Inflammation - the culprit behind all ills?

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CART 2018
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Acute inflammation normally occurs in response to physical injury such as a cut or sprain, or an infection. It is a protective immune response. Acute inflammation ends when the problem is resolved.
Ongoing inflammation is associated with many chronic diseases. These also include kidney problems, metabolic syndrome, dementia, and frailty.
HIV and immune response

- **Acute Phase**
  - Entry
  - Eclipse Phase

- **Chronic Phase**
  - Limit of detection of plasma HIV
  - Latent HIV Reservoir

- Plasma HIV RNA copies/ml
  - 100d
  - years

Pahwa, CART 2018
Acute HIV-1 Infection induces a cytokine storm, as reflected in plasma analyte levels

Andrea R. Stacey et al. J. Virol. 2009;83:3719-3733
Pahwa, CART 2018
Acute HIV and immune response

Acute Phase

Eclipse Phase

Latent HIV Reservoir

Chronic Phase

Limit of detection of plasma HIV

100d

years

Plasma HIV RNA copies/ml

Entry

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Chronic HIV and persistent immune activation

Acute Phase

Chronic Phase

Entry

Eclipse Phase

Latent HIV Reservoir

Limit of detection of plasma HIV

CD4 counts

Plasma HIV RNA copies/ml

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HIV: Common questions about inflammation

What causes it?

- Compromised intestinal integrity during acute inf;
- Gut Microbial Translocation
- Co-Infections: CMV, EBV
- Immune Dysregulation
- HIV Persistence

Why is it bad?

- End-organ disease, non-AIDS complications
- Fibrosis in lymph nodes
- Immune senescence
- Impaired immune function
- Viral Reservoirs

microbiome

Pahwa, CART 2018
Today’s discussion: Two studies related to inflammation/immune activation

**Immunity in HIV and Aging:**
US Cohort (on ART, Virally suppressed)

**HIV and Cardiovascular disease:**
Chennai Cohort (Includes patients not on ART and on ART)

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Immunity in HIV and Aging - Florah Project

(FLu Responses Of people in relation to Age and HIV)

Rationale:

• Natural biologic aging and HIV are both associated with increased incidence of infections

• Still debated if in treated HIV, immune senescence is hastened, also known as “accelerated aging”

Goals:

• Evaluate immunologic changes associated with healthy aging

• Evaluate impact of controlled HIV infection on immunity in young and old - commonalities and differences
Questions

1: Is the immune system of old “healthy” people worse than that of young

2. Does HIV lead to “accelerated aging” –how “early” can you detect it--- i.e. can you see it in young HIV infected?

3. Is the immune system of Old people with HIV worse than the immune system of Old people without HIV

4. Can the baseline immune parameters predict response to vaccine?
Immunologic Investigations

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

- **T cell immune activation**
  - CD38, HLA-DR
  - PD-1, Ki-67

- **Soluble Plasma Biomarkers**
  - Inflammatory (TNFa, IL-6)
  - Soluble receptors (TNFR, IL2R)
  - Microbial Translocation (sCD14, LPS)

**Outcomes**
- Age
- HIV status
- Serological Vaccine Response

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1. Is the immune system of old healthy people worse than that of young? **YES**

2. Does HIV lead to “accelerated aging” – can you see it in young HIV infected? **YES**

3. Is the immune system of Old people with HIV worse than that of Old people without HIV? (**???)

4. Can the baseline immune parameters predict response to vaccine? (?)
Paradoxical aging in HIV: immune senescence of B Cells is most prominent in young age

Stefano Rinaldi¹,*, Suresh Pallikkuth¹,*, Varghese K. George¹, Lesley R. de Armas¹, Rajendra Pahwa¹, Celeste M. Sanchez¹, Maria Fernanda Pallin¹, Li Pan¹, Nicola Cotugno⁴, Gordon Dickinson², Allan Rodriguez², Margaret Fischl³, Maria Alcaide², Louis Gonzalez¹, Paolo Palma⁴, Savita Pahwa¹

Combination antiretroviral therapies (cART) can lead to paradoxical aging in young age. People aged >50 yrs represent the fastest growing population. The immune system is associated with impaired humoral immunity, immune senescence, and T cell dysfunction.

In this study influenza vaccination was used to probe immune function in young HIV-positive (n=124), grouped by age as young (<40 yrs), middle-aged (40-59 yrs), and old (>60 yrs). d21 post-vaccination correlated inversely with age in PBMC demonstrated increased frequencies of double-negative (DN) cells in young HIV compared to young HC. Remarkably, young HIV were downregulated for CD38 and CD27 phenotype, influenza specific spontaneous (d7) or memory (d21) immune cell responses were not significantly associated with age. 

Figure 2: ex vivo B cells response in Y+ is similar to old

Spontaneous H1N1 specific ASC/million PBMC T1 (log2)

4096
1024
256
64
16
4
1
Y- O- Y+ O+

Stefano Pahwa, CART 2018
4. Can the baseline immune parameters predict response to vaccine?

Reevaluation of immune activation in the era of cART and an aging HIV-infected population

Lesley R. de Armas, Suresh Pallikkuth, Varghese George, Stefano Rinaldi, Rajendra Pahwa, Cristopher L. Arheart, and Savita Pahwa

Department of Microbiology and Immunology, and Department of Epidemiology and Public Health, Division of Biostatistics, University of Miami Miller School of Medicine, Miami, Florida, USA.

Biological aging is associated with immune activation (IA) and declining immunity due to systemic inflammation. It is widely accepted that HIV infection causes persistent IA and premature immune senescence despite effective antiretroviral therapy and virologic suppression; however, the effects of combined HIV infection and aging are not well defined. Here, we assessed the relationship between markers of IA and inflammation during biological aging in HIV-infected and -uninfected populations. Antibody response to seasonal influenza vaccination was implemented as a measure of immune competence and relationships between IA, inflammation, and antibody responses were explored using statistical modeling appropriate for integrating high-dimensional data sets. Our results show that markers of IA, such as coexpression of HLA antigen D related (HLA-DR) and CD38 on CD4+ T cells, exhibit strong associations with HIV infection but not with biological age. Certain variables that showed a strong relationship with aging, such as declining naive and CD38+ CD4 and CD8+ T cells, did so regardless of HIV infection. Interestingly, the variable of biological age was not identified in a predictive model as significantly impacting vaccine responses in either group, while distinct IA and inflammatory variables were closely associated with vaccine response in HIV-infected and -uninfected populations. These findings shed light on the most relevant and persistent immune defects during virological suppression with antiretroviral therapy.
Age associated changes in HIV compared to HC

Plasma Biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>HIV vs HC</th>
<th>Fold Change (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Dimer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFNγ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL17α</td>
<td></td>
<td></td>
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<tr>
<td>IL-6</td>
<td></td>
<td></td>
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<tr>
<td>IL-21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BNP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sCD14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sCD25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sICAM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sVCAM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neopterin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sCD163</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sTNFRI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS</td>
<td></td>
<td></td>
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<tr>
<td>sTNFRII</td>
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</tr>
</tbody>
</table>

Age (yrs)

≤40
41-59
≥60

CD38+HLADR + CD4+ T cells are higher in HIV than HC and show no relationship with Age

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

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>HIV Negative Correlation with Age</th>
<th>HIV Positive Correlation with Age</th>
</tr>
</thead>
<tbody>
<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>MCP1</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-17α</td>
<td>-0.26</td>
<td>0.0007</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.20</td>
<td>0.0036</td>
</tr>
<tr>
<td>Neopterin</td>
<td>0.25</td>
<td>6.86 x 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>

Young HIV (age <40 years) show most differences from HC

de Armas et al, JCI insight, 2017
Pahwa, CART 2018
Modelling using LASSO: discrimination of influenza vaccine responders and non-responders

T cell subset distribution
CD4 and CD8 absolute counts
Naïve, Memory, pTfh
B cell subsets
Monocyte subsets

T cell immune activation
CD38, HLA-DR
PD-1, Ki-67

Soluble Plasma Biomarkers
Inflammatory (TNFα, IL-6)
Soluble receptors (TNFR, IL2R)
Microbial Translocation (sCD14, LPS)

Healthy controls
PLSDA: Response Score

A

B

Influenza vaccine response score

Baseline immune characteristics can predict influenza vaccine responders (red) and non-responders (blue) in HC and HIV, but their immune signature is different, with some overlap

Li Pan

de Armas et al, JCI insight, 2017
Pahwa, CART 2018
HIV-related immune senescence is clearly evident in young HIV+ patients, is characterized by a core signature of increased immune activation of CD4+ T cells, and is distinct from biological aging.

Biomarkers predictive for Ab response to vaccination are not identical between HIV and Healthy Controls.

We hypothesize that immune activation and inflammation underlie immune dysfunction resulting in impaired vaccine response, but components most likely differ from those involved in comorbid health events.
HIV and Cardiovascular disease: Chennai Cohort

Indo-US collaboration R21 grant AI106373: Immune Activation in Virologically Suppressed Indian HIV-infected Patients

Members of YRG Care Team

B. Kausalya

N. Kumarasamy

S. Saravanan

Pahwa, CART 2018
1. What is the relationship of CD4 count on cardiac function as well as measures of immune activation, inflammation?

2. How do patients on ART differ from treatment naïve viremic patients?

3. Which immune measures correlate with cardiac function?
Study Design: Chennai Cohort, Mean Age 37.5 yr Cross-sectional study

HIV+ Untreated (RX naïve)

Group 1
N=102

1a. CD4<200
1b. CD4 200-350
1c. CD4 >350

HIV+ On ART

Group 2
N=172

2a
2b
2c

Pre- ART CD4

HIV- Not infected (Healthy controls)

Group 3
N=63

On ART status

★ ★ ★

vs. group 3; p<0.05
Pahwa, CART 2018
Cardiovascular status assessment

Cardiac function measures
- cardiac ejection time
- stroke volume and pulse rate
- cardiac output
- cardiac index
- stroke volume index

Arterial measures
- Systemic vascular resistance
- Large artery elasticity index
- Small artery elasticity index

HDI PulseWave™ CR-2000
Indicators of CVD in Study Population

- **Group 1**: HIV+ untreated
- **Group 2**: HIV+ on ART
- **Group 3**: HIV- Healthy

**Indicators**

- **A**, **B**, **C**, **D**
  - Cardiac Ejection Time
  - Cardiac output
  - Cardiac index
  - Stroke Volume

- **E**, **F**, **G**, **H**
  - Stroke Volume index
  - Systemic vascular resistance
  - Lg Artery Elasticity index
  - Sm Artery Elasticity index

**Group 1** has the most impairment in cardiac function.
Untreated with CD4<200 (gp 1a) had abnormal cardiac function (blue color)
Immunology: Study Design - Chennai Cohort

1. Markers of immune activation on T cells/subsets
   - HLA-DR, CD38
   - Immune senescence
     - CD28, CD57

2. Soluble Plasma Biomarkers
   - Inflammatory (TNFα, IL-6)
   - Soluble receptors (TNFR, IL2R)
   - Microbial Translocation (sCD14, sCD163, LPS)

3. Other immune subsets and exploratory biomarkers
   - Checkpoint Inhibitors
   - Monocytes

Outcome
- CVD

Pahwa, CART 2018
CD4+ T cell Cellular Immune Activation (IA) Markers

- IA is highest in treatment naïve groups and still evident post cART
- IA is greatest in Group 1a with CD4<200

Group 1: HIV+ untreated
Group 2: HIV+ on ART
Group 3: HIV- Healthy

Pahwa, CART 2018
Immune senescence: (CD57+CD28-) T cells

- Immune senescence more evident in CD8 T cells in untreated patients with low nadir CD4

Pahwa, CART 2018
Correlation of CD4 and CD8 T cells IA (HLA-DR+CD38+) with cardiac function

Immune activation of CD4 and CD8 cells is associated with reduced cardiac functions (blue)
Plasma biomarkers: Fold change in Cytokines in comparison to healthy controls

Group 1: HIV+ untreated
Group 2: HIV+ on ART
Group 3: HIV- Healthy

P<0.05

IFNα2
INFγ
IL-10
IL-17
IL-1β
IL-2
IL-6
IL-8
TNFα
TNFR1
TNFR2
sCD14
VCAM-1
ICAM-1

Group 1a 1b 1c 2a 2b 2c
Untreated Treated

Fold change over HC

Pahwa, CART 2018
Cardiac dysfunction (blue) is associated with multiple plasma cytokines and LPS

*Increased inflammatory cytokines are a result of Immune Activation*

Pahwa, CART 2018
Laboratory Study Design - Chennai Cohort

1. Markers of immune activation on T cells/subsets
   - HLA-DR, CD38
   - Immune senescence
     - CD28, CD57

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Outcome
- CVD

Pahwa, CART 2018
Immune checkpoint (ICP) molecules regulate immune activation

- PD-1 and CTLA-4: act at systemic level
- Tim-3, Lag-3, TIGIT: act at the tissue level; ligands expressed in tissues.
3. Immune Checkpoint Molecules PD1, LAG3, TIGIT and Tim3 on CD4+ T

Higher PD-1 and LAG-3 expressed in CD4+ T cells in Group 1

Group 1: HIV+ untreated
Group 2: HIV+ on ART
Group 3: HIV- Healthy

Pahwa, CART 2018
CD4 T cell CPI molecules in patient groups compared to HC

Group 1a (CD4<200) had highest CPI Molecules

Pahwa, CART 2018
Correlation of expression of LAG3 and PD1 on CD4 T cells with cardiac function

A
Gp1: $p = 0.0012$ $r = -0.33$
Gp2: NS
Gp3: NS

B
Gp1: $p< 0.0001$ $r = -0.43$
Gp2: NS
Gp3: NS

C
Gp1: $p< 0.0001$ $r = -0.4$
Gp2: NS
Gp3: NS

D
Gp1: $p = 0.001$ $r = -0.33$
Gp2: NS
Gp3: NS

E
Gp1: $p = 0.0009$ $r = -0.34$
Gp2: NS
Gp3: NS

LAG3+CD4

LAG3+PD1+CD4

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Summary of correlation of checkpoint inhibitor molecules on CD4 T cells and cardiac function

Summary:

- LAG3 and PD1 expressing CD4 T cells are correlated with impaired cardiac function in Group 1.
- The increase in CPI molecules is due to excessive IA.

Group 1: HIV+ untreated
Group 2: HIV+ on ART
Group 3: HIV- Healthy

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Monocytes in inflammation

- Accumulating evidence that monocytes are important players in inflammation
- Implicated in atherosclerosis and plaque formation
- Three major circulating subsets are inflammatory, classical, non-classical
### Markers and function of monocyte subsets

<table>
<thead>
<tr>
<th>Subsets</th>
<th>Surface markers</th>
<th>% in MNC</th>
<th>Chemokine receptors</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical</td>
<td>CD14^{++}CD16^{-}</td>
<td>80-95</td>
<td>CCR2^{high} CX3CR1^{low}</td>
<td>Phagocytosis</td>
</tr>
<tr>
<td>Inflammatory</td>
<td>CD14^{++}CD16^{+}</td>
<td>2-11</td>
<td>CCR2^{mid} CX3CR1^{high} CCR5^{+}</td>
<td>Pro-inflammatory</td>
</tr>
<tr>
<td>Non-classical</td>
<td>CD14^{+}CD16^{++}</td>
<td>2-8</td>
<td>CCR2^{high} CX3CR1^{low}</td>
<td>Patrolling</td>
</tr>
</tbody>
</table>

### CD14
- **CD14** is a receptor for endotoxins, lipopolysaccharides (LPS) and other bacterial wall components.
- Associated with development of innate immune system.
- CD14 determines the balance between Th1 versus Th2 cytokines.
- It is expressed on monocytes, macrophages, and neutrophils.

### CD16 (Fc receptor III)
- **CD16** activates degranulation, phagocytosis, and oxidative burst, which allows neutrophils to clear opsonized pathogens. Also involved in ADCC.
- It expressed on monocytes, macrophages, NK cells and neutrophils.

### CD11b
- **CD11b** is an integrin family member which pairs with CD18 to form the CR3 heterodimer.
- It expressed on monocytes, neutrophils, NK cells, granulocytes and macrophages.
- CD11b regulates leukocyte adhesion and migration to mediate the inflammatory response.
Gating strategy for monocyte and NK cell subsets

Total monocytes: CD45⁺CD3⁻CD56⁻HLADR⁺
Classical: (CM: CD14⁺⁺CD16⁻)
Inflammatory: (IM: CD14⁺⁺CD16⁺)
Non Classical: (NCM: CD14⁺⁻CD16⁺⁺)

Pahwa, CART 2018
Circulating inflammatory monocytes contribute to impaired influenza vaccine responses in HIV-infected participants

Varghese K. George, Suresh Pallikkuth, Rajendra Pahwa, Lesley de Armas, Stefano Rinaldi, Li Pan and Savita Pahwa

Objective: Antibody responses are often impaired in old age and in HIV+ infection despite virologic control with antiretroviral therapy but innate immunologic determinants are not well understood.

Design: Monocytes and natural killer cells were examined for relationships to age, HIV infection and influenza vaccine responses.

Methods: Virologically suppressed HIV+ (n = 139) and HIV− (n = 137) participants classified by age as young (18–39 years), middle-aged (40–59 years) and old (≥ 60 years) were evaluated preinfluenza and postinfluenza vaccination.

Results: Prevaccination frequencies of inflammatory monocytes were highest in old HIV+ and HIV−, with old HIV+ exhibiting higher frequency of integrin CD11b on inflammatory monocytes that was correlated with age, expression of C-C chemokine receptor-2 (CCR2) and plasma soluble tumor necrosis factor receptor-1 (sTNFR1), with inverse correlation with postvaccination influenza H1N1 antibody titers. Higher frequencies of CD11b+ inflammatory monocytes (CD11bhi, >48.4%) compared with low frequencies of CD11b+ inflammatory monocytes (<15.8%) was associated with higher prevaccination frequencies of total and inflammatory monocytes and higher CCR2 MFI, higher plasma sTNFR1 and CXCL-10 with higher LPS stimulated expression of TNFα and IL-6, concomitant with lower postvaccination influenza antibody titers. In HIV+ CD11bhi expressers, the depletion of inflammatory monocytes from PBMC resulted in enhanced antigen-specific CD4+ T-cell proliferation. Immature CD56hi natural killer cells were lower in young HIV+ compared with young HIV− participants.

Conclusion: Perturbations of innate immunity and inflammation signified by high CD11b on inflammatory monocytes are exacerbated with aging in HIV+ and negatively impact immune function involved in Ab response to influenza vaccination.
Chennai Cohort: Monocyte subsets

Frequencies of total monocytes and subsets did not differ between study groups

Group 1: HIV+ untreated
Group 2: HIV+ on ART
Group 3: HIV- Healthy

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CD11b<sup>Hi</sup> expression in three subsets of monocytes

- **Inflammatory Monocytes (IM)**
  - Group 1: HIV+ untreated
  - Group 2: HIV+ on ART
  - Group 3: HIV- Healthy

- **Classical Monocytes (CM)**
- **Non-classical Monocytes (NCM)**

Group 1 had higher frequencies and MFI of CD11b in all monocyte subsets, maximally in inflammatory monocytes

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CD11b^{hi} expression of Inflammatory and non-classical monocytes inversely correlated with CVD outcome in group 1 patients..... This may be a culprit in causing IA via TLR
Mechanism of Activation of Monocytes

Gut dysbiosis, altered microbiome, HIV and co-infections

HIV, viruses

Bacterial products

Toll like receptor (TLR) signaling

Inflammatory Monocytes

ACTIVATION

Pahwa, CART 2018
1. Untreated patients have significant immune dysfunction (immune activation, inflammation, increased checkpoint molecules and increased CD11b$^{\text{hi}}$ monocytes). This was most prominent in Group 1a, with CD4<200.

2. Patients starting ART at any nadir CD4 show immune recovery, but this is less in those who start at a lower nadir CD4.

3. Some immune dysfunction (sCD14, Immune activation) is evident even if ART started at higher CD4.

4. Cardiac dysfunction correlated with immune activation of CD4 T cells, increased inflammatory cytokines, increased checkpoint inhibitors LAG3 and PD-1 and increased CD11b$^{\text{hi}}$ inflammatory and non-classical monocytes. Maximum defects were in Group 1, especially in group 1a.
What causes it?
- Compromised intestinal integrity during acute inf; Gut Microbial Translocation
- Co-Infections: CMV, EBV
- Immune Dysregulation
- Viral Persistence

What determines which organ system is affected?
Specificity may be at tissue level:
- Receptors for inflammatory cytokines
- Ligands for Check point inhibitor molecules (e.g. LAG 3, PD1)
- Migration of effector cells and inflammatory monocytes

Why is it bad?
- End-organ disease, non-AIDS complications
- Fibrosis in lymph nodes
- Immune senescence
- Impaired immune function
- Viral Reservoirs

Conceptual framework on inflammation and HIV

Pahwa, CART 2018
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Thank you