Primate Models for a system biology approach to understanding AIDS pathogenesis
Non-human primate models for HIV/AIDS

Asian nonhuman primates (macaques):
- Macaque / SIVsm, SIVmac
- Pt Macaque / SIVagm.90
- Pt Macaque / SIVlhoest

African nonhuman primates: natural hosts of SIV (~40 species):
- Chimpanzee / SIVcpz
- Gorilla / SIVgor
- Sooty Mangabey / SIVsm
- African Green Monkey / SIVagm
- L’Hoest monkey / SIVlhoest
- Mandrill / SIVmnd

HUMANS

HIV-1
HIV-2

ASYMPTOMATIC

AIDS
AIDS
Major common and distinct features between non pathogenic (AGM, SM) and pathogenic (HUMAN, MAC) HIV/SIV infections

<table>
<thead>
<tr>
<th>Feature</th>
<th>SIVsm, SIVagm</th>
<th>HIV-1, SIVmac</th>
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<tbody>
<tr>
<td>High viral genetic variability</td>
<td>✔️</td>
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<tr>
<td>High viral load in blood and gut</td>
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<tr>
<td>Severe loss of CD4⁺ T cells in gut</td>
<td>✔️</td>
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<td>Anti-viral T and B cell responses</td>
<td>✔️</td>
<td>✔️</td>
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<tr>
<td>Chronic T cell activation</td>
<td>-</td>
<td>✔️</td>
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<tr>
<td>Progressive loss of blood CD4⁺T cells + AIDS</td>
<td>-</td>
<td>✔️</td>
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</table>


Natural host: excellent model for searching determinants of protection against bystander immune activation
Mechanism of protection against bystander immune activation in natural hosts of SIV?

Hypotheses from the literature:

- **Viral determinants** (CD3 down-regulation by NEF)
  (Schindler *et al*., Cell, 2006)

- **Specific CD4+ T cell characteristics** (low CCR5, CD4 down, Th17)

- **Preservation of tissues:**
  - lymph nodes (Beer *et al*., 1995; Diop *et al*., 2000; Cumont *et al*., 2008; Lederer *et al*., 2009)
  - intestinal barrier (Brenchley *et al*., Nat Med 2006; Li *et al*., JID 2008)

Rapid negative immunoregulation (Kornfeld *et al*., JCI 2005; Estes *et al* JI 2008; Favre *et al* 2009)
Gene expression profiles in CD4+ T cells (blood, lymph nodes) from African green monkeys (AGMs) and Rhesus Macaques (RM) before and after infection (day 1 - day 600)

Identification of genes whose expression is distinct between SIV-infected AGM and RM

Functional assay to study the regulation of these genes (in vitro)
Analysis of gene expression profiles in AGMs and RM s

Experimental approach

Ficoll → PBMC → Magnetic cell sorting → CD4+ → RNA → Gene Expression

Blood

-90-70  -40  -8  1  6  14  28  41  65  115  600

* Lymph Node
dilaceration

~13,000 genes expressed in AGM and RM CD4+ cells

Jacquelin et al, FASEB J. 2007

N = 6 AGM
N = 6 Mac (RM)

205 data sets
**Ontology:** Immunity and Defense subcategories (acute infection)

**Interferon Mediated Immunity**

AGM peripheral CD4+ cells
RM peripheral CD4+ cells
AGM LN CD4+ cells
RM LN CD4+ cells

* p < 0.01
** p < 0.001
*** p < 0.0001

Jacqulin et al, JCI, Dec 2009
Genes that were most upregulated: ISG

ISG (Interferon Stimulated Genes)

PRR signaling
Antiviral
Immunomodulatory

Blood CD4⁺

Induction of TRIMs
Tetherine
Mx1
OAS
IRF7
RIG-I
CXCL10
IDO
...

AGM and RM
Resolution after d28p.i. (p<0.001)

HIV-1/SIVmac

non-viremic
viremic

ISG

Bosinger et al, JI, 2004; Dandekar et al; Favre et al, 2009, and many others
Lymph Nodes: strong, transient induction of ISG in AGM

Species-specific up-regulations:
- only in RM: RANTES, TRAIL, IRF8
- chronic only in RM: CCL2, CCL8, CXCL9, CXCL10, CXCL11

Number of ISGs (type I)

<table>
<thead>
<tr>
<th></th>
<th>SIVmac/RM</th>
<th>AGM/SIVagm</th>
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<tbody>
<tr>
<td>Blood CD4⁺</td>
<td>43</td>
<td>67</td>
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<tr>
<td>Lymph node CD4⁺</td>
<td>63</td>
<td>44</td>
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Resolution after d28p.i. (p<0.001)
IFN-α plasma levels

**tight sampling...**

**AGM**
- SV045
- SV048
- SV042
- SV052
- SV046
- AGM Mean

**RM**
- M1076
- M1085
- M1099
- M1058
- M1073
- M1094
- RM Mean

**Graphs**

Plasma IFN-α: Associated with viral load \((p < 0.0001)\) and ISG profiles \((p < 0.002)\)
3 times lower in AGM than RM \((p < 0.005)\)
In some AGMs as high as in RM that progress to AIDS
Down-Regulation of Robust Acute Type I IFN Responses Distinguishes Non-Pathogenic SIV Infection of Natural Hosts from Pathogenic SIV Infection of Rhesus Macaques

AGM pDC are responsible for SIVagm-stimulated IFN-α production in vitro

The cells were labelled after 6h stimulation.

Similar to human and Mac PBMC, among AGM PBMC, only pDC were positive for IFN-α after stimulation with SIV.
IFN-α production by AGM pDC after *in vitro* stimulation with HSV and SIVagm

### IFN-α Production after 18h stimulation

<table>
<thead>
<tr>
<th>IFN-α supernatants (U/ml)</th>
<th>Medium</th>
<th>HSV</th>
<th>SIV</th>
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<tr>
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AGM (non-infected)  
N=12

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Similar IFN-α production by AGM and MAC pDC after *in vitro* SIV stimulation

### IFN-α production by AGM pDC after *in vitro* stimulation

<table>
<thead>
<tr>
<th>ng/ml SIV Gag p27</th>
<th>RM</th>
<th>1500</th>
<th>150</th>
<th>15</th>
<th>1500</th>
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<td>IFN-alpha (U/ml)</td>
<td>125</td>
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Species:  
RM  
AGM

Stimulus:  
SIVmac  
SIVagm

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Species</th>
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<tr>
<td>SIVmac</td>
<td>RM</td>
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<td>RM</td>
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<tr>
<td>SIVagm</td>
<td>AGM</td>
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IFN-α sufficient to induce ISG in AGM cells? Why strong ISG *in vivo* irrespective of plasma IFN-α levels?

Why some ISGs are not seen induced *in vivo*?

→ Low threshold for ISG induction

---> Efficient *in vitro* induction of TRAIL
Are cells of AGM more refractory to repeated stimulation than RM cells?

Design of repeated *in vitro* stimulations of PBMC:

- r-IFN-α
- no stimulus
- no stimulus or r-IFN-α
- cell harvest
- washing

![Graph showing gene expression](chart.png)

- RM
- AGM
- * p<0.01

Comparison of Mx1 gene expression (Log2Q) over time:
- 18h
- 46h
- 64h (w/o r-IFN-α)
- 64h (with r-IFN-α)
Do chronically infected AGMs respond less?

AGM SIV- (N=5)
AGM SIV+ (N=6)

Normal IFN-α production and ISG induction in PBMC from chronically infected AGMs \textit{in vitro} -> \textit{in vivo} regulation….
Immunosuppressive genes?

AGM (LN)

RM (LN)

Negative feed back loop genes?

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Genes:
- Indoleamine 2,3-dioxygenase (IDO)
- Galectin-3
- LACT3
- B-cell maturation antigen (BCMA)
- Cytotoxic T-lymphocyte antigen 4 (CTLA4)
- Leukemia inhibitory factor (LIF)
- FGF-2
- TGF-beta
- IL-10
- CD160

Immunosuppressive genes?

Negative feedback loop genes?
Which genes are associated with the control of the innate response in vivo?

- AGM
  - Before: Loss of «on» signals
  - Acute: Signals «off»
  - Chronic: «off» signals lacking

- MAC
  - Acute: Persistent «on»
  - Chronic: «off» signals lacking

Still too many genes...
Meta-analysis


A. Benecke
Non-pathogenic SIVagm infection in AGM

Immediate, strong and broad systemic IFN-I response
Immunoregulatory control at the transition from acute to chronic stage

Primary infection

A little inflammation in acute phase is essential for virus and host
→ Establishment of persistent infection
→ Partial control of viral replication

Chronic phase

→ Resolution of immune activation!
Beneficial for the host, but HOW is it achieved?
«Off» signals? Lack of second line «on» signals?
local environment...
Team »Early determinants of protection against AIDS »

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