpha \)-CD8 antibodies

Unstimulated

Stimulated

0.18

15.7

CD4

CFSE

CD4

CFSE
Adenovirus-specific but not Smallpox-specific CD4 T cells are detected in gut-mucosal tissues

Flow cytometric profiles of Adenovirus naturally infected/Smallpox vaccinated Subject #025

Cumulative data

Perreau et al, JVI 2011
Adenovirus-specific but not Smallpox-specific CD8 T cells are detected in gut mucosal tissues

Flow cytometric profiles of Adenovirus naturally infected subject #14 and Smallpox vaccinated Subject #006 Gated on CD3+CD8+ cells

Cumulative data

Adenovirus

<table>
<thead>
<tr>
<th>Location</th>
<th>Unstimulated</th>
<th>Ad5</th>
<th>Unstimulated</th>
<th>NYVAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>0.21</td>
<td></td>
<td>0.14</td>
<td>8.71</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.83</td>
<td></td>
<td>0.37</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Pox

<table>
<thead>
<tr>
<th>Location</th>
<th>Unstimulated</th>
<th>NYVAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>24.9</td>
<td></td>
</tr>
<tr>
<td>Ileum</td>
<td>9.49</td>
<td></td>
</tr>
</tbody>
</table>

Ad-specific CD8 T-cell proliferation

<table>
<thead>
<tr>
<th>Location</th>
<th>Percentage of responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>40</td>
</tr>
<tr>
<td>Rectum</td>
<td>40</td>
</tr>
<tr>
<td>Ileum</td>
<td>40</td>
</tr>
</tbody>
</table>

Pox-specific CD8 T-cell proliferation

<table>
<thead>
<tr>
<th>Location</th>
<th>Percentage of responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>5</td>
</tr>
<tr>
<td>Rectum</td>
<td>5</td>
</tr>
<tr>
<td>Ileum</td>
<td>5</td>
</tr>
</tbody>
</table>

Perreau et al, JVI 2011
Aims of the Study II

1. To investigate the anatomical distribution in blood and gut mucosal tissues of pre-existing T cells that might crossreact with Ad-derived or pox-derived vectors
   a. following natural adenovirus infection
   b. following smallpox vaccination

2. To investigate the anatomical distribution in blood and gut mucosal tissues of CD4 and CD8 T cells induced following DNA-C/NYVAC-C vaccine regimen encoding for gag/pol/nef and env clade C HIV-1 proteins
Methods II

To address this issue 6 HIV-uninfected individuals enrolled in the EV03 trial were recruited.

- Blood and gut biopsies collected at Weeks 0, 4, 8, 24, 2 years after administration of DNA-C NYVAC-C.
- Mononuclear cells were isolated from Blood, rectum, and ileum.
- Exposed to HIV-peptide pools/NYVAC vectors for 6 days.
- Stained with α-CD3, α-CD4, and α-CD8 antibodies.
- CFSE labeling.
NYVAC-specific and HIV-specific CD4 T cells home to gut-mucosal tissues

Flow cytometric profiles of DNA-C/NYVAC-C vaccinated Subject #1008U

Cumulative data

Perreau et al, JVI 2011
NYVAC-specific and HIV-specific CD8 T cells home to gut mucosal tissues

Flow cytometric profiles of DNA-C/NYVAC-C vaccinated Subject #1037F

Cumulative data

Perreau et al, JVI 2011
Adenovirus-specific and NYVAC-specific CD4 T cells express higher levels of $\alpha 4\beta 7$ integrins than Smallpox-specific CD4 T cells

Perreau et al, JVI 2011
Conclusions

1. Ad-specific T cells generated following natural adenovirus infection can be detected in periphery and at gut mucosal sites.

2. Pox-specific T cells generated following smallpox vaccination were detected only in peripheral blood, while DNA-C/NYVAC-C vaccine regimen induced detectable NYVAC-specific T cells in gut mucosal tissues.

3. The route of vaccination (Scarification vs IM), the delay between antigen exposure and the measurement of antigen-specific immune responses and the level of expression of $\alpha4\beta7$ integrins might partially explain the difference in the migratory capacity of pox-specific T cells.

4. DNA-C/NYVAC-C vaccine regimen induced HIV-specific T-cell immunity in gut mucosal tissues.
Acknowledgments

Division of Immunology
Hugh Welles
Alexandre Harari
Olivia Hall
Ricardo Martin
Pierre-Alexandre Bart
Giuseppe Pantaleo

External collaboration
Eric Kremer
Jim Tartaglia
Ralf Wagner
Mariano Esteban
Yves Levy

Division of Gastroenterology
Michel Maillard
Gian Dorta

Funding
EuroVacc
Swiss National Science Foundation
Adenovirus-specific but not Smallpox-specific CD4 T cells are detected in gut-mucosal tissues

Flow cytometric profiles of Adenovirus naturally infected/Smallpox vaccinated Subject #025

Cumulative data

Adenovirus

Ad-specific CD4 T-cell proliferation

P = 0.001

Perreau et al, JVI 2011