IMMUNE CORRELATES OF PROTECTION IN HIV DISEASE

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<table>
<thead>
<tr>
<th>Virus</th>
<th>Type of Vaccine</th>
<th>Vaccine-Induced protective immunity</th>
<th>Mechanisms of immune control during virus infection</th>
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<tbody>
<tr>
<td>Smallpox</td>
<td>Live</td>
<td>Antibodies ; CTL</td>
<td>CTL</td>
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<tr>
<td>Rabies</td>
<td>Killed virus</td>
<td>Antibodies</td>
<td>Antibodies ; CD4 ; CTL</td>
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<tr>
<td>Polio</td>
<td>Live ; Killed virus</td>
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<td>Antibodies ; CTL</td>
<td>Antibodies ; CD4 ; CTL</td>
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<td>Live</td>
<td>Antibodies</td>
<td>Antibodies</td>
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<td>Live</td>
<td>Antibodies</td>
<td>Antibodies</td>
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<tr>
<td>Varicella Zoster</td>
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<td>Antibodies ; CTL</td>
<td>Antibodies ; CTL</td>
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<tr>
<td>Influenza</td>
<td>Protein</td>
<td>Antibodies</td>
<td>Antibodies ; CD4 ; CTL</td>
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<td>Hepatitis A</td>
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<td>Hepatitis B</td>
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<td>Human papillomavirus</td>
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<td>CD4 ; CTL</td>
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<td>Hepatitis C</td>
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<td>CD4 ; CTL</td>
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<td>Cytomegalovirus</td>
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<td>CD4 ; CTL</td>
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<tr>
<td>Epstein-Barr Virus</td>
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<tr>
<td>Herpes simplex virus types 1 and 2</td>
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<td>CD4 ; CTL</td>
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<td>HIV-1/2</td>
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<td>CD4 ; CTL</td>
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<tr>
<td>Human Herpes Virus 6</td>
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<td>Antibodies ; T cells</td>
</tr>
</tbody>
</table>
Immunological Measures Associated with Control of HIV Replication - I

- **Functional profile**
  - Cytotoxic profile
    - Perforin/GrmB expression (Hersperger, PLoS Path, 2010; Migueles et al., Immunity 2008)
    - Inhibition of virus replication (Freeel et al., J Virol, 2010; Yang, JID, 2012)

- **TCR avidity** (Almeida et al., J Exp Med, 2008)

- **TCR clonotypes and protective role of HLA class I molecules** (Chen et al. Nat Immunol, 2012)

Proliferative Capacity of Virus-Specific CD8+ T-Cells Is Inversely Correlated with Plasma HIV Viral Load

Skewed representation of functionally distinct populations of virus-specific CD4 T cells in HIV-1–infected subjects with progressive disease: changes after antiretroviral therapy

Alexandre Harari, Stéphanie Petitpierre, Florence Valлеllian, and Giuseppe Pantaleo
Virus-Specific CD8 T-Cell Proliferation and Cytokine Profile

Zimmerli S C et al. PNAS 2005;102:7239-7244

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The Magnitude and Proportion of HIV-Specific CD8+ T-Cell Responses Positive for All 5 Functions Is Inversely Correlated with Viral Load

HIV-Specific CD8+ T-Cells in EC Demonstrate Enhanced Ability To Express Perforin Compared to CP

http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1000917
Inverse Relationship Between Viral Load and HIV-Specific Perforin Expression Is Not Rescued by HAART

http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1000917
Lytic Granule Loading of CD8+ T Cells Is Required for HIV-Infected Cell Elimination Associated with Immune Control

TCR CLONOTYPES MODULATE THE PROTECTIVE EFFECT OF HLA CLASS I MOLECULES IN HIV-1 INFECTION

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Selena Viganó 11.02.2012
PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression

Immunological Measures Associated with Control of HIV Replication - II

- **Epitopes targeted**
  - Conserved among strains (Turnbull, J Immunol, 2006)
  - Mutations having a fitness cost (Martinez-Picardo, J Virol, 2006)

- **Immune Activation (lack of immune activation)** (Many, Many Investigators)

- **Genetic Factors**
  - Long-term nonprogressors (LTNP) and ‘Elite controllers’ (EC): spontaneous control of HIV-1 replication and preservation of high CD4 T-cell counts in the absence of ART
  - HLA-B*57, HLA-B*27 and HLA-B*5801 genotypes are associated with viral control (Carrington M, Annu Rev Immunol 2003)
  - Genome-wide association studies indicate that:
    - The HLA-viral peptide interaction is the main determinant of HIV-1 control (Pereyra F, Science 2010)
    - Specific amino acids in the HLA-B peptide-binding groove are important in HIV-1 control (The international HIV controllers study, Science 2010)
Protective HLA Haplotypes and Correlates of Virus Control

- HLA-B*57, HLA-B*27 and HLA-B*5801 genotypes are associated to viral control

- Genome-wide association studies indicate that
  
  • The HLA-viral peptide interaction is the main determinant of HIV-1 control (Pereyra F, Science 2010)

  • Specific amino acids in the HLA-B peptide-binding groove are important in HIV-1 control (The international HIV controllers study, Science, 2010)

- HIV-1 control by HLA-B*27- and HLA-B*57-restricted responses is associated to the induction of escape mutations with viral fitness cost and to the generation of strong T-cell responses against emerging variants (Bailey JR, J Exp Med, 2006; Miura T, J Virol 2009)
Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA

CD8⁺ T-cell responses to different HIV proteins have discordant associations with viral load

Immunological Measures Associated with Control of HIV Replication

Based on the immunological measures associated with control of HIV replication in HIV infection, effective HIV-specific T-cell responses should comprise:

a) CD4 and CD8 T-cells with high proliferation capacity
b) polyfunctional T-cell responses
c) predominantly CD8 T-cells specific to Gag epitopes
d) CD8 T-cells with high perforin expression
f) protective TCR clonotypes
Why Does HIV Persist In Infected Individuals?

Two Main Hypotheses

Latent HIV Reservoir
- Size: $10^5$-$10^7$ cells
- Half life memory CD4 T cells: 43 months
- Estimated time for eradication: ~70 years under full virus suppression by ART
- Not susceptible to ART
- Not susceptible to the immune system

Residual HIV Replication
- Covert cellular reservoir
- Priveleged anatomic compartment
- Resistant to HIV cytopathic effect
- Poorly accessible to cytotoxic CD8 T cells
- Minimal virus spreading
- Replenishment of the latent cellular reservoir

TCM/TTM CD4 T cells

T_{CM}/T_{TM} CD4 T cells

CD4 T cells? Macrophages? DCs?
Follicular Helper T cells Serve as the Major CD4 T cell Compartment for HIV-1 Infection, Replication, and Production

Matthieu Perreau, Anne-Laure Savoye, Elisa De Crignis, Jean-Marc Corpataux, Rafael Cubas, Elias K. Haddad, Laurence De Leval, Cecilia Graziosi, and Giuseppe Pantaleo
Tfh Cells: Phenotype in HIV Infected Subjects

- 4 populations identified on the basis of CXCR5 and PD-1 expression
- Bcl-6 and ICOS are predominantly expressed in CXCR5^+PD-1^+ CD4 T-cells

AVIB#1024
46318 HIV RNA copies/mL

Gated on CD3^+CD4^+ cells
Tfh Cells Are Expanded in Lymph Nodes of Viremic HIV Infected Patients

- Significant expansion of CXCR5-PD-1+ CD4 T-cells and Tfh in viremic patients and significant reduction of both cell populations following suppression of virus replication by ART
Tfh Cells and CXCR5-PD-1+ CD4 T-Cells Are Enriched in HIV-Specific CD4 T-Cells

LNMCs → Intracellular staining → Aqua, CD3, CD4, CD8, CD45RA, CXCR5, PD1, IL-21, IL-2, TNF-α, IFN-γ

PTEs (Gag, Pol, Env)+α-CD28

Brefeldin A

CNA#2066 32250 HIV RNA copies/mL

Gated on CD3+CD4+CD45RA cells

Unstimulated

Gag #1

Gag #2

IL-2

TNF-α

Total HIV-specific responses (N=11)

\( P=0.0037 \)

\( P=0.0007 \)

\( P=0.04 \)

\( P=0.0040 \)

Percentage of HIV-specific memory CD4 T cells

PD-1

CXCR5
Tfh Cells and CXCR5-PD-1⁺ CD4 T-Cells Are Enriched in CD4 T-Cells Containing HIV DNA

DNA extraction → Taqman RT-PCR → HIV-1 gag gene

Gated on CD3+CD4+ cells

<table>
<thead>
<tr>
<th>PD-1</th>
<th>CXCR5</th>
<th>HIV DNA copies/10⁶ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>−</td>
<td>−</td>
<td>10⁴</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>10⁵</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>10⁶</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>10⁷</td>
</tr>
</tbody>
</table>

P-values:
- P=0.0006
- P=0.04
- P=0.003
- P=0.001
Tfh Cells and CXCR5-PD-1 CD4 T-Cells Are the Most Efficient in Supporting Production of HIV

High viremia
(>15000 HIV RNA copies/mL)

CNA#2132 57690 HIV RNA copies/mL

- CXCR5-PD-1
- CXCR5-PD-1
- CXCR5-PD-1

\* P<0.05

p24 detection

D2

D5

D0

Anti-CD3/CD28

Collection of SNs

High viremia
(>15000 HIV RNA copies/mL)
HIV Isolation from Patients with Low (<1000 HIV RNA copies/mL of plasma) in Different CD4 T-Cell Populations

- Tfh and CXCR5⁺PD-1⁺ but not CXCR5⁻PD-1⁻ and CXCR5⁺PD-1⁻ CD4 T-cells efficiently support virus isolation and production in patients with low viremia levels.
Tfh Efficiently Support HIV Production After *In Vitro* Inoculation
The Percentage of Tfh Cells Correlates with HIV Viremia Levels

- For CXCR5^PD-1^ CD4 T cells, the correlation is $R=-0.3900$, $N=23$, and $P=0.0658$.
- For CXCR5^PD-1^ CD4 T cells, the correlation is $R=0.1489$, $N=23$, and $P=0.4977$.
- For CXCR5^PD-1^ CD4 T cells, the correlation is $R=0.2543$, $N=23$, and $P=0.2417$.
- For Tfh cells, the correlation is $R=0.6035$, $N=23$, and $P=0.0023$. 
Events Occurring in the Germinal Centers in Viremic HIV-Infected Patients

- B-cell zone
  - SCS macrophage
  - BCR B-cell
  - primary follicle
  - antigen presentation
  - pre-GC TFH cells
  - TCR T-cell zone
  - DC
  - HIV-infected CD4-cells
  - pre-GC TFH cells
  - T_H cells

- Secondary follicle
  - GC B-cell proliferation
  - infected GC TFH cells
  - centroblasts proliferation
  - (immediate response)

- GC reaction
  - memory B-cells
  - centrocyte (HIV-specific)
  - GC B-cell
  - plasma cells (late response)
Events Occurring in the Germinal Centers in Viremic HIV-Infected Patients

- High levels of HIV replication and production within $T_{FH}$ cells. Correlation with viremia levels.

- Defective Tfh help to B-cells and decreased Ig production.
Events Occurring in the Germinal Centers in ART HIV Suppressed Patients – Non-HIV Ag Stimulation

B-cell zone

SCS macrophage

primary follicle

FDC

Non-HIV Ag stimulation

T-cell zone

BCR

B-cell

antigen presentation

pre-GC

T_{FH} cells

CD4 T cells proliferation

T_{H} cells

HIV

germinonal center

B-cell

B-cells proliferation

centroblasts proliferation

Secondary follicle

GC T_{FH} cells

plasma cells

(early response)
Transient bursts of HIV replication and production within $T_{FH}$ cells. Responsible for virus blips?
Combination Strategies to Purge the HIV Reservoir

Latent Reservoir

$T_{CM}/T_{TM}$ CD4 cells

Reactivation of replication

HDAC Inhibitors, SAHA, Prostratin, Bryostatin 1, 5-azathidine, anti-PD-1

HIV cytopathic effect

Killing by effector CD8 T cells

APOPTOSIS

Prostratin, Bryostatin 1, 5-azathidine, anti-PD-1

Therapeutic vaccine, anti-PD-1, Cytokines (IL-2, IL-7), Inhibitors of immune activation

Potentiation of immune response

APOPTOSIS

Covert Cellular Reservoir with Residual Replication

Potentiation of immune response

Selective targeting

Antibody coupled with toxins (Bi-specific CD4/PD-1)

APOPTOSIS
Acknowledgments

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