Aging in the era of ART and the impact on immunity

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Changing face of AIDS: HIV-infected population is aging

Why focus on Older Age Group?

The world’s population is Aging

In the US, the number of 65+ people is projected to grow rapidly

The Aging of the HIV Epidemic in the US

CDC Surveillance Data
Some Aging Phenotypes: Manifest sooner in ARV treated HIV

- Cardiovascular disease
- Cancers
- Osteoporosis & Fragility fractures
- Diabetes mellitus
- Frailty
- Neurocognitive decline

Unanswered Questions
- Do both groups share all “aging” phenotypes?
- Concept of accelerated aging- is this true for all phenotypes?
- Are underlying mechanisms the same?

Source: Peter Reiss, Univ of Amsterdam
Objective:

Evaluate healthy controls (HC) and ART-suppressed HIV in different age groups with the following aims:

1. To Investigate baseline immunologic biomarkers (e.g. immune activation and inflammation)
2. To develop predictors of immunologic aging and define rate of immunologic aging
3. To evaluate immune competence by investigating serologic response to seasonal influenza vaccine
4. To investigate immunologic determinants of influenza vaccine response
Immunity in HIV and Aging Project

Study Participants

**HIV+ (154)**
- Virally suppressed (plasma virus load < 50 copies/ml) on cART for ≥1 year prior to enrollment

**HIV negative (161)**
- Healthy controls (HC)

**Age Groups**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>HC</th>
<th>HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young (Y), 18-39 years</td>
<td>48</td>
<td>34</td>
</tr>
<tr>
<td>Middle (M), 40-59 years</td>
<td>62</td>
<td>70</td>
</tr>
<tr>
<td>Old (O), 60-80 years</td>
<td>51</td>
<td>50</td>
</tr>
</tbody>
</table>

**Mean ART Duration**
- Young (Y): 6 yr
- Middle (M): 11 yr
- Old (O): 13 yr

**Absol CD4**
- HIV:
  - Young (Y): 1272
  - Middle (M): 984*
  - Old (O): 872*
RNA Seq: Effect of HIV and Age on PBMC: Differentially Expressed Genes (DEG)

Top 50 DEGS in young vs old, (n=48; 12/group)

Total DEGs, young vs old: 10x more in HC than HIV

PBMC transcriptional background differs in Young and Old: Unlike HC, HIV Young show fewer differences from HIV Old

M Cameron, B Richardson: Case Western Reserve Univ.
S Williams, S Rinaldi: U Miami
Evaluation of Immunologic Biomarkers in HC and HIV

Eight 15 color FC panels +26 plasma cytokines

**Soluble Plasma Biomarkers**
- Pro-Inflammatory (TNFa, IL-6)
- Soluble Receptors (sTNFR, IL2R)
- Microbial Translocation (sCD14, LPS)

**Immune Cell Distribution**
- T cell subset distribution
  - CD4 and CD8 absolute counts,
  - Naïve, Memory, pTfh
- B cell subsets
- Monocyte subsets

**Immune Activation/Checkpoint**
- CD38, HLA-DR
- PD-1, Ki-67, etc

**Immune signatures of**
A. Age
B. Immune Competence
Reevaluation of immune activation in the era of cART and an aging HIV-infected population

Lesley R. de Armas,1 Suresh Pallikkuth,1 Varghese George,1 Stefano Rinaldi,1 Rajendra Pahwa,1 Kristopher L. Arheart,2 and Savita Pahwa1

HIV is associated with far greater immune activation than HC and this is independent of age

Frequency of cells co-expressing >1 immune activation markers is greater in HIV than in HC

(CD38, HLA-DR, PD-1, ICOS, Ki-67)
### Plasma Biomarkers: Correlation with Age in HIV and HC

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>HIV+</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Correlation with Age</strong></td>
<td><strong>Correlation with Age</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( r ) value</td>
<td>( P ) value</td>
</tr>
<tr>
<td>sCD25</td>
<td>0.26</td>
<td>0.0009</td>
</tr>
<tr>
<td>MCPI</td>
<td>0.25</td>
<td>0.001</td>
</tr>
<tr>
<td>sICAM</td>
<td>0.15</td>
<td>0.048</td>
</tr>
<tr>
<td>IL-17A</td>
<td>-0.26</td>
<td>0.0007</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.95</td>
<td>0.20</td>
</tr>
<tr>
<td>Neopterin</td>
<td>0.31</td>
<td>6.86 ( \times ) 10^{-5}</td>
</tr>
<tr>
<td>sVCAM</td>
<td>0.28</td>
<td>0.0003</td>
</tr>
<tr>
<td>sTNFRI</td>
<td>0.20</td>
<td>0.012</td>
</tr>
<tr>
<td>sTNFRII</td>
<td>0.20</td>
<td>0.01</td>
</tr>
<tr>
<td>sCD14</td>
<td>0.18</td>
<td>0.021</td>
</tr>
<tr>
<td>sCD163</td>
<td>0.19</td>
<td>0.018</td>
</tr>
</tbody>
</table>

IFN-\( \gamma \), IL-6, TNF-\( \alpha \), D-dimer, LPS, BNP, CRP, and IL-21 did not show a relationship with age in HC or HIV.

- **Red box**: Common for HIV and HC; **Green Box**: only in HC

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**Comparison of Plasma Biomarkers**

**HIV vs HC**

- Aging is associated with inflammation in HC and HIV
- Greatest increase in biomarkers is in young HIV vs young HC
### 14 parameter model

209 PID with 1357 markers

Linear Regression, 10X Cross Validation (CV)

**HC:** R squared: 76.88% RMSE 6.1 years  
**HIV:** R squared: 18.67% RMSE 11.23 years

<table>
<thead>
<tr>
<th>Key</th>
<th>PanelID</th>
<th>Key Name</th>
<th>Coefficient HC</th>
<th>Coefficient HIV</th>
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<tbody>
<tr>
<td>IM1</td>
<td>P1.68</td>
<td>Th2 non-pTfh</td>
<td>4.2303</td>
<td>-0.0468</td>
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<tr>
<td>Cyt1</td>
<td>Cyt17</td>
<td>Plasma Neopterin</td>
<td>3.1140</td>
<td>0.1050</td>
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<tr>
<td>IM2</td>
<td>P6.56</td>
<td>Th1/Th17 pTfh</td>
<td>3.1038</td>
<td>2.8408</td>
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<tr>
<td>IM3</td>
<td>P1.260</td>
<td>MFI of PD1 on CD8 TTM</td>
<td>3.0199</td>
<td>-1.9082</td>
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<tr>
<td>IM4</td>
<td>P5.81</td>
<td>CD57+CD28- Effector CD8</td>
<td>2.9525</td>
<td>2.4658</td>
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<tr>
<td>IM5</td>
<td>P6.14</td>
<td>CD4 (%CD3)</td>
<td>2.8922</td>
<td>0.6872</td>
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<tr>
<td>IM6</td>
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<td>MFI of PD1 on CD8 TCM</td>
<td>2.7821</td>
<td>2.7649</td>
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<tr>
<td>IM7</td>
<td>P8.11</td>
<td>Non classical monocytes</td>
<td>2.6930</td>
<td>-0.6723</td>
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<tr>
<td>Cyt2</td>
<td>Cyt25</td>
<td>Plasma D-Dimer</td>
<td>2.6418</td>
<td>2.6085</td>
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<td>IM8</td>
<td>P1.75</td>
<td>ICOS+ Th2 non-pTfh</td>
<td>-1.5351</td>
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<tr>
<td>IM9</td>
<td>P1.63</td>
<td>PD1+ Th1/Th17 non-pTfh</td>
<td>-2.1611</td>
<td>1.9488</td>
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<tr>
<td>IM10</td>
<td>P5.69</td>
<td>2B4+ CD8 TCM (% CD8 TCM)</td>
<td>-2.6969</td>
<td>-0.6618</td>
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<tr>
<td>IM11</td>
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<td>PD1+MEMORY TREG</td>
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<tr>
<td>IM12</td>
<td>P1.247</td>
<td>CD38+ CD8 TCM</td>
<td>-4.8441</td>
<td>-4.5514</td>
</tr>
</tbody>
</table>

**Total Y M O**

**HC**

<table>
<thead>
<tr>
<th></th>
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<th>Y</th>
<th>M</th>
<th>O</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>103</td>
<td>35</td>
<td>50</td>
<td>18</td>
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**HIV**

<table>
<thead>
<tr>
<th></th>
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<th>M</th>
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<tr>
<td></td>
<td>106</td>
<td>20</td>
<td>61</td>
<td>25</td>
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*Prediction power for HIV using 14 parameter model is poor*
25 parameter model

A. Correlation of predicted age/Age

HC: highly correlated (Spearman r=0.84, p<0.001),
HIV: moderately correlated (Spearman r=0.65, p<0.001)

B. Aging Rate (Predicted Age /Actual Age):
Not significantly different for HC and HIV (all age groups)
Young HIV age faster than young HC by 18.47%

C. Age Advancement (Predicted Age – Actual Age)
Young HIV age advanced 5.62 years than young HC
Assessment of response to influenza vaccine as a measure of immune competence
Risk for infection in elderly is very high; ~60% of influenza hospitalizations in 2017-18 were in adults >65 yrs

HIV are at greater risk than the uninfected

Yearly vaccination is recommended for elderly and immune compromised

Vaccine efficacy is variable; depends on the vaccine and host immune response; poorer response in HIV

Influenza vaccines can be used to probe components of the immune system
HIV and Aging affect Serologic Response to Flu vaccine

- Young HC have the best vaccine response
- Fewer vaccine responders in HIV+ than in HC

Vaccine Responders (VR):
> 4-fold increase in titers, T0 to T2

Vaccine Non-Responders (VNR):
< 4-fold increase in titers, T0 to T2

HIV negative

HIV+, ART suppressed, VL<50

Sample: PBMC, Plasma, Serum

Week

Influenza Vaccination

Time

T0 T1 T2 T3

% of Individuals

VR VNR Other

*** ** *

H1N1 Ab

H1N1, H3N2, B Ab

Time Points

H1N1 Geometric mean Titer

Young Old

128 256 512

% of Individuals

H1N1, H3N2, B Ab

Time Points

T0 T1 T2 T3

Vaccine Responders (VR):

Pallikkuth
AIDS 2018
Immunologic Determinants of an Antibody Response

CXCR5+ CD4 T cells subset: T follicular helper cells (Tfh) in LN
Peripheral (p)Tfh in blood
Quantitative and qualitative assessment of H1N1 antigen-specific CD4 pTfh cells

- Phenotype and Co-stimulatory molecules
- Function (ICS): IL-21, IL-2, TNF, IL-17

Identification of antigen-specific pTfh (CD40L+CD69+)

Antigen-induced proliferation of pTfh (cell trace dye dilution)

Multi-parameter 15 Color Flow Cytometry

PBMC

12 hrs

5 days

+/- H1N1 peptide + αCD28

Brefeldin A Last 4 hours
Functional characteristics of H1N1-specific pTfh at T2 are distinct in vaccine responders and non-responders

**VR** = Vaccine responder  
**VNR** = Vaccine-non responder

**ICS in H1N1-specific pTfh (CD40L+CD69+) at T2**

### Frequency (%)

- **CD40L**
- **IL-21**
- **IL-2**

### Proliferation

- **pTfh**
- **ICOS**
- **TNF**
H1N1-specific pTfh at T2: Impact of Age and HIV

In VR, HIV- have better quantity and quality than HIV+, Young HIV- are best; No correlation with age.
IL-21 and ICOS pTfh at T2: Impact of Age and HIV

Y- show higher responses than all others, while Y+ similar to O+
IL-21+ and ICOS+ pTfh correlate inversely with age in HC VR
IL-2 and TNF Ag.pTfh at T2: Impact of Age and HIV

VNR do not differ across age or HIV infection status
Can we improve the B cell function?

PBMC (HIV+, HC) from VR, VNR at T2

Mem B cells (CD20+CD27+)

pTfh
CD3+CD4+
CD45RO+CXCR5+

APC
CD3-CD8-CD56-

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FACS Cell sorting

Co-culture x 7d
+/- H1N1 Ag +/- IL-21+anti-IL-2+antiTNFa (cytokines)

Analysis

IgG in culture supernatants

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Can we improve the B cell function?

**Antagonism of IL-2 and TNF in vitro enhances Ab response in VR and partially rescues VNR in experimental model**
Summary: Determinants of flu vaccine-induced Ab Response

**Monocytes: Detrimental at T0**
Inflammatory monocytes with ↑CD11b (in HIV)

**Favorable, Post-vaccine (Ag.pTfh)**
- Proliferation
- ↑IL-21+, ICOS+
- ↓IL-2, TNF and IL-17
- Ag-specific IgG+ in B cell co-cultures

**Detrimental, pTfh at T0:**
- ↓frequency
- ↑CD38+HLA-DR
- ↑PD-1

Post-vaccine (Ag.pTfh)
- ↑IL-2, TNF and IL-17

**B cells: Detrimental**
- T0: ↑PDL1+ B cells in HIV
- ↑DN B cells
- T2: ↑PTEN expression in HIV

- Pallikkuth J Immunol 2011; JACI 2011; Blood 2012;
- Rinaldi Aging 2017
- de Armas JCI Insight 2018
- George AIDS 2018;
- de Armas Sci Rep, 2019
Overall Summary: Aging, HIV and Immunity

- Immunologic signature of aging in HIV+ is not identical to HC; differences in immunologic phenotype most evident in young
- Precocious Immunologic aging in Young HIV+ is by 5.6 yr
- A higher state of CD4 immune activation pre-vaccination adversely influences Ab response to flu vaccine
- Old age and HIV+ have less VR for flu vaccine (frequency and magnitude) than HC; best flu response is seen in young HC
- Quantity and quality of pTfh influence vaccine response status; good response features of pTfh best seen in young HC
- pTfh in HIV and HC VNR exhibit similar features that are age-independent
- Understanding mechanism of pTfh impairment may help in developing interventions to improve immune responses
Acknowledgements

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