Therapeutic Immunization with Autologous DC Pulsed with Autologous Inactivated HIV-1 Infected Apoptotic Cells

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HIV and DC Vaccines

- During seroconversion, there is an equilibrium between viral replication and immune control.
- HIV immune response fail to control virus once viral escape mechanisms prevail.
- HAART results in enhancement of CD4 T cell reactivity but not to HIV\(^1\)
- Enhancing host immune response may be a valid strategy to overcome lack of viral control in chronic HIV infection.
- Trials of treatment interruption (\textit{auto-vaccination}\(^2\)) fail to enhance viral control\(^3\)
- Strategy of "therapeutic vaccination" to enhance strength and extent of HIV-1 cellular immune response could be effective for viral control.

\(^1\)Rinaldo JID 179:329; \(^2\)Ortiz J Clin Invest 104:R13; \(^3\)Carcelain J Virol 75:234
DC loaded with inactivated autologous HIV

- Lu et al. (Nat Med 12/04)
  - 18 untreated HIV infected patients vaccinated with autologous DCs pulsed with AT-2 inactivated, autologous HIV

- Major Results:
  - HIV RNA ↓ 80% (median) at day 112
  - ≥ 90% suppression in 8/18 at one year
  - Peripheral CD4+ cell count increased significantly from days 28-112 but thereafter returned to baseline
  - HIV-specific IL-2 and \( \gamma \)-IFN-expressing CD4+ increased significantly, peaking at day 112, this increase correlated with viral load decrease.
Autologous Inactivated HIV-DC vaccine

- Garcia et al (JID 5/05)
  - 12 HIV+, ART treated received 5 doses of autologous, heat inactivated HIV pulsed DC at 6 week intervals.

- Results:
  - $\geq 0.5 \log$ decrease in viral load in 4/12 subjects
  - Significant increase in mean viral load doubling time
  - Increase in CTL (CD8+, granzyme B+) which correlated with increase in viral load doubling time
Phase I/II Evaluation of Therapeutic Immunization with Autologous Dendritic Cells Pulsed with Autologous, Inactivated HIV-1 Infected, Apoptotic Cells

- **Hypothesis:** Therapeutic vaccination with autologous DC pulsed with autologous HIV-infected apoptotic cells will be safe and result in a decrease in the HIV-1 RNA set-point when given to HIV-infected adults
Objectives

- **Primary objective:**
  - To define the safety and tolerability of the ApB Dendritic Cell vaccine in HIV-1 infected adults.

- **Secondary objectives:**
  - To explore the effect of ApB DC vaccination on the viral load set-point 12 weeks after analytic treatment interruption as compared with the pretreatment baseline.
  - To determine if there are immunologic markers that correlate with vaccine response as measured by a decrease of at least $0.5 \log_{10}$ copies/mL in HIV-1 RNA from baseline to week 12 post-ATI.
Study Design

- Phase I/II, single arm, single site, N=16 evaluable subjects
- Three phases/steps:
  - **STEP 1**: Pre-vaccination Phase
    - Viral isolation from autologous blood
    - Initiation of ART until plasma HIV-1 RNA <50 cp/mL.
  - **STEP 2**: Immunization Phase
    - Leukapheresis for autologous DC and PBMC for vaccine production
    - Three doses of ApB DC vaccine at 2 week intervals (~10 million cells /dose SC).
  - **STEP 3**: ATI and Follow Up Phase
    - Stop ART 6 weeks after 3rd vaccine dose
    - 4th vaccine dose 2 weeks after start of ATI
    - Follow up for total of 48 weeks post-leukapheresis. Re-initiate ART based on clinical criteria or at discretion of investigator or primary care physician.
Major Inclusion/Exclusion Criteria

- **Inclusion criteria:**
  - HIV-infected adults
  - ART naïve
  - CD4 ≥ 350 cells/mm³
  - Plasma HIV RNA 5,000-100,000 copies/mL
  - Acceptable baseline laboratory tests; no chronic HBV or HCV
  - Willingness to interrupt ART for 12 weeks

- **Exclusion criteria**
  - Prior or current AIDS defining condition
  - Immunosuppressive/immunomodulatory treatment
  - Recent investigational therapy/treatment of any type
  - Pregnancy or breastfeeding
  - Lack of adequate venous access
Statistical Considerations

- **Primary Endpoint:** safety of autologous HIV-1 ApB DC vaccine as defined by:
  - Two consecutive HIV RNA ≥400 copies/mL following any vaccination (prior to ATI), or
  - Any grade 3 or 4 clinical or laboratory abnormality

- **Secondary Endpoints:**
  - Mean of log_{10} HIV RNA at end of ATI (average of week 11 and 12 post-ATI) minus the mean of log_{10} HIV RNA at the 2 visits preceding ART initiation
  - Immunologic response in ELISPOT defined as 2-fold increase in reactive cells over baseline

- **Safety endpoint:** with 16 evaluable subjects, the probability of detecting at least 2 subjects with toxicity is 67% if the true rate is 10% and 18% if the true rate is 5%

- **Virologic endpoint:** the study has >80% power to detect 0.6 log_{10} difference in means before and after vaccination (estimated sd 0.5, alpha 0.05) using 2-sided Wilcoxon signed rank test
Vaccine Preparation

Virus Isolation

PBMC → Autologous Virus Isolation → Viral supernatant produced
CD8 depleted

PBMC → Autologous Virus Isolation → Viral supernatant produced

CD4 superinfected and UV inactivated
BANKED
Goal: 25 ng/ml
50-100 ml

CD8 depleted
Leukapheresis
Lymphocytes Harvested

CD4 superinfected and UV inactivated
CD8 depleted
Leukapheresis
Lymphocytes Harvested

Monocytes Harvested
Cultured with IL4 and GM-CSF

Apopotic bodies
Co-culture & Maturation
Immature DCs

ApB DC Vaccine
Pre-clinical vaccine development

- 4 patients, HIV+, not on ART
- Blood collected weekly for viral isolation
- Full-scale vaccine production (n=2)
Isolation of HIV-1 from PBMC

p24 levels in supernatants of CD4+ cell co-cultures (virus isolation)

<table>
<thead>
<tr>
<th>“Feeder CD4+cells”</th>
<th>HIV-1+ Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#1</td>
</tr>
<tr>
<td>Allo (normal donor)</td>
<td>38</td>
</tr>
<tr>
<td>Auto</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Auto + CD3/CD28 beads</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

Data are in ng/ml as measured in ELISA assays. The supernatants were harvested on day 29.
Viral Isolation

Isolation of virus from autologous PBMC

<table>
<thead>
<tr>
<th>Subject</th>
<th>p24 (ng/ml)</th>
<th>TCID_{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>337</td>
<td>31,623</td>
</tr>
<tr>
<td>#2</td>
<td>227</td>
<td>215</td>
</tr>
<tr>
<td>#3</td>
<td>39</td>
<td>14,678</td>
</tr>
</tbody>
</table>

Co-culture supernatants were repeatedly tested on various days (d 4-37). The highest titers were obtained on days 10-19. The p24 levels were measured by ELISA. TCID-50 was measured in a colorimetric assay with an indicator cell line TZM-bl.
Superinfection of normal CD4+T cells

<table>
<thead>
<tr>
<th>Culture day</th>
<th>p24+cells</th>
<th>p24 in cells</th>
<th>p24 in sup</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>ng/10^5 cells</td>
<td>ng/mL</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0.06</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>0.12</td>
<td>10.8</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>0.80</td>
<td>19.7</td>
</tr>
<tr>
<td>Pos cont</td>
<td>51</td>
<td>3.60</td>
<td>16.6</td>
</tr>
<tr>
<td>Neg cont</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

% p24+ cells was determined by flow cytometry; cell associated p24 in lysed CD4+cells and in CD4+ cell supernatants by ELISA. Viral sup of Pt. #1 was used for infection of CD4+ T cells (98% purity) separated by positive selection on immunobeads from PBMC of a normal donor.
Subject Q -05-131 p24 PE intracellular staining in CD4+ cells

Negative and Positive Controls for p24 PE Staining

P24 Expression on CD4 cells after Superinfection
Superinfection of autologous CD4+ T cells

<table>
<thead>
<tr>
<th>Culture day</th>
<th>p24+cells %</th>
<th>p24 in cells ng/10^5 cells</th>
<th>p24 in sup ng/mL</th>
<th>TCID_{50} per mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>4</em></td>
<td>25</td>
<td>0.11</td>
<td>0.10</td>
<td>3,162</td>
</tr>
<tr>
<td><em>5</em></td>
<td>18</td>
<td>0.08</td>
<td>0.02</td>
<td>10,000</td>
</tr>
<tr>
<td>Pos cont</td>
<td>52</td>
<td>0.03</td>
<td>0.09</td>
<td>3,162</td>
</tr>
<tr>
<td>Neg cont</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

% p24+ cells was determined by flow cytometry; cell associated p24 in lysed CD4+ cells and in CD4+ cell supernatants by ELISA. Viral sup of Pt. #3 was used for infection of CD4+ T cells (95% purity) separated by positive selection on immunobeads from PBMC of Pt #3.
Psoralen/UV inactivation of HIV-1 (Cell associated HIV IIIb)

Irradiation time (min) | TCID-50
--- | ---
0 | 1,000,000
10 | 10,0000
12 | 468
14 | 234
15 | 32
16 | 23
17 | 9
18 | 6

% inactivation

irradiation time (min)

98.40 98.60 98.80 99.00 99.20 99.40 99.60 99.80 100.00


Slide 17
Psoralen/UV inactivation of autologous HIV-1

<table>
<thead>
<tr>
<th></th>
<th>TCID&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated cells</td>
<td>14,678</td>
</tr>
<tr>
<td>Treated cells</td>
<td>3</td>
</tr>
<tr>
<td>Positive con (not treated)</td>
<td>3,162</td>
</tr>
</tbody>
</table>

CD4+ T cells of Pt.# 3 were superinfected with the autologous virus (HIV-1+ supernatant) and on day 5, they were treated with psoralen (20ug/mL) and UVB (3mW/m<sup>2</sup>) for 30 min.
Effects of UVB/psoralen treatment on CD4+ T-cell viability: 30 min of treatment is sufficient to induce apoptosis (Annexin+/7-AAD+)
# Leukapheresis Product

<table>
<thead>
<tr>
<th></th>
<th>Cell counts</th>
<th>Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial WBC count</td>
<td>3.9 x 10^{10}</td>
<td></td>
</tr>
<tr>
<td>ELUTRA™ fraction 2</td>
<td>1.2 x 10^{10}</td>
<td>99</td>
</tr>
<tr>
<td>(CD45^+CD14^-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELUTRA™ fraction 5</td>
<td>5.6 x 10^{9}</td>
<td>77</td>
</tr>
<tr>
<td>(CD45^+CD14^+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-CliniMACS</td>
<td>9.8 x 10^{8}</td>
<td>95</td>
</tr>
<tr>
<td>CD4^+CD8^- T cells</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Characteristics of DC generated from monocytes (HIV-1+ subject)\textsuperscript{b}

Phenotype:

<table>
<thead>
<tr>
<th>Marker</th>
<th>iDC</th>
<th>αDC-1 alone % positive\textsuperscript{a}</th>
<th>αDC-1 + ApB</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DR</td>
<td>86</td>
<td>94</td>
<td>97</td>
</tr>
<tr>
<td>CD80</td>
<td>50</td>
<td>94</td>
<td>93</td>
</tr>
<tr>
<td>CD83</td>
<td>4</td>
<td>60</td>
<td>49</td>
</tr>
<tr>
<td>CD86</td>
<td>19</td>
<td>86</td>
<td>91</td>
</tr>
<tr>
<td>CD11c</td>
<td>97</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>CD40</td>
<td>86</td>
<td>96</td>
<td>97</td>
</tr>
<tr>
<td>CCR7</td>
<td>10</td>
<td>45</td>
<td>42</td>
</tr>
</tbody>
</table>

Recovery: 57%

Viability: 87% after co-incubation with apoptotic CD4+CD8- T cells

Sterility (14 d); mycoplasma, endotoxin: negative

IL-12 production: 473 pg/mL

TCID-50/mL: untreated CD4+CD8- T cells = 1,467,799

mDC + ApB = 3

Stability: 4 h at room temperature
Screen and Enroll
CD4 350; ART naïve

Autologous Viral Isolation
p24 = 25 ng/ml
50-100 ml

4-8 weeks
Initiate HAART
HIV RNA < 50 cp/ml

Vaccine Preparation
Superinfect harvested CD4 cells with viral supernatant & inactivate. Pulse iDCs with ApB

Grow DCs
GM-CSF & IL4

Leukapheresis
Harvest monocytes

2 weeks
Vaccination
3 vaccines 2 week intervals

4-8 weeks
Treatment Interruption

8-12 weeks
Observation and Follow-up
Follow-up for 36 wks from start of ATI. Restart ART as indicated. Follow viral load/CD4/immune markers.
Study Status

- Total no. of patients screened = 7
- Total enrolled = 3
- Screened out = 3
- Pending results = 1
- Successful viral isolation = 3/3
- Subject 1 now on 3rd month of ART; HIV RNA <50 copies; Leukopheresis mid-June
- Subjects 2 & 3 on ART
The supernatants from culture day 7 onwards were combined and tested for p24 by ELISA and the TCID-50 was determined.

- Supernatant volume = 89 ml
- p24 ELISA = 173.9 ng/ml
- TCID-50 = 31,623

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 13</th>
<th>Day 21</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>p24 ng/ml</strong></td>
<td>0.2</td>
<td>87.7</td>
<td>450.3</td>
<td>590.0</td>
<td>274.4</td>
</tr>
</tbody>
</table>
Acknowledgements

- Funding from NIH IPCP (NIAID) and PACT (NHLBI)
- Theresa Whiteside
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- Beej Macatangay
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