Induction of protective innate immune responses:
Lessons learned from HIV-2

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Current clinical strategy for HIV worldwide

- Need to put all infected individual on therapies

- Need for a Cure due to several limitations of current drugs:
  - adherence to treatment
  - renal toxicity
  - neuropsychic effects
  - cost

Source: Kaise family / UNAIDS 2014
HIV vaccine as a cure

**Therapeutic (functional cure)**
- Induce functional cytotoxic CD8+ T cells to kill actively infected cells
- Control viral load to prevent transmission
- Produce neutralizing antibodies to the virus

**Prophylactic**
- Induce functional cytotoxic CD8+ T cells residing at mucosal sites
- Produce neutralizing antibodies at mucosal surfaces

**Main current vaccine strategies**
- **Non-HIV viral vectors (Ad, MVA)**
  - Limits: Dilution of HIV epitopes by vector epitopes
  - Quality of the immune response dictated by the vector

- **Viral peptides coupled to x**
  - Limits: Limited functional CD8/CD4 response
  - Limited breadth to viral epitopes

- **Viral proteins to raise bnAbs**
  - Limits: Method of induction is unknown
  - gp41/gp120 antigens
INNATE IMMUNITY IN HIV INFECTION

• unchecked induction of innate immune responses → Pathogenesis

• avoidance of innate sensing pathways → Immune evasion

Cell-extrinsic / Cell-intrinsic sensing in CD4+ target cells

Silvin & Manel, Current Opinion in Immunology, 2015
INNATE IMMUNITY IN HIV INFECTION

- unchecked induction of innate immune responses → Pathogenesis
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Cell-extrinsic / Cell-intrinsic sensing in CD4+ target cells

Silvin & Manel, Current Opinion in Immunology, 2015
DCs do not get activated in response to HIV-1 in vitro
"DCs do not sense HIV-1"

Day 0
- Isolate CD14+ monocytes from adult blood
- DC differentiation

Day 4
- Infection

Day 6
- Analysis

Control
- CD86: 4.22
- GFP: 193.7
- Analysis: 0.97

HIV-1
- CD86: 2.57
- GFP: 92.5
- Analysis: 1.05

No machinery in DCs?
or Viral escape?
Primate lentiviruses

Sharp/Hahn 2011
HIV-2 encodes the Vpx protein, absent in HIV-1
DCs sense HIV-1 when replication blocked is removed

\[ \text{SIVmac/HIV-2} \]

\[ \text{Control} \quad \text{HIV-1} \quad \text{Vpx} \quad \text{HIV-1+Vpx} \]

\[
\begin{array}{cc}
\text{CD86} & \text{GFP} \\
93.7 & 0.97 \\
92.5 & 3.9 \\
92.7 & 1.06 \\
1.99 & 11 \\
\end{array}
\]

\[ 4.22 \quad 1.15 \]
[2.57 \quad 1.05 \]
[4.69 \quad 1.5]
[9.74 \quad 77.3]

→ existence of a DC-intrinsic, cytosolic, sensing machinery of HIV-1

Manel et al., Nature 2010
HIV-2 is less pathogenic than HIV-1

HIV-1
>-98% AIDS
<-2% control

HIV-2
<-25% AIDS
>-75% control

Apparent contribution of the immune system to control infection (reviewed in Rowland-Jones et al., Nature Immunology 2007)

Inability of the immune system to control the virus
HIV-2 is not an attenuated virus, but infected patients naturally exhibit characteristics of a desired response to an effective therapy against HIV.

- Behave as Long-Term Non Progressors
- Low or no viral load detectable
- Proviral load is controlled
- Neutralizing antibodies for >15 years (Silva/Weiss JVI 2011)
- CTLs are more polyfunctional
- Larger breath of targeted epitopes
- Partial cross-protection against HIV-1 pathogenesis:

Esbjörnsson et al., NEJM, 2013
HIV-2 infects the DCs and DCs respond to the infection

- Isolate CD14+ monocytes from adult blood
- DC differentiation
- Infection
- Treatment
- Analysis

Day 0  Day 4  Day 6

Control  HIV-1  Control  HIV-2

CD86  GFP  CD86  GFP  CD86  GFP  CD86  GFP

Manel & Littman, *Cell* 2011
Stimulation of dendritic cells

HIV-2 or HIV-1+Vpx

Degradation of SAMHD1

Infection

Increase in viral antigens

Protection of CD4+ T cells

Antigen presentation to CD4+ and CD8+ T cells

Rice et al., Nature Genetics 2009
Hrecka et al., Nature 2011
Lagouge et al., Nature 2011
Goldstone et al., Nature 2011
Lahoussa et al., Nat Imm 2012

Manel et al. Nature 2010
Gao et al. Science 2013
Lahaye et al. Immunity 2013
Molecular mechanism of HIV sensing by dendritic cells

Manel & Littman, Cell 2011
cGAS = cyclic GMP-AMP synthase

second messenger
2'3'-cGAMP

DNA

extracellular

cGAS

ATP+GTP
cyclic dinucleotide

DNA(U) cGAS(A) DNA(E)
cGAS(C)

Wu et al., Science 2012
Ablasser et al., Nature 2013
Diner et al., Cell Reports 2013
Zhang et al., Mol Cell 2013

STING

NFKB
IRF3

Type I IFN

Dendritic cell
activation
cGAS transduction activates MDDCs

Lentivector cGAS/control +/- Vpx

Monocytes → Dendritic Cells

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CD86 BFP

Gentili et al., Science 2015
Is cGAMP packaged into viral particles and transmitted to target cells?

c[G(2'-5')pA(3'-5')p]
Detection of cGAMP presence in viral particles

→ Extract small molecules → Mass spec

Gentili et al., Science 2015
cGAMP is present in viral stocks of enveloped DNA viruses

Modified Vaccinia Ankara (MVA) (bar 100 nm)
Gallego-Gómez, JVI 2003

mCMV (bar 100 nm)

Gentili et al., Science 2015
Transfer of innate immune signaling by packaging of cGAMP in viral particles

- Enveloped viruses function as paratenic hosts for cGAMP
- Packaging of a DAMP in viral particles – "tagging" viruses as danger
- Spreads an immune signal that leads to DC maturation and antiviral protection
- HIV-1 normally escapes cGAS stimulation
- Provides a vectorization mean for cGAMP

Gentili et al., Science 2015
cGAMP-containing HIV VLPs an “all-in-one” HIV vaccine?

Bypassing viral replication to activate cGAS-STING pathway

Priming of HIV-specific CD4 and CD8 T cells
- antiviral context
- large breadth of epitopes

MHC-peptide

HIV ANTIGENS

cGAS → cGAMP → STING → cytokines IFN → costimulation CD86 → MHC-peptide

IFNAR

CD28

TCR

ADJUVANT
Capsid-cyclophilin A interactions play a critical role at the intersection of replication vs. innate sensing.

What are the associated host factors?
Cyclophilin A: Host protein that binds the HIV capsid

Identification: Luban et al., Cell 1993

Multiple regulatory activities on the virus:
- Uncoating
- Reverse transcription
- Nuclear entry
- Integration targeting
- Susceptibility to DNA sensors

Mechanism unclear & no host factor essential for CypA activities
The viral capsid determines innate immune sensing of the virus.

**Mutant capsid:** Genetic dissociation between productive infection and innate sensing.
HIVac mutant capsids are blocked at nuclear import and increase cGAS sensing

Lahaye et al., Immunity 2013
Restriction of HIVac capsids is mediated by CypA and can be rescued.

Conserved restriction of HIV nuclear import by CypA in human, macaque and murine cells.

However, CypA is not sufficient (not shown).

Lahaye et al., Cell Reports 2016
SUN2 is a candidate regulatory host factor of HIV at the nuclear envelope

Burke & Stewart, NRMCB 2013
SUN2 participates to the CypA restriction

Lahaye et al., Cell Reports 2016
SUN2 is an essential host factor of HIV infection in CD4+ T cells and mediates CypA activities

Lahaye et al., Cell Reports 2016
Identification of SUN2 as a new player in capsid-cyclophilin A interactions

Lahaye et al., Cell Reports 2016

SamHD1

Capsid

SSRNA

dNTPs

dsDNA

Cyclophilin A

SUN2

Regulations of innate sensing by SUN2?

Viral expression

Viral expression
Innate immunity intersects innate sensors with other factors of viral replication.

**Innate immunity**
- Immune sensors
- Restriction factors (intrinsic immunity & IFN effectors)

**Adaptive immunity**
- MHC presentation and antibodies (in vivo only)

**Fitness of Replication**
- Viral replication

Flowchart:
- Innate immunity
- Adaptive immunity
- Restriction factors

Diagram:
- Viral replication
- Fitness of Replication
- Innate immunity
- Immune sensors
- Restriction factors (intrinsic immunity & IFN effectors)
- Adaptive immunity (MHC presentation and antibodies)
Restriction factors, innate & adaptive immunity are in interactions (genetic or biochemical)

- Antagonism (competition)
  - e.g. SAMHD1 restricts viral replication but limits innate sensing
- Synergism (cooperation)
  - e.g. Tetherin restricts viral budding and induce an innate signal
- Neutral
Cooperation and Conflict in Antiviral Immune Responses

Viral genotypes landscape:

- **Innate immunity**
  - Escape from innate sensors

- **Adaptive immunity**
  - Escape from CTLs

- **Lethal susceptibility to CTL and antiviral effectors**

- **Lethal susceptibility to innate immunity & antiviral effectors**

- **Optimal replication**

- **Replication fitness**
  - Escape from antiviral effectors
  - Optimal use of host dependency factors

Adapted from Chae et al., PLoS Pathogens 2016
Cooperation and Conflict in the Plant Immune System
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