Hacking HIV: Creating and Assessing a novel CTL-based HIV-1 Vaccine

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Immunity Project
Waag Society, Amsterdam, NL
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Immunity Project

Immunity Project is a non-profit initiative dedicated to developing an Open-Access HIV vaccine.

We are a non-profit initiative of Flow Pharma, Inc. (Business hack)
EPIDEMIOLOGY

Global summary of the AIDS epidemic  |  2013

<table>
<thead>
<tr>
<th>Number of people living with HIV in 2013</th>
<th>Total 35.0 million [33.1 million – 37.2 million]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>31.8 million [30.1 million – 33.7 million]</td>
</tr>
<tr>
<td>Women</td>
<td>16.0 million [15.2 million – 16.9 million]</td>
</tr>
<tr>
<td>Children (&lt;15 years)</td>
<td>3.2 million [2.9 million – 3.5 million]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>People newly infected with HIV in 2013</th>
<th>Total 2.1 million [1.9 million – 2.4 million]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>1.9 million [1.7 million – 2.1 million]</td>
</tr>
<tr>
<td>Children (&lt;15 years)</td>
<td>240 000 [210 000 – 280 000]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AIDS deaths in 2013</th>
<th>Total 1.5 million [1.4 million – 1.7 million]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>1.3 million [1.2 million – 1.5 million]</td>
</tr>
<tr>
<td>Children (&lt;15 years)</td>
<td>190 000 [170 000 – 220 000]</td>
</tr>
</tbody>
</table>

*Data from WHO, UNAIDS and UNICEF

More than 1.1 million people in the United States are living with HIV infection, and almost 1 in 6 (15.8%) are unaware of their infection.
### Immune Response to HIV

- **Humoral** - antibody responses to virus are difficult due to high mutation frequency (old vaccines)

- **Cellular** - the infected body makes CD8+ CTL responses to viral infection, but these cells are down regulated during infection.

- Unless you’re a controller!
CONTROLLER

• **Controllers** have the natural ability to prevent advancement of HIV into AIDS.

• This is accomplished by the production of T cells with the ability to kill HIV infected cells (Killer T Cells).

• All people with HIV produce killer T cells, but in **non-controllers**, these cells are deleted by HIV-programmed cells.

• Different from **slow progressors**.

• We want to turn everyone into controllers!
### The Vaccine

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA</td>
<td>98.5%</td>
</tr>
<tr>
<td>CpG</td>
<td>0.25%</td>
</tr>
<tr>
<td>Peptide</td>
<td>0.05% (100ng/mL)</td>
</tr>
<tr>
<td>Mannose</td>
<td>1.25%</td>
</tr>
</tbody>
</table>

**Poly(lactic-co-glycolic acid)**

![Diagram of vaccine components](image)
HACKING CTL PEPTIDES

• Identification of immunogenic proteins

• Identification of peptides that possibly bind to Class I molecules (based on HLA restriction)

• Creating databases of peptide binding (syfpeithi.de)

• Designing peptides for inclusion in our vaccine
A2, B57 AND B44

- Based on these databases, we chose two peptides specific for class I HLA: SL09 (SLYNTVATL) and KF11 (KAFSPEVIPMF)

- We performed a SYFPEITHI analysis to determine the binding probability of HIV-1-derived epitopes to class I molecules used in our studies.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>SL09 score</th>
<th>KF11 score</th>
<th>Positive Control Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>31</td>
<td>9</td>
<td>31</td>
</tr>
<tr>
<td>B44</td>
<td>14</td>
<td>20</td>
<td>17 (228-239, vimentin)</td>
</tr>
</tbody>
</table>
IN VITRO ASSAY (PART I)

DAY

0 5 6 7 8

CD14+ MONOCYTE

IL-4+GM-CSF

CpG

PLGA

ERLA

mature DC

VACCINATE

dendritic cell

endosome

vaccine degradation & endocytosis

antigen processing

CTLA-4

antigen signals

MHC I

T-cell

transport of assembled MHC I

PRESENTATION TO T-CELL

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IN VITRO ASSAY (PART II)

DAY

24

CD4+ T-cells from blood

infect

HIV

p24

25

HIV EPITOPE

programmed CD8+ T-cells

IFNγ

CD4+ HIV+ T-cells

apoptosis
DENDRITIC CELL UPTAKE
**Dendritic Cell Maturation**

- Flow Cytometry

<table>
<thead>
<tr>
<th>CD11c+CD86+</th>
<th>CC</th>
<th>DY</th>
<th>EE</th>
<th>FW</th>
<th>JeB</th>
<th>KT</th>
<th>PG</th>
<th>PL</th>
<th>TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>71.3</td>
<td>83.2</td>
<td>69.4</td>
<td>66.9</td>
<td>56.5</td>
<td>30.0</td>
<td>72.3</td>
<td>76.9</td>
<td>56.4</td>
</tr>
</tbody>
</table>

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Activation

Figure a) shows the percentage of CD4+CD25+ cells in SL09 MPS, KF11 MPS, and DC+T conditions. The data indicates no statistically significant difference (NS).

Figure b) displays the percentage of CD8+CD25+ cells. There is a statistically significant difference (***p=0.0005) between SL09 MPS and KF11 MPS, with a trend towards significance between KF11 MPS and DC+T (***p=0.0001) and SL09 MPS and DC+T (NS).

Figure c) presents the percentage of CD4+CD25+ cells. Similar to Figure a), there is no statistically significant difference (NS).

Figure d) illustrates the percentage of CD8+CD25+ cells. Again, there is no statistically significant difference (NS), but a trend towards significance is observed (p<0.05) between SL09 MPS and DC+T conditions.
TCR UPREGULATION

• On day 15, programmed CD8+ T cells were assessed for their expression of TCR that recognize SL09 in the context of HLA A*02 using A2:SL09 Pentamer (ProImmune).
IN VITRO ASSAY (PART II)

DAY

24

CD4+ T-cells from blood

\( \text{infect} \) HIV

\( \rho 24 \)

25

HIV EPITOPE

programmed CD8+ T-cells

IFN\( \gamma \)

apoptosis

CD4+ HIV+ T-cells
CTL-MEDIATED KILLING

- HIV-infected autologous CD4+ T cells were co-cultured with CD8+ T cells 5:1 (E:T)
- CD4+ T cells were assessed for apoptosis and death.
- CD8+ T cells were assessed for their ability to produce IFNγ in the presence of targets.
INTERFERON GAMMA

- CD8+ Effector T cells were co-cultured with autologous HIV+ CD4+ T cell targets and assessed for their production of Interferon gamma (IFNg) on day 30.
P24 SUPPRESSION

- p24 ELISA was performed to assess the amount of HIV in the supernatant of CTL assay co-cultures.
- A decrease in p24 in tissue culture supernatant means that the T cells are effective at inhibiting HIV.
Future Directions

• We are designing pre-clinical experiments in Monkeys.

• We are working on clinical trials…

• We need a High Throughput Assay (that doesn’t take 30 days to perform).
DANK JE WEL!

- Dank je wel Pieter van Boheemen en de Waag Society!
- Co-Founders:
  - Dr. Reid Rubsamen
  - Naveen Jain
- Scientists
  - Charlie Herst
  - Vikram Paranjpe (former Intern)
- Interns:
  - Rohun Patel
  - Hannah Hoban
- COMMUNITY!
Today’s Experiment

• Ouchterlony (Double Diffusion)

  • A test for reactivity of an antigen (protein) and antibody.

  • Precipitation reaction occurs and determines whether or not the antibody recognizes antigen.

  • This assay can be used in the field to determine whether or not a patient has come into contact with a virus containing a known antigen.
Precipitation

Figure 1

Supernatants
Excess Ab
Excess Ag

Antibody Precipitated

Ab-Excess Zone
+ + + + +

Equivalence Zone
0 0 0 0

Ag-Excess Zone
+ + + +

Antigen added

Figure 2

Antibody-Excess Zone
Equivalence Zone
Antigen-Excess Zone

Antibody
Antigen

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Precipitation in two dimensions

- Add antigen and antibody to wells and assess precipitation...
Today’s Experiment (Cont.)

• The Ouchterlony procedure allows us to measure different reactivities.
PROCEDURE

• Pour plates (5mL of molten 1% agarose in Borate Buffer, pH 7.4)

• Allow to cool (5-10min)

• Punch holes in each plate (x3) and remove plug.

• Add antigen and antibody to each plate.

• Incubate overnight at 37C, in humid chamber.
**Plate Setup**

**Tiny Holes**
- 10uL of antibody
- 10ul of antigen

**Large Holes**
- 30uL antibody
- 30uL antigen

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**Plate 1**
- Center well: antiserum to the fluid containing antibodies (Tube A)
- Left upper well: Whole serum (Tube B)
- Right upper well: Whole serum (Tube B)
- Left lower well: Whole serum (Tube B)
- Right lower well: Whole serum (Tube B)

**Plate 2**
- Center well: antiserum to the fluid containing antibodies (Tube A)
- Left upper well: Whole serum (Tube B)
- Right upper well: albumin (Tube C)
- Left lower well: albumin (Tube C)
- Right lower well: Whole serum (Tube B)

**Plate 3**
- Center well: antiserum to the fluid containing antibodies (Tube A)
- Left upper well: IgG (Tube D)
- Right upper well: albumin (Tube C)
- Left lower well: albumin (Tube C)
- Right lower well: IgG (Tube D)