Early HIV-specific CD8⁺ T cell responses in treated and untreated hyper acute HIV infection in the FRESH cohort

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Outline

• HIV incidence in young women in KwaZulu-Natal and in sub-Saharan Africa

• The FRESH cohort- a unique cohort to address gaps in HIV prevention, pathogenesis and cure research

• Insights on early CD8 and CD4 T cell responses during untreated and treated hyperacute HIV infection

• Implications for HIV prevention and cure strategies in resource-limited settings
Eastern and southern Africa: high new HIV infections among young women aged 15-24 years

- There is a need to better understand the reasons for high incidence among young women
- Current strategies are suboptimal- new biomedical interventions such as vaccines are needed
Acute HIV-1 infection- what lessons can we learn?

Viral set point is a predictor for:
- Rate of disease progression
- Risk of transmission

Key questions:
• What behavioural, socioeconomic and biomedical factors are responsible for such high incidence among young women?

• What is the nature of the transmitted/founder virus?

• What do immune responses in acute HIV-1 infection look like and why do they ultimately fail in most cases?
FRESH study cohort

• **FRESH**: Females Rising through Education, Support and Health

• Recruit women 18 to 23 at very high risk of HIV infection

• **Objectives**

1. Provide an intensive empowerment, life-skills and job readiness curriculum.

2. Identify persons in the earliest stages of acute infection.
   - Study antiviral immune mechanisms
Study setup and sample collection

• Phase I: Surveillance
  – Twice weekly HIV RNA PCR testing via finger stick blood draws
  – Quarterly blood and mucosal sampling of the female genital tract

• Phase II: Acute Infection

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WEEK: 0-152
Empowerment Program

FRESH Placements (n=248)

- Employment
- Internship/Learnership
- High School Diploma Course
- University
- Not yet placed
- Small Business
Acute infections detected (N=32)

As of Sept 29, 2015:
- 699 enrolled
- 32 acute infections (397 uninfected / active)
- **Incidence 8.5** (95% CI=5.8-12.0) per 100 p/y

- Median: 124 days; range 0-684
Viral and cellular dynamics in hyperacute HIV infection

127-33-0097-079

Days

Plasma RNA (log_{10} copies/ml)

CD4 Count (cells/mm^3)

RNA

CD4

CD4 Count (cells/mm^3)
FRESH studies

- The FRESH study design allows us to address critical questions in:
  - HIV prevention research-
  - Innate immune responses (Kløverpris et al, 2016, *Immunity*
  - Early B cell responses
  - Early CD8 T cell responses (Ndhlovu et al, 2015, *Immunity*)
Dynamics of T cell responses in primary HIV infection: experimental approach

Study subjects- 12 FRESH seroconverters

Longitudinal analysis of HIV induced T cell responses

Recently activated T cells (CD38+, HLA-DR+)

Recently stimulated cycling T cells (Ki67+, Bcl-2 negative)

HIV antigen-specific T cells (Elispot, ICS and tetramer)
HIV infection induces massive activation (CD38+HLA-DR+) of CD8+ T cells

Pre-infection

Day 3
Log VL 6.3

Day 10
Log VL 7.1

Day 17
Log VL 5.3

Day 56
Log VL 4.2

B

Percent CD38+HLA-DR+

Days following onset of plasma viremia (DFOPV)
The timing and strength of the initial CD8+ T cell responses impact viral load set point.

- Spearman $r = 0.8$, $p = 0.003$
- Spearman $r = -0.8$, $p = 0.006$
Hyperacute CD8+ T cells are highly proliferative and pro-apoptotic (Ki67+BCL2low).

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<th>Day</th>
<th>Log VL</th>
<th>Percent Ki67</th>
<th>Percent BCL-2</th>
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<td>Day 56</td>
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Spearman r = 0.7, p = 0.03

Log10 viral load set point vs. Percent Ki67 high, BCL-2 low, CD8+ cells
Paucity of IFN-\(\gamma\) production in hyperacute infection

A

Pre infection
Day 3
Log VL: 5.1

Day 7
Log VL: 7.1

Day 14
Log VL: 6.8

Day 28
Log VL: 3.8

Proportion of responding CD8+ T cells

HIV-specific?
Tetramer staining CD8+ T cells at peak activation

- A*6802 EL9 gp41: 3.8
- A*6802 EA10 int: 3.9
- B*5801 ISW9 p24: 10.5
- B*5801 TW10 p24: 8.6
- B*5801 QW9 p24: 9.2

CD8, CD38, HLA-DR analysis.
HIV specific CD8 T cells are defective in IFN-gamma secretion during acute HIV infection

% of teramer+ cells secreting IFN-γ

P=0.01
Assessment of bystander activation during hyperacute HIV infection

Donor 093

HIV-specific

CMV-specific

Flu-specific

CD8 T cells

HIV

CMV

EBV

Flu

Kruskal-Wallis p=0.001

CD38+HLA-DR+Tetramer+ CD8 T cells
HIV-specific CD8 T cells fail to upregulate survival molecules during acute HIV infection

Kruskal-Wallis p = 0.001

P = 0.01

P = 0.02
Marked apoptosis of HIV-specific CD8 T cells during hyperacute HIV infection

% AnnexinV+PI+ CD8 T cells

Pre infec | Peak activ | Post activ

P=0.02 | P=0.03

HIV-specific

CD8

HIV tetramer

Annexin V

CMV-specific

CMV tetramer

Apoptosis
CD8 responses in hyperacute HIV Infection

**Activation**
- Tetramer
  - CD8
- CD38
  - HLA-DR

**Bystander Activation**
- CD8+ T cell activation over time
  - HIV, CMV, EBV, Flu

**Impact on viral replication**
- Days to peak CD8+ T cell activation
  - Log_{10} viral load set point

**Apoptosis**
- HIV-specific
  - HIV tetramer
  - CD8 and Annexin V
- CMV-specific
  - CMV tetramer
  - CD8 and Annexin V

**Poor survival**
- CD127+Tetramer+ CD8 T cells
  - HIV, CMV, EBV, Flu

Ndhlovu et al Immunity 2015
CD4 T cell responses in hyperacute HIV infection
CD4+ T cell activation during acute HIV infection

Pre-infection

Day 3 Fiebig I

Day 10 Fiebig I

Day 17 Fiebig I

Day 56 Fiebig V1

CD38

HLA-DR

LogVL: 4.1

LogVL: 6.9

LogVL: 6.11

LogVL: 5.3

Percent activated CD4+ cells

CD38+HLA-DR+

Days following onset of plasma viremia (DFOPV)
CD4+ T proliferation during hyperacute HIV infection

- Day 3
  - Fiebig I
  - LogVL: 4.1
- Day 10
  - Fiebig I
  - LogVL: 6.9
- Day 17
  - Fiebig I
  - LogVL: 6.11
- Day 56
  - Fiebig VI
  - LogVL: 5.3

- Pre-infection
- Ki67
- BCL-2

Percent Ki67^high BCL-2^low CD4+ cells

Days following onset of plasma viremia (DFOPV)
Dynamics of HIV-specific CD4+ T cell responses during acute HIV infection

Days post Fiebeg 1

% IFN-γ+ CD4+ T cells

- Gag
- Nef
- Env
Comparison between activated CD4+ T cells and gamma secreting cells
Number of HLA-DRB1 variants that can present each peptide

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Class II tetramer staining
Activation profile of HIV-specific CD4 T cells

Acute HIV

Chronic HIV
Phenotypic profile of HIV-specific CD4$^+$ T cells in acute and chronic HIV infection

Acute HIV peak CD4 activation

Chronic HIV infection
Conclusions

• CD8+ T cell immune responses during acute HIV-1 infection is broader than previously thought (Ndhlovu et al, 2015, *Immunity*)
  – Acute HIV infection rapidly induces massive activation and proliferation of CD8+ and CD4+T cells
  – The magnitude and timing of CD8+ T cell activation impact viral load set point
  – A high proportion of activated CD8+ cells are HIV-specific

• HIV-specific CD8+ and CD4+ T cells are defective for cytokine secretion, memory generation and prone to apoptosis during acute HIV infection following ex-vivo stimulation

• There is measurable expansion of HIV-specific peripheral TfH during hyperacute HIV infection
The impact of early treatment on generation and maintenance of HIV-specific T cell responses?
Treatment during hyperacute phase blunts peak viremia

Peak Viral Load

Log10 RNA copies/ml

Untreated (n=14) | Treated (n=20)
Raltegravir intensification reduces time to full suppression

Viral Load
(p-value .0014)

Log\text{10} RNA copies/ml

Sampling Timepoints (weeks)

- Untreated (n=14)
- Early HAART (n=11)
- Early HAART + RAL (n=9)
Impact of early ART on T cell responses

Investigate if very early ART treatment suppresses the subsequent development of HIV specific CD8 cell responses by removing the antigenic stimulus, OR.

If early ART treatment might help preserve HIV specific CD4 to CD8 and B cell responses.
Very early ART limit CD4 T cell loss

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<th>Nadir CD4</th>
<th>Rebound CD4</th>
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<td>Untreated</td>
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CD4 Count (cell/mm$^3$)

- Early treated: p=0.01 vs. Preinfection, ns vs. Nadir CD4, ns vs. Rebound CD4
- Untreated: p=0.001 vs. Preinfection, p=0.0001 vs. Nadir CD4, p=0.01 vs. Rebound CD4
CD8+ T cell responses in early treated subject

127-33-0628-451

Plasma RNA (log_{10} copies/ml)

RNA

CD4

Days

Treatment initiation

Pre-infection

Day 4
Log VL 3.5

Day 21
Log VL <2.0

Day 56
Log VL <2.0

Tetramer

CD8

CD38

HLA-DR

0.0

5.7

9.8

5.7

70.3

69.5

62.4

Gate %

TetPE 3.56e-3

CD8 23.1

TetPE 5.67

CD8 46.3

TetPE 9.8

CD8 43.1

TetPE 5.7

CD8 34.4
Frequency of individual tetramers$^+$ CD8$^+$ cells in early treated and untreated subjects

$p=0.01$
Increased CD127 expression on CD8$^+$ T cells during early treated hyperacute HIV infection.
-specific CD8+ T cells in early treated subjects have a more functionally competent
Conclusions

• Very early treated individuals have *measurable T cell responses that have a pro-survival phenotype*

• Prompt antigen withdrawal results in stable HIV-specific CD8 T cell clonal repertoire.
Future directions
Vaccinate FRESH subjects with Ad26 mosaic vaccine

The Ad26 mosaic vaccine yielded many more epitope-specific responses than did the Ad26 M consensus, clade B + clade C, or optimal natural clade C vaccines

Assays to measure HIV Persistence

Proteinase K Lysis
Cells
Pre-amplification from cell lysate

Total HIV DNA
Integrated HIV DNA

3'LTR  gag  pol  env  3'LTR
Alu  Alu

Single well pre-amplification of HIV and CD3

2-LTR circles

Frequency of cells harbouring HIV DNA

HIV copy number
2 X CD3 copy number

TaqMan Probes
(sensitivity: 1 HIV DNA copy)

Real-time nested PCR of pre-amplified products

HIV
CD3

adapted from Vandergeeten et. al., 2014, JVI
Conclusions I

- The FRESH study design allows us to address critical questions in HIV prevention research—such as the impact of cervicovaginal microbiota on female genital inflammation (Anahtar et al, 2015, *Immunity*) and impact of IPC on HIV acquisition risk (Byrne, Anahtar et al, *Lancet ID*, in press).
- FRESH study participants initiated on cART during hyperacute HIV infection may offer new insights on long-term viral remission.
- Understanding of immune responses following cART initiation may be useful for future intervention studies.
Acknowledgements

- **Ragon Institute**
  - Dr. Bruce Walker
  - Galit Alter
  - Xu Yu

- **HIV Pathogenesis Programme/UKZN**
  - Prof. Thumbi Ndung’u
  - Krista Dong
  - Amber Moodley
  - Nikoshia Mewalal
  - Karyn Pretorius
  - Philomena Kamya
  - Sannie Mahungela
  - Nasreen Ismael
  - **FRESH Participants**