CD4+ T-cell-inducing HIV vaccines may have an impact on viral control. Clues from cytokines produced by CD4+ T-cells from HIV-infected patients and vaccinated seronegative volunteers.

Eva Van Braeckel 1, Frédéric Clement 1, Linos Vandekerckhove 2, Dirk Vogelaers 2, Geert Leroux-Roels 1

1 Center for Vaccinology and 2 AIDS Reference Centre, Ghent University and Hospital
After 30 years of research, no effective HIV vaccine is yet available. An effective HIV vaccine should either:
- Prevent infection (prophylactic)
- Generate an immune response that helps to control viral load in HIV-infected subjects (disease-modifying or therapeutic)

Obstacles: viral hypervariability, immune evasion strategies

Paradox: HIV elicits a broad immune response, but natural immunity is not protective

Many questions remain unsolved:
- Which epitopes to include in a vaccine, from which viral subtypes?
- Should a vaccine induce neutralizing antibodies or T-cells?
- What are the correlates of protection?
Subpopulations of HIV-infected patients have better disease outcomes: long-term nonprogressors (LTNPs), viral controllers (VCs). Are CD4+ T-cell responses correlating with better disease outcome? Does a CD4+ T-cell inducing HIV vaccine elicit the desired immune response? A comparative study of HIV-specific CD4+ T-cell responses to different HIV-1 antigens induced by (1) Natural infection in patients with different disease courses (2) HIV-1 vaccination in healthy volunteers.
<table>
<thead>
<tr>
<th></th>
<th>Long-term nonprogressors (LTNPs)</th>
<th>Recently infected (RI)</th>
<th>Typical progressors (TPs)</th>
<th>Viral controllers (VCs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV diagnosis (years)</td>
<td>14.6 [9.7]</td>
<td>1.5 [0.7]</td>
<td>1.8 [1.1]</td>
<td>4.1 [4.4]</td>
</tr>
<tr>
<td>Viral load (copies/mL)</td>
<td>1123 [2087]</td>
<td>29462 [54454]</td>
<td>35034 [47872]</td>
<td>&lt;50 [113]</td>
</tr>
</tbody>
</table>
METHODS

- HIV-1-vaccinated, uninfected volunteers (n = 50):
  - Healthy volunteers aged 18-40 years
  - Vaccinated on day 0 and day 30
  - Blood samples from day 60

- Study vaccine F4/AS01 (GSK Bio):
  - Adjuvant system AS01: MPL + QS21

Van Braeckel E et al., Clin Infect Dis, in press
METHODS

- Intracellular cytokine staining (ICS):
  - Subject’s PBMCs are isolated and cryopreserved
  - Thawed PBMCs are restimulated overnight with HIV-1 antigens (overlapping peptide pools): p17, p24, RT, Nef, (Tat, gp120)
  - Extracellular staining for membrane surface markers: CD4, CD8, (CD45RA, CD27)
  - Intracellular staining for activation markers: IL-2, IFN-γ, TNF-α, CD40L
- 8-color flowcytometric analysis (LSR II)

Expression of CD40L, IL-2, IFN-γ and TNF-α by CD4+ T-cells in response to HIV-1 p17, p24, RT and Nef
RESULTS

- All HIV-infected patients:
  - Lower viral load was correlated with specific CD4+ functionalities:
    - p24-specific CD4+ T-cells co-expressing 4 markers (CD40L, IL-2, IFN-γ and TNF-α) \( (p < 0.001) \)
    - p17, p24 and RT-specific CD4+ T-cells co-producing 3 cytokines \( (p \leq 0.005) \)
    - p24 and RT-specific CD4+ T-cells co-producing IL-2 and TNF-α \( (p \leq 0.005) \)

- No significant correlations between higher CD4+ count and cytokine expression

Statistics: Spearman correlation
Among HIV-1-infected patient groups:
- Viral controllers had significantly more CD4+ T-cells co-producing IL-2, IFN-γ and TNF-α than other patient categories.
- Differences between patient groups became smaller as polyfunctionality decreased (production of single or double cytokines), except for combined production of IL-2 and TNF-α after p24, RT and Nef stimulation.

RESULTS

HIV-1-infected versus vaccinated volunteers: Polyfunctional cytokine production profiles correlating with viral control in natural HIV-1 infection, were also induced by the F4/AS01 vaccine in uninfected volunteers. Almost all CD4+ T-cell responses induced by the F4/AS01 vaccine in healthy volunteers were higher than those observed in naturally infected patients.

Statistics: Kruskal Wallis and Mann-Whitney U test
CONCLUSIONS

- HIV-1 viral controllers have more HIV-specific CD4+ T-cells exhibiting a polyfunctional cytokine production profile (IL-2, IFN-γ and TNF-α) than other patient groups.

- Vaccination of healthy HIV-uninfected volunteers with the adjuvanted polyprotein vaccine F4/AS01 induced polyfunctional CD4+ T-cell responses to p17, p24, RT and Nef comparable to those observed in HIV-infected patients who spontaneously control the virus.

- CD4+ T-cell inducing vaccines may have an impact on viral control: in a prophylactic, disease-modifying or therapeutic setting, in a stand-alone approach or in combination with other vaccine strategies.
ACKNOWLEDGEMENTS

All study volunteers
Center for Vaccinology (CEVAC), Ghent University
Prof. dr. Geert Leroux-Roels
Frédéric Clement
Lab technicians
Study nurses and physicians
HIV vaccine R&D team at GlaxoSmithKline Biologicals
Gerald Voss
Marguerite Koutsoukos
Patricia Bourguignon
AIDS Reference Center, Ghent University Hospital
Prof. dr. Dirk Vogelaers
Dr. Linos Vandekerckhove
Tessa James
Prof. Dr. Stijn Blot
Dr. Filip Van Wanzeele
Dr. Bea Vander Gucht
Dr. Jolanda Pelgrom
AIDS Reference Lab, Ghent University
Prof. dr. Chris Verhofstede