Association of Subclinical CMV DNA and Immunological Markers of Cardiovascular Disease

Ankita Garg [1*], Sara Gianella[2], Masato Nakazawa[2] and Stephen A. Spector[1,3]

[1]Departments of Pediatrics[1] and Medicine[2], Divisions of Infectious Diseases, University of California San Diego, La Jolla, California, USA, and [3]Rady Children's Hospital, San Diego, California, USA;

** Current Address: National Institute of Biomedical Genomics, Kalyani, West Bengal, INDIA*

Background

- Asymptomatic CMV reactivation can lead to chronic inflammation due to the high levels of IFNγ and other inflammatory cytokines.
- Chronic inflammation during HIV infection is associated with increased risk of cardiovascular disease (CVD) and atherosclerosis, mediated by the CX3CR1-CX3CL1 (fractalkine) axis.
- In HIV-CMV co-infection, expanding CMV specific CX3CR1$^+$ T cells release IFNγ and TNFα that can result in increased expression of CX3CL1 by endothelial cells causing endothelial cell damage.
- Previously, we have shown that: i) CD4$^+$ T cells from CMV+ healthy donors respond to CMV antigen presented by endothelial cells and produce IFNγ and TNFα *in vitro*; and ii) cells from CMV high responders (HR) compared to CMV low responders (LR) induce higher levels of CX3CL1 in endothelial cells resulting in endothelial cell damage.

Objective

To determine: i) if a subset of HIV-infected individuals maintain persistently high levels of IFNγ producing CMV-specific CX3CR1 bearing T cells; and ii) if levels of CMV DNA detected over time are associated with the magnitude of the CMV-specific IFNγ response.

Methods

- 80 paired PBMC samples were collected from 40 CMV-seropositive, HIV-infected men who started ART a median of 3 months from estimated date of infection, and achieved suppressed HIV RNA a median of 3 months on ART.
- PBMC were obtained ≥12 months apart (1st sample a median of 34 weeks post-ART initiation).
- CMV IFNγ response was determined by ELISPOT using a peptide pool of CMVpp65 antigen; CMV specific memory T cells were identified by flow cytometry. CMV and EBV DNA levels were measured by ddPCR.
- Data were analyzed using a mixed effects regression model to predict associations between CMV shedding, IFNγ production and CX3CR1-expressing CD4$^+$ and CD8$^+$ T cells over time. Bayesian hierarchical models were used to quantify individual differences in CMV and EBV replication over time.
- Participants were classified as low (LR)- or high-responders (HR) according to IFNγ production (≤ or >100 SFU/10^5 cells).

Results

Figure 1

CMV High Responders (HR) and Low Responders (LR) maintain their response category over time

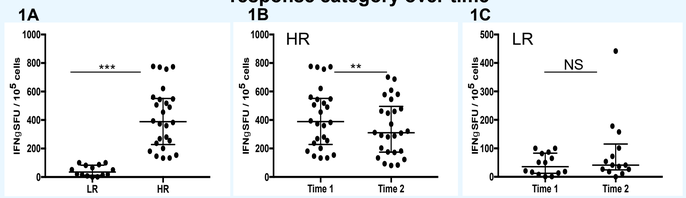


Figure 1: Panel 1A shows comparison between HR and LR at the first time-point. Longitudinal changes in IFNγ response over time are also shown for HR (**Panel 1B**) and LR (**Panel 1C**).
Dots represent individuals; the bars represent 25th, median, 75th percentile values. **p<0.01, ***p<0.001.

Figure 2

Low CMV shedders have a greater increase in IFNγ over time than high CMV shedders

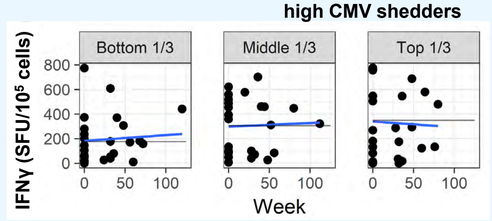


Figure 2: Participants are grouped into low CMV shedders (bottom 1/3 shedders), middle 1/3 shedders, and high CMV shedders (top 1/3 shedders). The IFNγ increase was greater in the bottom shedders than the high shedders (p<0.01). The lines represent regression lines; black horizontal lines represent baseline values.

Figure 3

Increased IFNγ production is associated with increased CMV-specific CX3CR1$^+$CD4$^+$ and CD8$^+$ T cells (greatest at the early time-point)

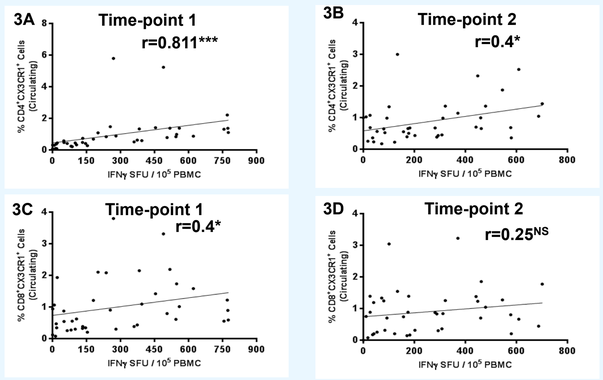
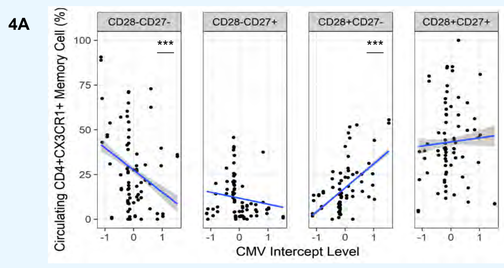


Figure 3: Correlation between CMV-induced IFNγ production (%) and CMV-specific CX3CR1$^+$ CD4 and CD8 T cells (%) at Time-point 1 **(Panel 3A and 3C)** and Time-point 2 **(Panel 3B and 3D)**. Each dot represents an individual donor. *p<0.05, ***p<0.001

Figure 4

Increased expression of CX3CR1$^+$ in circulating memory CD4$^+$ T cells of CD28$^+$CD27$^-$ subtype are associated with higher levels of CMV replication and more IFNγ production



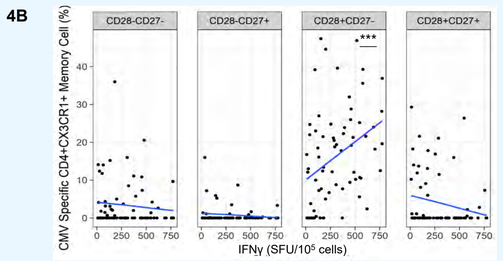


Figure 4: Panel 4A shows correlation between CMV-specific CX3CR$^+$ CD4$^+$ T cells subsets and CMV DNA levels, **panel 4B** shows associations between cellular subsets and frequency of cells producing IFNγ. For all graphs, Each dot represents an individual donor. Blue lines represent regression lines and the grey shades indicate 95% confidence intervals. ***p<0.001.

Figure 5

PD-1 is associated with IFNγ but not with CX3CR1$^+$ memory CD4$^+$ or CD8$^+$ T cell activity or subclinical CMV replication

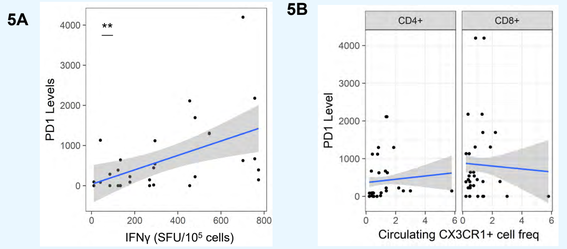


Figure 5: Panel 5A displays the positive correlation between PD1 levels and IFN-γ. **Panel 5B** shows PD1 levels by frequency of CX3CR1$^+$ memory CD4+ (Left) and CD8+ (Right) cells. **Panel 5C** shows PD1 levels by CMV Intercept level in CX3CR1$^+$ memory CD4 and CD8 T cells. Each dot represents an individual donor. Blue lines represent regression lines and the grey shades indicate 95% confidence intervals. **p<0.01.

Conclusions

- **IFNγ HR and LR CMVpp65 in HIV-CMV co-infected individuals on suppressive ART maintain their response category over time.**
- **Levels of IFNγ response to CMV (but not CMV DNA levels) are positively associated with the frequency of circulating memory CD4$^+$CX3CR1$^+$ T cells.**
- **Levels of CMV DNA (and IFNγ responses) are associated with CD45RA$^-$CD4$^+$CX3CR1$^+$CD28$^+$CD27$^-$ T cell memory subsets**
- **Using a similar interaction model, EBV DNA was not associated with any of these findings (data not shown).**
- **CD4$^+$CX3CR1$^+$ and CD8$^+$CX3CR1$^+$ memory T cell responses to CMV are not associated with PD-1**
- **A subgroup of HIV-infected CMV IFNγ HR has been identified that may be at increased risk for chronic inflammation and CVD as a result of CMVpp65 specific "inflated memory" CD4$^+$ T cells.**

Acknowledgements

This work was supported in part by R56 HL125028 and the IMPAACT Network NIAID-U01 AI068632 (SAS) and R24 AI106039 Primary Infection Resource Consortium and R21 AI127132 (A.G.); AI68636 (ACTC-supplements) and AI027763 (Supplement-CMV driven mechanisms, S.G.). Portions of this research were performed with support of Flow Cytometry Core at The UC San Diego Center for AIDS Research (P30 AI036214), the VA San Diego Health Care System and the San Diego Veterans Medical Research Foundation.

University of California San Diego, Stein Clinical Research Building Room 434, 9500 Gilman Drive, La Jolla CA 92093-0672, USA ● email.ajaiswal@ucsd.edu saspector@ucsd.edu ● Phone: +1 (858) 534 7170 ● Fax: +1 (858) 534 7411