The Use of Flow Cytometry To Study Acute HIV-1 Infection

5th INTEREST Workshop

Dar es Salaam, Tanzania

10-13 May 2011

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Center for AIDS Research (CFAR)
Duke Human Vaccine Institute (DHVI)
International AIDS Training Research Program (IATRP)
Duke Global Health Institute (DGHI)
Flow Cytometry is a Quantitative Method That Measures Single Events (Cells)

**Light Amplification by Stimulated Emission of Radiation**

Lasers generate intense beams of coherent light.

wwwbdbiosciencescom/immunocytometry_systemsupport/training/online
Basic Aspect of a Flow Cytometer

Analog
Flow Cytometry: A Quantitative Assay Platform

Level of Expression
The Achilles’ Heel of Flow Cytometry: Signal Spillover

Presented at the 5th INTEREST workshop – 10 – 13 May 2010, Dar-es-Salaam, Tanzania
The Achilles’ Heel of Flow Cytometry: Signal Spillover

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The Achilles’ Heel of Flow Cytometry: Signal Spillover

Non Compensated

Compensated

Parameter 1

Parameter 2

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Flow Cytometry Applications in Acute HIV-1 Infection

• Clinical Applications

• Research Applications
Clinical Application: Patient Management
1 Assay for CD4 Count and Rx Adherence

CD4 Count:
Clinical Stage

CD8 Activation ⇔ Virus Load
Virus Load ⇔ Rx Adherence
CD8 Activation ≈ Rx Adherence

Glencross et al. “CD8/CD38 activation yields important clinical information of effective antiretroviral therapy”. Cytometry (2008) vol. 74B (S1) pp. S131-S140

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Please see Posters

• P_10: Moodley K. et al.: “CD38 Activation (MFI) Assessment by Flow Cytometry in HIV+ and HIV- Patients with or without TB Co-Infection”
What is the level of CD8 activation during acute HIV-1 infection?

How does early initiation of HAART impact the activation of CD8\(^+\) T cells?
Levels of Total CD8 T cells Activation during Acute HIV-1 Infection (n=61)

Does not Reset Following Initiation of HAART


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Flow Cytometry Applications in Acute HIV-1 Pathogenesis

• Clinical Applications

• Research Applications:
  1. Frequency of Immune Cellular Subsets (T, B, NK, DC subsets);
  2. Memory Phenotype and Maturation Stage of Cellular Subsets;
  3. Cellular Functions: Cytokine production, Cytotoxic Activity, Ab production;
  4. Proliferative Capability of Ag-specific and non-Ag-specific Cells.
Basic Aspect of a Flow Cytometer

Analog

Digital
Detection of 8 parameters to identify all possibility for 4 combined parameters:
4-color Cytometer = 25 tubes = 25 million cells = 25 ml of blood
8-color Cytometer = 1 tube = 1 million cell = 1 ml of blood

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ICS Methodology

1. Stimulate
   + Brefeldin A
   6 h
   ↓
   EDTA
   ↓
   cytokine
   lymphocyte
   erythrocyte

2. Viability & Surface Staining
   ↓
   Wash
   ↓
   IFN-γ
   + α-IFN-γ FITC
   + α-CD3 APC
   Ag presenting cell
   T cell

3. Permeabilize
   ↓
   Wash
   ↓
   + α-CD8 PE

4. Stain
   ↓
   Wash
   ↓
   + Brefeldin A

5. Acquisition

6. Analysis


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Gating Strategy for the ICS Assay Analysis

I. Live Lymphocytes Subsets

- Singlets
- Live CD3+
- Lymphocytes

CD8+

- Naive
- Memory

CD8+ Memory

- CD27+
- CD57+
- CD45RO+

memory CD8 Functions

- CD107a
- IFN-γ
- IL-2
- MIP-1α
- TNF-α

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What is the level of Ag-specific CD4 and CD8 activation during acute HIV-1 infection?
Antigen-specific CD4 cells were significantly more activated, as per CD38 expression, when compared to the total memory compartment in both acute and chronic infection
Multiparameter ICS to Identify Functional CD8+ Memory Subsets

memory CD8 Functions

- CD107a
- IFN-γ
- IL-2
- MIP-1β
- CD107a

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Boolean Logic in Flow Cytometry

The Boolean logic in Flow Cytometry allows to identify populations that fell in different gates according to the “And”, “Or”, or “Not” principles.
Multiparameter ICS to Identify Functional CD8+ Memory Subsets

CD107a
IFN-γ
IL-2
MIP-1β
TNF-α

Functional Family
5+
4+
3+
2+
1+

Boolean Analysis

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Comparison of Ontogeny of Functional CD4⁺ and CD8⁺ Memory T Cell Responses following Acute HIV Infection

**CD4⁺ Responses**

<table>
<thead>
<tr>
<th>Week</th>
<th>Freq.</th>
<th>Funct.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>4-8</td>
<td>1.24</td>
<td></td>
</tr>
<tr>
<td>12-16</td>
<td>0.54</td>
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<tr>
<td>28-34</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>54-78</td>
<td>0.79</td>
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</table>

**CD8⁺ Responses**

<table>
<thead>
<tr>
<th>Week</th>
<th>Freq.</th>
<th>Funct.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>4-8</td>
<td>1.60</td>
<td></td>
</tr>
<tr>
<td>12-16</td>
<td>1.87</td>
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<tr>
<td>28-34</td>
<td>2.23</td>
<td></td>
</tr>
<tr>
<td>54-78</td>
<td>1.52</td>
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</tr>
</tbody>
</table>

G. Ferrari, Gray CM, et al. Unpublished Data

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What functionality is displayed by the Ag-specific CD8 driving escape during acute HIV-1 infection?
We ranked the mapped epitopes within each patients according to the order of appearance of escape mutants

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>HIV Antigen/aa position</th>
<th>Sequence</th>
<th>Allele restriction</th>
<th>Week</th>
<th>Mutation</th>
<th>Epitope</th>
<th>Order of escape within PID</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH040</td>
<td>Rev 49-66</td>
<td>QRIQRISERILSTYLER</td>
<td>A*0201</td>
<td>4</td>
<td>e-ME</td>
<td>a</td>
<td>1</td>
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<tr>
<td></td>
<td>Vif 113-129</td>
<td>DCFSESAIRAILGRIVS</td>
<td>A*3101</td>
<td>4</td>
<td>e-ME</td>
<td>b</td>
<td>2</td>
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<tr>
<td></td>
<td>Env 830-847</td>
<td>IEVQORACAILHIPRI</td>
<td>undetermined</td>
<td>12</td>
<td>I-ME</td>
<td>c</td>
<td>3</td>
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<tr>
<td></td>
<td>Env 765-682</td>
<td>LFHYRLDLLLVTRIV</td>
<td>A*0201</td>
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<td>nME</td>
<td>d</td>
<td>4</td>
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<tr>
<td></td>
<td>Gag 481-498</td>
<td>KELYPLASLRSFNGDPS</td>
<td>B*4001</td>
<td>12</td>
<td>nME</td>
<td>e</td>
<td>4</td>
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<tr>
<td></td>
<td>Pol 824-841</td>
<td>VKTHTDNGSFTSTTVK</td>
<td>undetermined 2</td>
<td>24</td>
<td>nME</td>
<td>f</td>
<td>4</td>
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<tr>
<td>CH058</td>
<td>Env 581-596</td>
<td>LALERYLDQQILLGIW</td>
<td>B<em>1402/Cw</em>0702</td>
<td>2</td>
<td>e-ME</td>
<td>a</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Gag 236-253</td>
<td>IAGSTSTLQEQGWM'TSN</td>
<td>B*5701</td>
<td>2</td>
<td>e-ME</td>
<td>b</td>
<td>2</td>
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<tr>
<td></td>
<td>Gag 140-157</td>
<td>GQMVHQAISPRTLNAWVK</td>
<td>B*5701</td>
<td>4</td>
<td>I-ME</td>
<td>c</td>
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<tr>
<td></td>
<td>Nef 113-130</td>
<td>WYYHTQGYFDPDQNYTPG</td>
<td>B*5701</td>
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<td>d</td>
<td>4</td>
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<td>CH077</td>
<td>Env 350-368</td>
<td>HVVDKLRQFRNKTIVFNH</td>
<td>Cw*0401</td>
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<td>e-ME</td>
<td>a</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Gag 236-253</td>
<td>IAGSTSTLQEOVQWM'TSN</td>
<td>B*5701</td>
<td>4</td>
<td>e-ME</td>
<td>b</td>
<td>2</td>
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<tr>
<td></td>
<td>Env 334-351</td>
<td>SGEDWNTLSHVVDKLRE</td>
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<td>4</td>
<td>e-ME</td>
<td>c</td>
<td>3</td>
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<tr>
<td></td>
<td>Env 605-622</td>
<td>TTVTPWNVSWSNKSLNEI</td>
<td>B*5701</td>
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<td>d</td>
<td>4</td>
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<tr>
<td>MM33</td>
<td>Pol 81-98</td>
<td>DTGADTVLEEMNLPGRW</td>
<td>B*4402</td>
<td>2</td>
<td>e-ME</td>
<td>a</td>
<td>na</td>
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<tr>
<td>MM39</td>
<td>Nef 65-82</td>
<td>EVGFPRPQPQPRLPMTYK</td>
<td>A*0301</td>
<td>1</td>
<td>I-ME</td>
<td>a</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>Gag 17-34</td>
<td>EKIRLPRGGKKKYKLHI</td>
<td>A*0301</td>
<td>1</td>
<td>I-ME</td>
<td>b</td>
<td>na</td>
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<tr>
<td>MM42</td>
<td>Nef 185-202</td>
<td>FDSRLAFHHIARELHEY</td>
<td>A*0201</td>
<td>4</td>
<td>e-ME</td>
<td>a</td>
<td>na</td>
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<tr>
<td>MM43</td>
<td>Vif 113-130</td>
<td>DCFSSEAIRGAILGHIVS</td>
<td>undetermined</td>
<td>4</td>
<td>e-ME</td>
<td>a</td>
<td>1</td>
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<tr>
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<td>Nef 177-194</td>
<td>EKEVFLEWPDTIAHHRR</td>
<td>A*0201</td>
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<td>e-ME</td>
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<td>2</td>
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<tr>
<td></td>
<td>Env 6-20</td>
<td>NYQHILWGGMLLWGRIM</td>
<td>undetermined</td>
<td>6</td>
<td>e-ME</td>
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<tr>
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<td>Nef 89-106</td>
<td>FFLKEKGGELEIHSQKR</td>
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<tr>
<td></td>
<td>Pol 633-650</td>
<td>ELQAIHLALQDSGLEVNV</td>
<td>A*0201</td>
<td>6</td>
<td>I-ME</td>
<td>e</td>
<td>4</td>
</tr>
</tbody>
</table>


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MIP-1β production dominates early responses...
Frequency of Epitope-specific MIP-1\(\beta\) Functional CD8\(^+\) T cells Correlates with order of Appearance of Escape Mutants

<table>
<thead>
<tr>
<th>Earliest Positive ICS</th>
<th>Total Freq</th>
<th>Fxn 1</th>
<th>Fxn 2-5</th>
<th>MIP-1(\beta)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>p =</strong></td>
<td>0.03</td>
<td>0.11</td>
<td>0.07</td>
<td>0.006</td>
</tr>
</tbody>
</table>
Flow Cytometry does not have to be highly complex and can be implemented in research-limited setting if focused and aimed to study any of the parameters thus far presented.
What is needed to maximize the utilization of Flow Cytometry in limited resource setting?
Way forward:
1. To mentor young scientists
2. Create a network of young scientists
3. To implement education of clinical staff
Participants

- We received 101 applications from the countries indicated on the map.
- Twenty-four (24) applicants were selected for the symposia and 12 more for the African Flow Cytometry Workshops that followed the Symposium.
Organizing Committee *
Scientific Committee and Faculty *
2009 Infectious Diseases in Africa: Measurement of Immune Responses &
3rd African Flow Cytometry Workshop

Presented at the 5th INTEREST workshop – 10 – 13 May 2010, Dar-es-Salaam, Tanzania
2011 Infectious Diseases in Africa: Measurement of Immune Responses &
4th African Flow Cytometry Workshop

University of Cape Town, Cape Town 11-13 November 2011

Visit: http://www.immunopaedia.org.za/

Topics of the Symposium:

1. Faculty Seminars on Adaptive and Innate Immune Responses to HIV, Malaria, and TB;

2. 12 Presentations by Young Investigators on HIV, Malaria, and TB; 2 will be re-invited from the previous event because were the top performers

   2. How to present your data.

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2011 Infectious Diseases in Africa: Measurement of Immune Responses &
4th African Flow Cytometry Workshop

University of Cape Town, Cape Town 11-13 November 2011

Topics of the Workshop: TBD

1. Instrument optimization for BD Calibur and LSR II
2. Panel Optimization
3. Assay standardization
4. Software training for DIVA, FlowJo, PESTLE, and SPICE
Evaluation of the event’s impact:

1. Daily questionnaire reviewing the topic of the day. Qs&As were discussed by different groups the following day (8 attendees with 2-3 faculty);

2. Final questionnaire to review all of the topics with scores;

3. The scores were used to identify the recipients of two fellowships to attend an International Meeting or Technical Workshop.
Way forward:

1. To mentor young scientists
2. Create a network of young scientists
3. To implement education of clinical staff: open clinical/research laboratory to medical student
Medical Education Partnership Initiative (MEPI)

- Goal to sustainably enhance and support medical education through partnerships between African and US schools of medicine
- Jointly sponsored by NIH and PEPFAR
- Eleven awards announced October 2010
- Kilimanjaro Christian Medical College-Duke University School of Medicine awarded $10,000,000 over 5 years (Prof. Moshi and Dr. Bartlett P.I.)
The Patients.

CLINICAL CORE
Mike Cohen (UNC, US)
Marybeth McCauley (FHI, US)
Joe Eron (UNC)
Charles Hicks (Duke)
Cindy Gay (UNC)
Kara McGhee (Duke)
Ian Williams (St Mary, UK)
Perry Pellegrino (St Mary, UK)

T CELL CORE
Michael Liu
Nilu Goonetilleke
Emma Turnbull
Stephen Moore
Rachel Tanner
Kati Digleria
Tim Rostron
McM CHAVI LAB
Persephone Borrow
Andrew McMichael (Oxford, UK)

VACCINE RESEARCH CENTER/NIH
Mario Roederer

U. of Pennsylvania
Michael Betts

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Maria Gonzalez-Salazar
Brandon Keele
Beatrice Hahn
George Shaw (UAB, US)

Jennifer Kirchner
Chunlai Jiang
Feng Gao (Duke, US)

Florette Treurnicht
Carolyn Williamson (UCT, S. Africa)

MANAGEMENT CORE
Kelly Soderberg
Tom Denny
Liz Petzold
Bart Haynes (Duke, US)

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Clive Gray (NICD, South Africa)

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The Senior and Junior Faculty and the Participants.


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