Inflammation, HIV and Aging

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University of Miami Miller School of Medicine

HIV DART, Miami
28 NOVEMBER 2018
The HIV infected population is aging

Why focus on Older Age Group?

Aging population is increasing worldwide

Top 10 states for new residents age 65+

In the US, Florida has the highest percentage of elderly people (19.4%)

The Aging of the HIV Epidemic in the US

CDC Surveillance Data

- Number of People Living with HIV: 1.25 Million
- Age 50 and Older In 2011: 37%
- Age 50 and Older In 2015: 50%
- Age 50 and Older In 2020: 70%
Features of Natural Aging and of HIV

Chronologic Aging - associated with
• Inflammation---termed inflammaging –driver of comorbidities
• Immune senescence- declining immunity – increased susceptibility to infections

Chronic HIV (on ART) - associated with
• Inflammation and immune activation
• Earlier development of comorbidities- ?Premature aging
• Immune compromised state- ?Premature aging
Prevailing questions:

A. What are independent and combined effects of each state (i.e. aging and HIV) on measurements of IA and inflammation?

B. How does each state (and combined) affect immune competence?
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

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<th>Age Group</th>
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Evaluation of Immunologic Biomarkers

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

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

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

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**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, Kristopher L. Arheart,2 and Savita Pahwa1

Plasma Biomarkers: Correlation with Age in HIV and HC

Table 3. Correlation of plasma biomarkers with age in study participants

<table>
<thead>
<tr>
<th></th>
<th>HIV Negative</th>
<th>HIV Positive</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Correlation with Age</td>
<td>Correlation with Age</td>
</tr>
<tr>
<td></td>
<td>r value</td>
<td>P value</td>
</tr>
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</tr>
<tr>
<td>Neopterin</td>
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</tr>
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</tr>
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IFN-γ, IL-6, TNF-α, 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
Reevaluation of immune activation in the era of cART and an aging HIV-infected population

Lesley R. de Armas, Suresh Pallikkuth, Varghese George, Kristopher L. Arheart, and Savita Pahwa

Plasma Biomarkers: Correlation with Age in HIV and HC

Aging is associated with inflammation in HC and HIV, with more cytokines associated with healthy aging. However, differences between HC and HIV more evident in young age.

<table>
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<th>Protein</th>
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<tr>
<td>sCD25</td>
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IFN-γ, IL-6, TNF-α, D-dimer, LPS, BNP, CRP, and IL-21 did not show a relationship with age in HC or HIV.

Young HIV (age <40 years) show most differences from HC.
Frequency of cells co-expressing >1 immune activation markers is greater in HIV than in HC

(PD1, CD38, HLA-DR, ICOS, Ki-67)

de Armas et al, JCI insight, 2017
Immune Activation: Correlation with Age in HIV and HC

HIV is associated with far greater immune activation than HC, most of which is age-independent.

Immune markers include CD38, HLA-DR, PD-1, ICOS, and Ki-67.

de Armas et al, JCI insight, 2017
Immunity in HIV and Aging
Evaluation of Immune Competence

HIV and Aging have independent and combined effects on IA and inflammation

How does each state (and combined) affect immune competence?
FLORAH Project (FLu Responses Of people in relation to Age and HIV): Study Design

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

**HIV negative (161) healthy controls (HC)**

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Influenza Vaccination* (2012-2015)

- Day 0 (T0)
- Day 7 (T1)
- Day 21-28 (T2)
- Month 5-7 (T3)

Trivalent Influenza vaccine (TIV):
- H1N1/09*
- H3N2
- B

Sample (T0):
- PBMC
- Plasma
- Serum

Sample (T1):
- PBMC
- Plasma
- Serum

Sample (T2):
- PBMC
- Plasma
- Serum

Sample (T3):
- PBMC
- Plasma
- Serum

*H1N1 strain included in vaccine since 2009 epidemic
HIV and Aging affect Serologic Response to Flu vaccine

**Vaccine Responders (VR):** ≥ 4-fold increase in titers from pre-vaccination (T0) to post-vaccination (T2)

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

H1N1 Ab

H1N1, H3N2, B Ab

% of Individuals

Y-: Young, HIV-  O-: Old, HIV-
Y+: Young, HIV+  O+: Old, HIV+

Suresh Pallikkuth

_Pallikkuth, AIDS 2018_
**HIV and Aging affect Serologic Response to Flu vaccine**

**Vaccine Responders (VR):** ≥ 4-fold increase in titers from pre-vaccination (T0) to post-vaccination (T2)

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

**Suresh Pallikkuth**

Fewer vaccine responders in HIV+

Difference from HC most prominent in young HIV+

**Y-:** Young, HIV-
**O-:** Old, HIV-
**Y+:** Young, HIV+
**O+:** Old, HIV+

*Pallikkuth, AIDS 2018*
Immunologic Components of an Antibody Response

- APC
- ICOSL
- MHCII
- IL-12R
- IL21
- Tfh
- Ascl2
- BCL-6
- MAF
- IL4
T follicular helper cells (Tfh)

CD4 T cell

T follicular helper cell
CD4+CD45RO+CD27+CXCR5+

Tfh
Th1
Th2
Th17
Treg

Mature memory CD4 T cell

CD4 T cell subsets

Th2: CXCR3\textsuperscript{neg}CCR6\textsuperscript{neg}CCR4\textsuperscript{+}

Th1: CXCR3\textsuperscript{+}CCR6\textsuperscript{neg}CCR4\textsuperscript{neg}

Th17: CXCR3\textsuperscript{neg}CCR6\textsuperscript{+}CCR4\textsuperscript{neg}

Th1/Th17: CXCR3\textsuperscript{+}CCR6\textsuperscript{+}CCR4\textsuperscript{neg}

Tfr: FOXP3\textsuperscript{+}

Tfh subsets
Antigen-Induced Tfh and B Cell interaction to develop Antibody secreting cells

- Somatic Hypermutation
- Class switch Recombination

GC reaction

Switched Memory B cells

Plasmablast

Plasma cell

Ab secreting cells

Short lived plasmablast

Experimental Protocol: quantitative and qualitative assessment of Flu antigen-specific CD4 pTfh cells

+/- flu peptide + αCD28
Brefeldin A Last 4 hours

12 hrs
5 days

• Phenotype: Activation/exhaustion, co-stim molecules
• Function (ICS): IL-21, IL-2, TNF, IL-17

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

for quantitative and qualitative assessment if Ag specific pTfh

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

PBMC
Gating strategy for Flu antigen specific CD4 pTfh cells

Ag-specific pTfh
Frequencies of Antigen specific pTfh at T2 post-vaccination are greater in VR

![Graph showing comparison between VR and VNR groups in frequencies of CD40L+CD69+pTfh and CD40L+CD69+pTfh. The VR group shows significantly higher frequencies compared to the VNR group.](image)
Molecular interactions of Tfh and B Cells in the GC: Role of ICOS

Nutt SL, Nat Immunol 2011
Summary: Immunologic components and determinants of a successful antibody response in Vaccine responders

B cell features post-vaccination: Increases occur in
- Plasmablasts and spontaneous Ab secreting cells (ASC) at T1
- Memory B cells and switch memory at T2
- IL-21R on memory B cells, T2
- Plasma cells at T2

pTfh cell features post-vaccination:
- Expansion of bulk pTfh and Ag+ pTfh at T1, T2
- IL-21 production at T2
- Upregulation of ICOS at T1, T2
- Ag-specific IgG in pTfh:B cell co-culture at T2

Pallikkuth J Immunol 2011; JACI 2011; Blood 2012; Rinaldi Aging 2017 de Armas JCI Insight 2018 George AIDS 2018;
Summary: Features of immune cells that negatively influence influenza vaccine responses in **Vaccine non-responders**

- **B cells**
  - PDL1 at T0*
  - DN B Cells at T0*

**Monocytes:**
- Inflammatory monocytes
- CD11b high at T0

**pTfh phenotype and function:**
- CD38+HLA-DR at T0
- PD-1 high at T0
- ICS showing TNF, IL-2, IL-17 NOT IL-21 at T2.
Summary of Cellular Basis of Flu Ab responses

• We have identified what constitutes good and bad features of immune cells responding to flu vaccine antigens

• Best vaccine responses are associated with generation of antigen-specific pTfh cells that show:
  o Proliferation in response to flu antigens
  o Induction of ICOS and IL-21 secretion post-vaccination
  o Low basal activation (HLADR, CD38, and PD1)
  o Low to absent secretion of TNF and IL-2 following antigen stimulation
The Effects of Aging and HIV infection on Tfh:B cell Responses to Influenza Vaccination

HIV infected young individuals resemble old individuals making the differences between young and old less evident
Acknowledgements

Pahwa Lab

Study Participants

University of Miami
Margaret Fischl, MD
Gordon Dickinson, MD
Allan Rodriguez, MD
Maria Alcaide, MD
Katherine Klose

THE MIAMI CENTER FOR AIDS RESEARCH

UNIVERSITY OF MIAMI MILLER SCHOOL OF MEDICINE

NATIONAL INSTITUTES OF HEALTH

National Institute of Allergy and Infectious Diseases
Thank you