Immune Correlates of Protection to HIV

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Overview

- Immune correlates of protection vs. correlates of control (in virus-infected individuals)

- Correlates of protection in virus-infected subjects vs. correlates of protection in immunized subjects (efficacy trials)

- Issues raised from the RV144 and STEP trials
Immune Correlates of Protection vs. Immune Response Profiles of Virological Control (correlates of control)

**Immune correlates of protection:**
The effector components of the immune response which are actively and directly involved in the suppression of HIV replication

**Immune profiles of virological control:**
The immunological profiles of the immune response which reflect optimal virological control
Immune correlates of protection are mostly derived from studies performed in nonprogressive HIV-1 infection

**Rationale:** Immune correlates of protection are needed to identify attributes of vaccine-induced T-cell responses leading to substantial reduction of virus replication.
Immune correlates of protection

• Cytokines profile
• Cytotoxic capacity
• Proliferation capacity
• T-cell differentiation
• TCR avidity (Antigen sensitivity)
• Breadth or durability of the response
• ...

Pt#1010:

Prototypic example of a patient showing effective virus control
Evolution of Viral Load and CD4 T-cell Count Over Time in Patient #1010

- HIV exposure on 15.03.1999.
- Emergency room admission on 15.03.1999.
- Start of HAART and CsA 2 days later.
- Viral Load: <50 copies/ml (17.06.2009).

**Highlights**:
- Cyclosporine A: 26,500,000
- Stavudine + Lamivudine + Nelfinavir + saquinavir
- Stavudine + lamivudine + efavirenz
- Hypersensitive Amplicor Assay (LOD: 5 c/mL)
- No Treatment
Evolution of Viral Load shortly after treatment interruption in Patient #1010

(Experiments performed by Dr. Tae-Wook Chun, NIAID, NIH)
Patient #1010 – Viral Load

(Experiments performed by Dr. Tae-Wook Chun, NIAID, NIH)

Real time PCR for HIV-1 LTR

- 1.7 HIV DNA copies per 1 microgram genomic DNA or 11 HIV DNA copies per $10^6$ blood CD4 T-cells

- Infectious viral load of enriched CD4 T-cells from patient #1010 is estimated 0.0027 infectious unit per million CD4 T-cells; no virus has been isolated from 360 million stimulated blood CD4 T-cells (10 million cells per well x 36 wells)

- 4.5 HIV DNA copies per $10^6$ gut CD4 T-cells
Analysis of HIV-1-specific T-cell responses in Pt#1010
Functional Patterns of HIV-1-Specific T-Cell Responses and Control of Virus Replication and Disease


- Functional heterogeneity of CD4 T cell responses in different conditions of antigen exposure and persistence (Harari et al. J. Immunol., 2005)

- HIV-specific CD8 T-cell proliferation is coupled to perforin expression and is maintained in nonprogressors (Migueles et al. Nat. Immunol., 2002)

- HIV-1-specific IL-2/IFN-γ secreting CD8 T cells support CD4-independent proliferation of HIV-1-specific CD8 T cells (Zimmerli et al. PNAS, 2005, Emu et al., J Virol 05 and 07)

- HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells (Betts et al. Blood, 2006)

- ...
Cytokine Profile of Virus-Specific T-Cells
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 Valletian, and Giuseppe Pantaleo
HIV-1-specific IFN-γ/IL-2-secreting CD8 T cells support CD4-independent proliferation of HIV-1-specific CD8 T cells

Simone C. Zimmerli, Alexandre Harari, Cristina Cellera, Florence Valellean, Pierre-Alexandre Bart, and Giuseppe Pantaleo*
HIV-1-Specific CD8 T-Cell Responses During Primary Infection in Patient # 1010

Baseline (prior to ART)

Unstimulated Pool GAG

2 years after primary infection and 3 months after spontaneous treatment interruption

Unstimulated Pool GAG

3 years after primary infection and 15 months after spontaneous treatment interruption

Unstimulated Pool GAG

Gated on CD8 T cells

IL-2

IFN-γ
Functional Profile of HIV-1-Specific CD8 T-Cells Over Time in Patient #1010

Gated on CD8 T-cells

---|---|---|---
Unstimulated | 0 | 0 | 0 | 0
B*0702 GPSHKARVL gag | 0.02 | 2.15 | 1.24 | 0.01
B*0702 IPRRIRQGL env | 0 | 2.65 | 1.51 | 0.86
A*0301 HMYISKKAK vif | 0.01 | 0.09 | 0.08 | 0.07

IL-2 vs. IFN-γ
HIV-1-Specific CD8 T-Cell Responses in Patient #1010

IL-2

GPGHKARVL (B*0702-gag)

Neg

TNF-α

IPRRIRQGL (B*0702-env)

IFN-γ
Functional Profile of HIV-1-Specific CD8 T-Cell Responses in Pt. # 1010 as compared to LTNP
HIV-1-specific CD8 T-cell responses in Pt#1010 are more polyfunctional than the average responses observed in LTNP.
Functional Profile of HIV-1-Specific CD8 T-Cell Responses in Patients #1010, 1017 and 1023 recognizing the same epitopes (B*0702-gag and -env)
Within different patients, CD8 T-cell responses directed against the same epitopes do not necessarily have the same cytokine profile.
In an HIV-1 infected patient (#1010) with optimal virological control, the cytokine profile is:

highly polyfunctional
(>3/4 of CD8 T-cells secreting IL-2)
Cytotoxic Profile of Virus-Specific CD8 T-Cells
Lytic Granule Loading of CD8+ T Cells Is Required for HIV-Infected Cell Elimination Associated with Immune Control

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Distinct Profiles of Cytotoxic Granules in Memory CD8 T Cells Correlate with Function, Differentiation Stage, and Antigen Exposure

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Distribution of Perforin and Granzymes (A, B and K) in Virus-Specific CD8 T Cells

Harari A et al, J. Virol, 2009
Correlation between Perforin/Granzymes and Cytotoxicity

**Perforin**
- % of perforin expression on virus-specific CD8 T cells
- Percentage of specific lysis
- R = 0.97
- P < 0.001

**Granzyme A**
- % of Grz A expression on virus-specific CD8 T cells
- Percentage of specific lysis
- R = 0.36
- P > 0.05

**Granzyme B**
- % of Grz B expression on virus-specific CD8 T cells
- Percentage of specific lysis
- R = 0.66
- P < 0.05

**Granzyme K**
- % of Grz K expression on virus-specific CD8 T cells
- Percentage of specific lysis
- R = 0.59
- P < 0.05

Distribution of Perforin and Granzymes (A, B and K) in HIV-Specific CD8 T Cells in Patients 1010, 1017 and 1023

Gated on Tet+ CD8 T-cells

Pt# 1010
B*0702-GPGHKARVL

Pt# 1017
B*0702-GPGHKARVL

Pt# 1023
B*0702-GPGHKARVL
Distribution of Perforin and Granzymes (A, B and K) in Virus-Specific CD8 T Cells in Pt 1010 as compared to Flu, EBV and CMV
Distribution of Perforin and Granzymes (A, B and K) in HIV-Specific CD8 T Cells in Pt# 1010 directly ex vivo and after 7 days of in vitro expansion.
In an HIV-1 infected patient (#1010) with optimal virological control, the cytotoxic profile is:

lack of expression of cytotoxic granules such as perforin and GrmB critical for the cytotoxic activity of CD8 T-cells
TCR avidity of virus-specific T-cell responses
TCR Avidity of the Different CD8 T-Cell Response

- **B*0702 - GPGHKARVL (gag)**
- **B*0702 - IPRRIRQGL (env)**
- **A*0301 - HMYISKKAK (vif)**

**EC₅₀** values:
- **0.035**
- **0.002**
- **0.038**

Peptide concentration (µg/ml)

% of response

Peptide concentration (µg/ml) range from 1 to 1E-06.
TCR Avidity of the Different CD8 T-Cell Response
In an HIV-1 infected patient (#1010) with optimal virological control, TCR avidity is:

Low to intermediate
Proliferation capacity of virus-specific T-cell responses
HIV-1-specific CD4 and CD8 T-cell proliferation in Pt#1010
Differentiation of virus-specific CD8 T-cell responses
Differentiation Stage of HIV-1-Specific CD8 T-Cell Responses in Patients #1010, #1017 and #1023
Conclusions

The prototypic immunological profile of an HIV-1-infected patient (#1010) with optimal virological control in the absence of ART, i.e. very low DNA load in blood and gut CD4 T-cells (about 10 HIV DNA copies per $10^6$ cells, no isolation of infectious virus, viremia <5 HIV RNA copies), has the following characteristics:

- **Cytokine profile**: highly polyfunctional (>3/4 of CD8 T-cells secreting IL-2)

- **Cytotoxic profile**: lack of expression of cytotoxic granules such as perforin and GrmB critical for the cytotoxic activity of CD8 T-cells

- **Proliferation profile**: extensive CD4 and CD8 T-cells proliferation capacity

- **Differentiation stage**: >70% of CD8 T-cells CD45RA⁻, CD127⁺, CCR7⁺, CD27⁺ and CD28⁺ (Central memory differentiation stage):

- **TCR avidity**: Low to intermediate
Conclusion

A polyfunctional (cytokines + proliferation but not cytotoxic), Quiescent, Non Effector, Immunological Profile of the HIV-Specific T-Cell Response

Represents

the Best Immune Response Profile of Optimal Control of HIV Replication
Functional profiles represent optimal markers of virological controls.

Whether they represent the effector components of the immune response which are actively and directly involved in the suppression of HIV replication remains to be determined.
Issues raised from the RV144 and STEP efficacy trials

The STEP study assessed the efficacy of a T cell-based vaccine. It induced polyfunctional T-cell responses, the magnitude of responses was high and/but the majority of T-cell responses were directed against 1 epitope.

Based on the functional profile, vaccine-induced T-cell responses were consistent with those observed in nonprogressive HIV-1 infection. Unfortunately, no protection or reduction of viral load were observed.

In the RV144 trial, cellular immune responses were characterized in a minority of subjects. Preliminary analyses report different range of immunogenicity (18% ELISpot, 33% CD4 ICS, 90% LPA). It is too premature to make conclusions about the impact of these responses. However, a significant level of protection was observed.

Therefore, mechanisms other than cellular immunity were involved in protection.
Issues raised from the RV144 and STEP trials

The two efficacy trials suggest that the current immunological markers of controls of virus replication identified in patients with established HIV infection are not predictive of vaccine effectiveness.
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