Ongoing immune activation in treated HIV infection

Robert Maughan PhD
HIV Molecular Research Group, UCD

• Multidisciplinary team of 14: academic ID physicians, post-doctoral researchers, research nurses, a research pharmacist, a data manager and a laboratory technician
• Our research: translational research into long-term comorbidities associated with aging and HIV
HIV Molecular Research Group

Talk Outline

1. Immune reconstitution with antiretroviral therapy
2. Incomplete immune normalisation?
3. Mechanisms of ongoing immune activation
4. Inflammation and mortality/morbidity in treated HIV:
   a) Predictors
   b) Cardiovascular disease
   c) Bone disease
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Turn of the tide

Number of Global AIDS-related deaths vs Antiretroviral therapy coverage

- 18.2 million of 36.7 million people infected with HIV were accessing therapy in June of 2016
- AIDS-related deaths have fallen by 45% since the peak in 2005

2016 Global AIDS update, UNAIDS
Improving Life Expectancy

Danish Cohort - Legarth/Obel, JAIDS, 2016
Immune reconstitution with therapy

Kaplan et al., JAIDS 2012

Start Treatment
HIV i-Base

Viral Replication
Immune reconstitution

CD4+ T cell count
HIV RNA copies/mL

Expected age at death*

Men

UK LE 78 years

Women

UK LE 82 years

* Expected age at death for a person aged 35 years with different durations of antiretroviral therapy according to current CD4 count and viral load suppression

May M, et al. AIDS 2014
Immune reconstitution with therapy

**Expected age at death**

*Men

- UK LE 78 years

**Women

- UK LE 82 years

<table>
<thead>
<tr>
<th>Years since ART</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*VL ≤400: CD4 ≥350 CD4 200–349 CD4 <200

* VL >400: CD4 ≥350 CD4 200–349 CD4 <200

* Expected age at death for a person aged 35 years with different durations of antiretroviral therapy according to current CD4 count and viral load suppression

May M, et al. AIDS 2014

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**In general, initiation of ART dramatically reduces inflammation & immune activation:**

- ↓ IL-6 [1, 2]
- ↓ IL-2 [4]
- ↓ IL-10 [2, 4]
- ↓ TNF [2, 4]
- ↓ MCP1 [2]
- ↓ sCD163 [2, 4]
- ↓ Activated CD4+ T cell number (CD38+DR+) [1, 3, 5]
- ↓ Activated CD8+ T cell number (CD38+DR+) [1, 3, 5]
- ↓ CD4+ T cell proliferation (Ki67) [1, 3]
- ↓ CD8+ T cell proliferation (Ki67) [1, 3]

1. Gandhi R et al., CROI 2017
2. Kaplan R et al., JAIDS 2012
3. Funderburg N et al., PLOS ONE 2013
4. Wada NI et al., AIDS 2015
Incomplete Normalisation

However, many markers of immune function do not normalise in comparison to the HIV negative population...

Systemic Inflammation

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Participants 33–44 years of age</th>
<th>Participants 45–76 years of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>hsCRP, µg/mL</td>
<td>140</td>
<td>2.13 (0.77–6.26)</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>138</td>
<td>1.89 (1.15–3.42)</td>
</tr>
<tr>
<td>D-dimer, µg/dL</td>
<td>140</td>
<td>0.21 (0.15–0.46)</td>
</tr>
<tr>
<td>Cystatin C, mg/dL</td>
<td>96</td>
<td>0.90 (0.76–0.97)</td>
</tr>
</tbody>
</table>

NOTE: Data are the median level and interquartile range (IQR), CARDIA, Coronary Artery Development in Young Adults; Diff., difference; MESA, Multi-Ethnic Study of Atherosclerosis; NA, not available; SMART, Strategies for Management of Anti-Retroviral Therapy.

Neuhaus et al., JID 2010

Incomplete Normalisation

T Cell Activation:

*CD4+ and CD8+ T cells that are CD38+HLA-DR+

Hunt P et al., JID, 2003
Incomplete Normalisation

Monocyte activation

O’Halloran J et al. HIV Med. 2015

Data from Jaworowski A et al., Presented by Crowe S at CROI 2016

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   a) Predictors
   b) Cardiovascular disease
   c) Bone disease
Mechanisms

Mechanisms for ongoing immune activation despite suppressive antiretroviral therapy

- Persistent & Low level viral replication
- Gut microbial translocation
- Antiretroviral toxicity
- Lifestyle factors & co-infections

Ongoing Immune activation
Mechanisms

HIV DNA and transcription is still detectable in PBMCs after 6 years suppressive ART

Viral reservoirs:
Cells harbouring replication competent HIV despite ART

• Resting, central memory CD4+ T cells
• Hematopoietic progenitor cells?
• CNS immune cells and microglia?
• Adipose tissue resident immune cells?

Extremely slow decay of latent reservoir:
Finzi D et al., Nat. Med. 1999
Gandhi R et al., CROI 2017

Mechanisms

HIV infection disrupts intestinal immune homeostasis leading to intestinal barrier disruption

“Leaky Gut” & microbial translocation
Mechanisms

HIV infection disrupts intestinal immune homeostasis leading to intestinal barrier disruption

"Leaky Gut" & microbial translocation

Microbial translocation increases with HIV progression and correlates with immune activation

Microbial translocation remains elevated despite suppressive ART

Brenchley J, Nat. Med. 2006
Funderburg N, PlosOne. 2013

Effect of ongoing immune activation

Chronic Inflammation – Root of all evil!
Effect of ongoing immune activation

Danish Cohort - Legarth/Obel, JAIDS, 2016

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Inflammation predicts mortality in HIV

A single measurement of IL-6 or D-dimer predicts morbidity/mortality over next 10y

Kaplan-Meier estimates of the cumulative proportion of participants who experienced serious non-AIDS disease or death (SNA/death), by quartiles of IL-6

Grund et al., PLoS One 2016

CD4:CD8 ratio

- HIV-induced CD4+ T cell loss is accompanied by increases in CD8+ T-cells, resulting in an inverted CD4:CD8 ratio
  - Normal ratio: ~2:1 (or 2) CD4:CD8
  - HIV: <1:1 (or <1) CD4:CD8
- Initiation of ART partially restores the CD4:CD8 ratio, through CD4+ T cell recovery
  - But not to normal levels due to persistent CD8+ T cell elevation
- CD4:CD8 ratio, a measure of immune restoration, may serve as a marker for the identification of those at risk of morbidity/mortality in treated HIV infection
Incremental Association between CD4:CD8 Ratio and Incidence of non-AIDS Events

P McGettrick¹,², W Tinago¹, A Lacey¹, A Macken¹, A Ni Fhlaitheartaigh¹, S Tennant¹, G Sheehan¹,², J S Lambert¹,² and PWG Mallon¹,²

1. HIV Molecular Research Group, School of Medicine, University College Dublin, Dublin, Ireland
2. Department of Infectious Disease, Mater Misericordiae University Hospital, Dublin

CROI 2016, Abstract 710

Biomarkers and outcome - CD4:CD8 ratio

- MMUH ID Cohort Study
- 550 PLWH started ART since Jan 2001
- 135 first time non-AIDS defining events
- 2557 person years of follow up (5.3 /100 PYFU)

Subject Characteristics:

<table>
<thead>
<tr>
<th></th>
<th>N=550</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>317 (58%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>299 (54%)</td>
</tr>
<tr>
<td>CD4+ current (cells/mm³)</td>
<td>545 (389-717)</td>
</tr>
<tr>
<td>CD4+ nadir (cells/mm³)</td>
<td>187 (80-284)</td>
</tr>
<tr>
<td>CD4:CD8 ratio current</td>
<td>0.7 (0.39-0.92)</td>
</tr>
</tbody>
</table>

Median (IQR)

McGettrick P et al., CROI 2016
Biomarkers and outcome - CD4:CD8 ratio

Factors associated with a non-AIDS event
(HR, 95% CI)

- Gender
  - Female (ref.)
  - Male
- Age at ART initiation
  - (per 10 yrs)
- Ethnicity
  - Caucasian (ref.)
  - African
  - Other/Unknown
- Mode of HIV transmission
  - Non-IDU (ref.)
  - IDU

Pre event CD4+/CD8+ ratio
0.26
0.27-0.43
0.44-0.59
0.60-0.86
≥0.87 (ref.)

<table>
<thead>
<tr>
<th>Gender</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.03</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>0.02</td>
</tr>
<tr>
<td>Mode of transmission</td>
<td>0.12</td>
</tr>
</tbody>
</table>

McGettrick P et al., CROI 2016

Clinical, immunological and treatment-related factors associated with normalization of CD4+/CD8+ T-cell ratio: effects of naïve and memory T-cell subsets

Tinago W1, Coghlan E1, Macken A1, McAndrews J2, Doak B,2 Fuller-Prior C2, Lambert J1,3, Sheehan G1,3, Mallon P1,3

1HIV Molecular Research Group, School of Medicine and Medical Sciences, University College Dublin, Ireland
2Department of Immunology, Mater Misericordiae University Hospital, Dublin, Ireland
3Department of Infections Diseases, Mater Misericordiae University Hospital, Dublin, Ireland

PLOS ONE May 9 2014; 9(5):e97011
The Mater Immunology Study

- Prospective cohort of HIV+ patients attending MMUH ID clinics
- Compared naïve and memory CD4+ and CD8+ T-cells with CD4:CD8 ratio

<table>
<thead>
<tr>
<th>Subject Characteristics:</th>
<th>N=190</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42 (36-48)</td>
</tr>
<tr>
<td>Gender: Male</td>
<td>123 (64%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>124 (65%)</td>
</tr>
<tr>
<td>Nadir CD4+ count (cells/mm³)</td>
<td>200 (112-309)</td>
</tr>
<tr>
<td>Current CD4+ count (cells/mm³)</td>
<td>465 (335-607)</td>
</tr>
<tr>
<td>CD4:CD8 ratio</td>
<td>0.6 (0.4-1.0)</td>
</tr>
<tr>
<td>CD4:CD8 ratio &gt;1</td>
<td>50 (26.3%)</td>
</tr>
</tbody>
</table>

Median (IQR)

*Tinago W et al., PlosOne 2014*
### Multivariable Regression Analysis for factors associated with CD4:CD8 ratio

<table>
<thead>
<tr>
<th>Factor</th>
<th>Coefficient</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
<th>Coefficient</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cumulative ART exposure (years)</td>
<td>+0.014</td>
<td>0.0042, 0.025</td>
<td>0.006</td>
<td>+0.014</td>
<td>0.0022, 0.026</td>
<td>0.021</td>
</tr>
<tr>
<td>Nadir CD4+ T-cell count (per 10cells/mm³ increase)</td>
<td>+0.011</td>
<td>0.0081, 0.014</td>
<td>&lt;0.001</td>
<td>+0.013</td>
<td>0.0084, 0.017</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Absolute CD8+ T-cell count (per 10cells/mm³ increase)</td>
<td>-0.0044</td>
<td>-0.0056, -0.0033</td>
<td>&lt;0.001</td>
<td>-0.0052</td>
<td>-0.0068, -0.0036</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>%CD4+ effector memory (CD45RO+CD62L-)</td>
<td>-0.0036</td>
<td>0.0074, 0.00006</td>
<td>0.054</td>
<td>-0.0057</td>
<td>-0.010, -0.0012</td>
<td>0.014</td>
</tr>
<tr>
<td>%CD8+ naive (CD45RO-CD62L+)</td>
<td>+0.0088</td>
<td>0.0044, 0.013</td>
<td>&lt;0.001</td>
<td>+0.0080</td>
<td>0.0028, 0.013</td>
<td>0.003</td>
</tr>
</tbody>
</table>

1Adjusted for age, gender, ethnicity, Hepatitis C status, and HIV RNA,  
2Adjusted for age, gender, ethnicity, Hepatitis C status  
NB: Coefficients of %CD4+, %CD8+ T-cells and their subsets are per 1% increase
CD4:CD8 Ratio - Summary

- Patients on suppressive ART with lower CD4:CD8 ratios have a higher risk for non-AIDS morbidity
  - MMUH ID Cohort
  - Serrano-Villar S et al., PLoS Pathog 2014

- Correlation analysis:

  ![CD4:CD8 Ratio Diagram]

  - Subjects with a higher %CD8 naive are more likely to attain a normalized CD4:CD8 ratio

Mortality in treated HIV

Causes of death in a successfully ART-treated population?
Mortality in treated HIV

Causes of death in a successfully ART-treated population:

SMART/ESPRIT: causes of death in N=3,280 HIV-infected persons receiving suppressive cART with CD4 counts ≥350 cells/mm³

% deaths

* = non-AIDS malignancy
** = accident, suicide or violent death

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Monocytes and CVD in HIV

- Aberrant monocyte/macrophage function is pivotal in the development of atherosclerosis and cardiovascular disease

Early pathogenesis of atherosclerosis

- Does persistent monocyte activation in treated HIV result in a heightened risk of CVD?

The Expression of Cholesterol Metabolism Genes in Monocytes From HIV-Infected Subjects Suggests Intracellular Cholesterol Accumulation

Eoin R. Feeney,¹,² Nuala McAuley,¹ Jane A. O’Halloran,² Clare Rock,² Justin Low,² Claudette S. Satchell,¹ John S. Lambert,² Gerald J. Sheehan,² and Patrick W. G. Mallon¹,²

¹HIV Molecular Research Group, School of Medicine, University College Dublin, Dublin, Ireland
²Department of Infectious Disease, Mater Misericordiae University Hospital, Dublin

JID 2013 Feb 15;207(4):628-37
Differences in monocyte gene expression with HIV

Cholesterol efflux genes

Regulators of cholesterol metabolism

The Monocyte Intracellular Cholesterol (MONIC) Study


1 HIV Molecular Research Group, School of Medicine and Medical Sciences, University College Dublin, Ireland
2 University of California Los Angeles, Los Angeles, CA, USA
3 Mater Misericordiae University Hospital, Dublin, Ireland

CROI 2017, Abstract 613
Differences in monocyte gene expression with HIV

Developed novel assay to measure monocyte cholesterol efflux \textit{ex vivo}

Functional confirmation of dysregulated monocyte cholesterol metabolism

\textit{O'Halloran et al., CROI 2017}

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### Prevalence of osteoporosis in HIV

Meta-analysis: Prevalence of osteoporosis in HIV-infected patients is > 3.5 times greater than in uninfected controls

<table>
<thead>
<tr>
<th>Study</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ameer (2004)</td>
<td>5.03 (91.47, 17.27)</td>
</tr>
<tr>
<td>Brown (2004)</td>
<td>4.28 (0.22, 2.84)</td>
</tr>
<tr>
<td>Bruera (2003)</td>
<td>4.51 (0.26, 79.27)</td>
</tr>
<tr>
<td>Dolan (2004)</td>
<td>2.11 (0.54, 8.28)</td>
</tr>
<tr>
<td>Huland (2002)</td>
<td>3.52 (0.15, 81.92)</td>
</tr>
<tr>
<td>Knobel (2001)</td>
<td>5.13 (1.80, 14.60)</td>
</tr>
<tr>
<td>Loiseau-Peres (2002)</td>
<td>4.28 (0.46, 53.81)</td>
</tr>
<tr>
<td>Madeddu (2004)</td>
<td>25.84 (1.80, 484.92)</td>
</tr>
<tr>
<td>Tebas (2000)</td>
<td>2.40 (0.19, 61.57)</td>
</tr>
<tr>
<td>Tolchman (2003)</td>
<td>17.41 (0.97, 313.73)</td>
</tr>
<tr>
<td>Yin (2005)</td>
<td>2.37 (1.09, 5.16)</td>
</tr>
<tr>
<td><strong>Overall (95% CI)</strong></td>
<td><strong>3.68 (2.31, 5.84)</strong></td>
</tr>
</tbody>
</table>

Odds ratio = odds of osteoporosis (T-score ≤ -2.5) in HIV-infected patients vs HIV-uninfected controls.

*Figure adapted from Brown TT, et al. AIDS 2006*

### Understanding the Pathology of Bone Disease in HIV-infected Patients

**HIV UPBEAT Study**

HIV Molecular Research Group

*Cotter AG et al., AIDS 2014; 28(14):2051-60*

*Tinago W et al., AIDS 2017, Mar 13;31(5):643-652*
HIV UPBEAT

• Prospective cohort with annual visits
• HIV+ and HIV- subjects from similar demographic backgrounds
• Bone assessments:
  • Questionnaires - bone health, fracture history, falls
  • Dual X-ray Absorptiometry (DXA) scan – bone mineral density (BMD)

Subject Characteristics:

<table>
<thead>
<tr>
<th></th>
<th>HIV+ (n=210)</th>
<th>HIV- (n=264)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Male</td>
<td>123 (58.6)</td>
<td>115 (43.6)</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>39 (33, 46)</td>
<td>42 (34, 49)</td>
</tr>
<tr>
<td>African ethnicity</td>
<td>83 (39.5)</td>
<td>65 (24.6)</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>26 (23, 30)</td>
<td>27 (24, 30)</td>
</tr>
<tr>
<td>Smoker</td>
<td>73 (34.8)</td>
<td>44 (16.7)</td>
</tr>
</tbody>
</table>

* Median (IQR)

HIV UPBEAT

Change in BMD over 96 weeks

• No significant differences in rate of BMD decline in HIV+ vs HIV-
• BMD decline in HIV+ was associated with:
  • Starting ART in previous 3 months
  • Being naive to ART

* Tinago W et al., AIDS 2017
Bone disease and HIV – role of inflammation

Emerging data have highlighted the importance of immune function to bone turnover balance and the development of osteoporosis*

HIV UPBEAT - Year 5 (in progress)

Objectives:
• To compare the immunophenotype (T-cell activation and innate immune activation) of HIV-positive persons versus HIV-negative persons
• To assess the effect immune activation on bone health outcomes (BMD, TBS and fracture)

*Mcginty T et al., Curr Opin HIV AIDS 2016

Bone disease and HIV – role of inflammation

HIV UPBEAT - Year 5

Methods
• Bone health: DXA scans and questionnaires
• Flow cytometry: monocyte and T-cell activation in cryopreserved PBMCs
• Multiplex Immunoassays: cytokines and inflammatory markers

To be continued...
Bone disease and HIV – role of inflammation

• Emerging data have highlighted the importance of immune function to bone turnover balance and the development of osteoporosis*

HIV UPBEAT - Year 5 (in progress)

Objectives:
• To compare the immunophenotype (T-cell activation and innate immune activation) of HIV-positive persons versus HIV-negative persons enrolled in the HIV UPBEAT study
• To assess the effect immune activation on bone health outcomes (BMD, TBS and fracture).

*Mcginty T et al., Curr Opin HIV AIDS 2016

Summary

• Despite the success of ART in extending life expectancy, immune activation persists with suppressive therapy

• The causes of ongoing immune activation are multifactorial with persistent viral reservoirs and microbial translocation likely mechanisms

• In a growing HIV+ population that is aging, using inflammatory markers to identify those at risk of mortality/morbidity will become increasingly important

• Ongoing immune activation provides a conceptual framework to understanding common comorbidities in HIV: CVD and bone disease
But… progress brings its own challenges

Oversteegen L et al., Nat Rev. Drug Discov 2007

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- Dr Jack Lambert

MMUH Immunology
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- Charlotte Prior-Fuller

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- Prof Alan Landay (Rush U)
- Dr Theodoros Kelisidis (UCLA)
- Therese Herlihy (UCD)