CMV co-infection in HIV elite controllers: importance for disease progression and inflammation

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Introduction

Cytomegalovirus (CMV) infection is highly prevalent in the general population, and even more frequently in people living with HIV (PLWH). We recently demonstrated that CMV co-infection was associated with a lower CD4/CD8 ratio, and high levels of gut damage, microbial translocation and inflammatory markers in PLWH under antiretroviral therapy (ART). (Ramendra, *Clinical infectious Diseases* 2020) Elite controllers (ECs) are a rare subgroup of PLWH who maintain undetectable plasma HIV viral load without ART. Several mechanisms for control of HIV replication have been identified and include protective HLA alleles enhancing CD8 T-cell and innate immune responses. While ECs can spontaneously control viral replication, chronic inflammation, gradual CD4 decay and increased incidence of non-AIDS comorbidities such as cardiovascular disease and cancer have been reported (Crowell, *Journal of Infectious Diseases* 2015; Hunt, *Journal of Infectious Diseases* 2008). However, it is not yet understood which factors are associated with loss of HIV viral control or CD4 T-cell decay when HIV viral replication remains undetectable (El-Far, *Scientific Reports* 2016). Identifying such factors will be helpful to determine the optimal time for ART initiation in this rare PLWH. Herein, we sought to investigate whether CMV co-infection is associated with CD4 T-cell decay, a key marker for HIV disease progression, and inflammation in ECs.

Methods

Blood samples and clinical parameters were collected from 25 ART-naïve ECs of the Canadian Slow progressor cohort (CIHR/CTN 247) with viremia <50 copies/ml. Longitudinal CD4 count were extracted from study medical charts and A CD4 count vs. date slope was created and a linear regression analysis performed to calculate annual change in CD4 count.

We categorized EC regarding their expression of protective HLA alleles (B*27, B*57, B*58) (El-Far, *Sci Rep* 2016).

At the end of study follow up: ELISA were performed to assess anti-CMV IgG levels (GenWay), gut damage marker Intestinal fatty acid binding protein (I-FABP), and microbial translocation markers lipopolysaccharide (LPS) (R&D and Cusabio respectively).

Participant characteristics

	Age	Gender	Protective HLA	CD4 count (cells/µL)	CD8 count (cells/µL)	CD4/CD8	HIV VL (copies/mL)	Follow up (years)	Annual CD4 variation (cells/year)	CD4 variation
1	47	F	No	581	211	2.8	<40	16.5	-32.2	Decay
2	54	F	No	639	689	0.9	<50	7.9	-14.3	Stable
3	32	M	Yes	634	630	1	<50	2.6	-86.8	Stable
4	44	M	Yes	920	750	1.2	<40	3.3	29	Stable
5	36	F	Yes	576	990	0.6	<40	8.7	-13.9	Stable
6	72	M	Yes	740	960	0.8	<40	10.1	19.3	Increase
7	44	M	No	550	450	1.2	<50	8.8	20	Increase
8	49	M	No	710	1010	0.7	<40	25.6	-7.8	Stable
9	35	M	No	650	260	2.5	<40	7	5.7	Stable
10	41	M	No	928	1566	0.6	<50	3.2	36.3	Stable
11	56	M	Yes	771	252	3.1	<50	13.2	16.4	Increase
12	55	M	No	680	500	1.4	<40	13.2	-40.7	Stable
13	45	M	Yes	570	800	0.7	<50	12.3	-11.2	Stable
14	26	M	Yes	1040	1090	1	<50	9.6	14.4	Stable
15	40	M	Yes	710	450	1.6	<50	5.9	0.6	Stable
16	51	M	No	460	630	0.7	<50	5.3	-52.6	Decay
17	49	F	No	640	580	1.1	<50	17.7	-31.6	Decay
18	60	M	No	746	1344	0.6	<40	1.7	-10.8	Stable
19	49	M	No	1220	734	1.7	106	24.7	2.3	Stable
20	57	M	Yes	290	390	0.7	<40	6.9	-44.5	Decay
21	46	M	No	750	730	1	<40	3.7	-73	Stable
22	47	F	Yes	510	800	0.6	<40	6.6	-21.1	Stable
23	36	M	No	560	520	1.1	<40	5.3	-68.5	Decay
Median	47			650	689	1	<40	7.9	-11.2	

Results

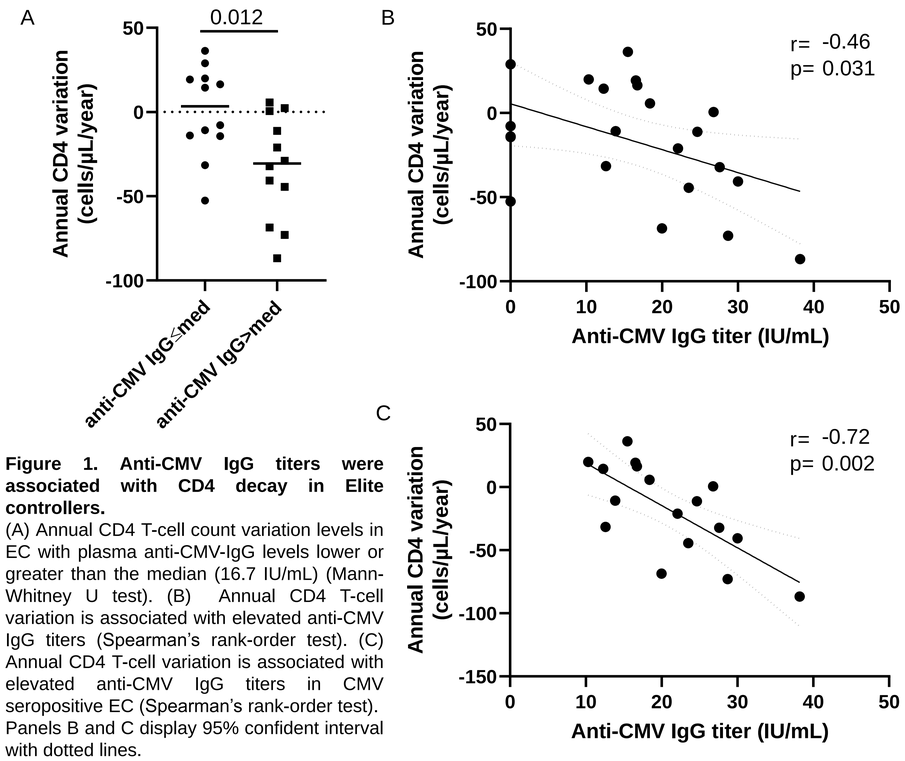

Figure 1. Anti-CMV IgG titers were associated with CD4 decay in Elite controllers.
(A) Annual CD4 T-cell count variation levels in EC with plasma anti-CMV-IgG levels lower or greater than the median (16.7 IU/mL) (Mann-Whitney U test). (B) Annual CD4 T-cell variation is associated with elevated anti-CMV IgG titers (Spearman's rank-order test). (C) Annual CD4 T-cell variation is associated with elevated anti-CMV IgG titers in CMV seropositive EC (Spearman's rank-order test). Panels B and C display 95% confident interval with dotted lines.

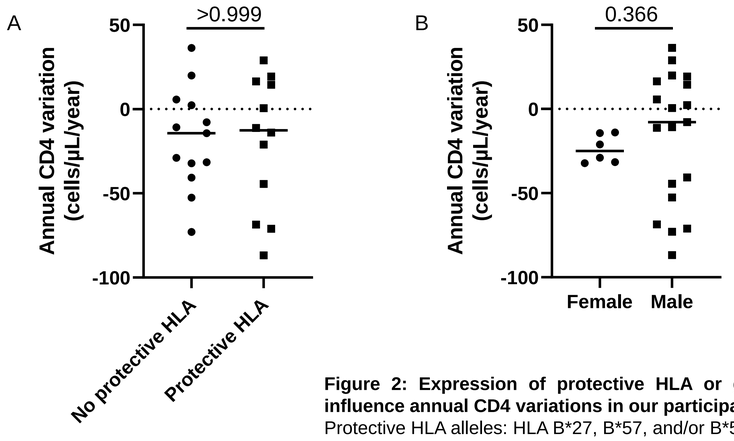

Figure 2: Expression of protective HLA or gender didn't influence annual CD4 variations in our participants.
Protective HLA alleles: HLA B*27, B*57, and/or B*58.

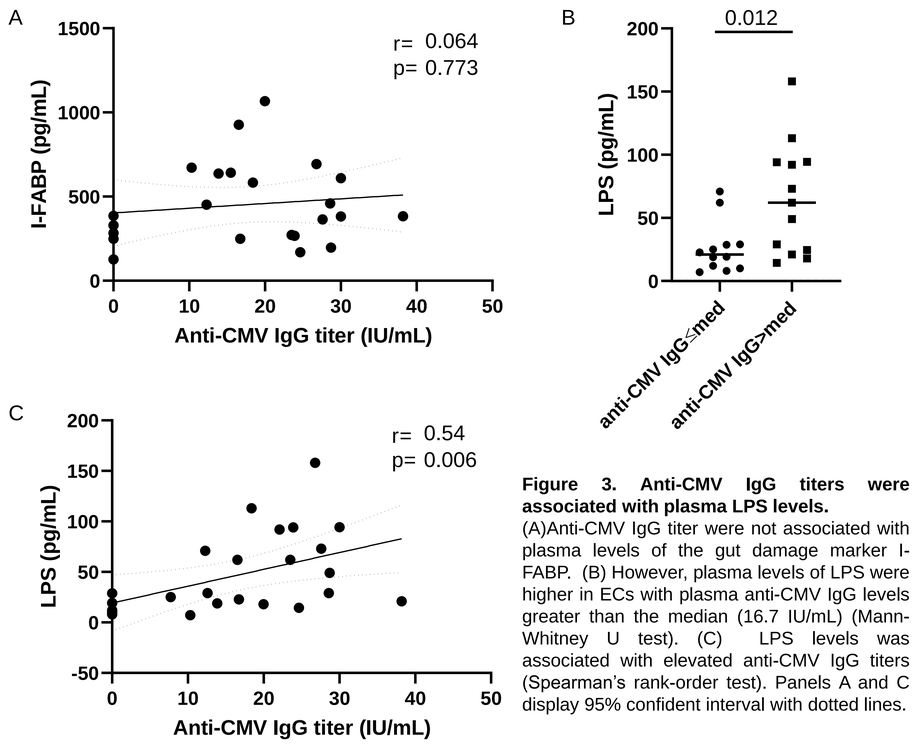

Figure 3. Anti-CMV IgG titers were associated with plasma LPS levels.
(A)Anti-CMV IgG titer were not associated with plasma levels of the gut damage marker I-FABP. (B) However, plasma levels of LPS were higher in ECs with plasma anti-CMV IgG levels greater than the median (16.7 IU/mL) (Mann-Whitney U test). (C) LPS levels was associated with elevated anti-CMV IgG titers (Spearman's rank-order test). Panels A and C display 95% confident interval with dotted lines.

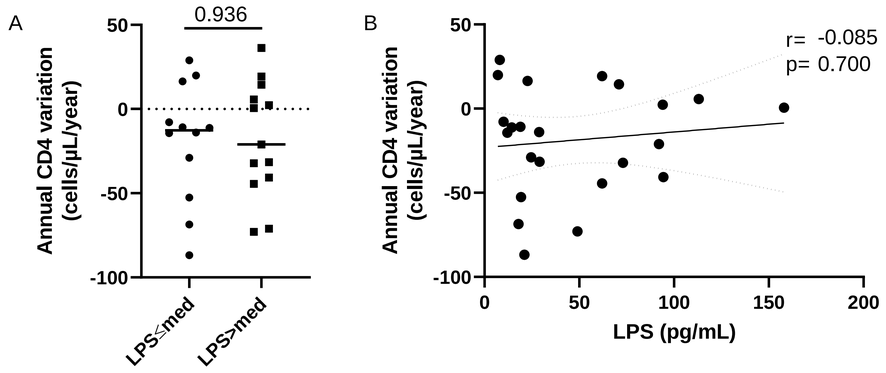

Figure 4. Plasma LPS levels were not associated with CD4 decay
(A) Annual CD4 variation were not different in ECs with plasma levels of LPS greater than the median (28.8 IU/mL) (Mann-Whitney U test). (B) LPS levels were not associated with annual CD4 variations (Spearman's rank-order test). Panels B display 95% confident interval with dotted lines.

Conclusions

Altogether, our results showed a negative association between anti-CMV IgG levels and annual CD4 T-cell count decay in ECs, independently of age, sex or protective HLA expression. Also, we found that plasma LPS in correlation with anti-CMV IgG titers, although we didn't detect increased levels of the gut damage marker I-FABP. As such CMV co-infection may enhance epithelial gut damage and microbial translocation contributing to CD4 T-cell decay over time, as it was seen in ART-treated progressors. Hence, we propose that in ECs, CMV co-infection contributes to CD4 T-cell decay and thus an increased risk of developing non-AIDS comorbidities. Moreover, elevated CMV IgG levels may help determine the appropriate timing of ART initiation in ECs (Isnard*, Ramendra* and Routy, *Clinical Infectious Diseases*, under review)

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