The Breadth and Polyfunctionality of T cell Responses to CMV Differ by HIV Status

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BACKGROUND

- Chronic cytomegalovirus (CMV) infection elicits large T cell responses in both HIV-infected (HIV+) and -uninfected (HIV-) people, which vary greatly across individuals[1-4]
  - CMV-specific T cell responses were strongly correlated with serum levels of several inflammatory markers, depending on HIV status[5]
  - CMV-specific T cells producing IFN-γ, but not TNF-α or IL-2, predicted both onset and maintenance of frailty, positively in HIV-, but negatively in HIV+, men[6]
  - CMV antigens that elicited IFN-γ-only CD4 responses were different between HIV- and HIV+ men[4]
- CMV reactivation or low-level replication has been implicated in sustaining CMV-specific T cell responses[6]
- The frequency and extent of CMV reactivation may differ by HIV status[7]
- The specificity, magnitude, and functionality of T cell responses depend on exposure to antigens[8].
- Therefore, **we hypothesized that the breadth and polyfunctionality of T cell responses to CMV may differ by HIV status**

METHODS

Study population: 20 HIV- and 22 virologically suppressed HIV+ men from the Baltimore-Washington DC center of the Multicenter AIDS Cohort Study (MACS)[3]

Assessment of CMV-specific T cell responses:

- Production of IFN-γ, TNF-α, and/or IL-2 by T cells in response to peptide pools spanning 19 CMV open reading frames (ORFs) (listed in Table 1) was assessed by intracellular cytokine staining flow cytometry[2,3]
- The cut-off value for positive responses was 0.05% of CD4 or CD8 T cells
- Polyfunctionality of the T cell responses was expressed as a polyfunctionality index, calculated by the following formula (ranged 0.33-1)[9]:

$$\sum_{i=1}^{n}(F_i \times \frac{i}{n})$$

i: numbers of cytokines produced by each CMV-responsive T cell subset,
n: total numbers of cytokines studied (in this study, n=3),
Fi: the percentage of cells producing i cytokines among total CD4 or CD8 T cell responses (i = 1, 2, or 3)

- CMV ORFs were classified as Early or Late based on when they are known to be expressed during the CMV replication cycle (Table 1)

Statistical analysis of difference by HIV status:

- Non-parametric Mann-Whitney test for differences in continuous variables
- Fisher's Z test for differences between Spearman's correlation coefficients

Table 1. Time of Expression of CMV ORFs Tested in This Study*

ORF	Time of expression	Category	ORF	Time of expression	Category
US24	Early	Early	UL55 (gB)	Late	Late
UL36	Early	Early	UL86	Late	Late
UL123 (IE-1)	Immediate Early	Early	UL153	Early-Late	Late
US3	Immediate Early	Early	UL32 (pp150)	Late	Late
UL122 (IE-2)	Immediate Early	Early	UL48	Late	Late
UL28	Early	Early	UL82 (pp71)	Late	Late
UL151	Unknown	Unknown	UL83 (pp65)	Late	Late
			UL94	Late	Late
			UL99 (pp28)	Late	Late
			UL103	Late	Late
			US29	Late	Late
			US32	Late	Late

*Time of expression according to [1,10,11]

- **CD8 T cells from *HIV*+ men responded to more CMV antigens than those from *HIV*- men**
- **Polyfunctionality of CMV-specific CD4 responses was lower in *HIV*+ men than in *HIV*- men**
- **The correlations between numbers of ORFs responded to and magnitude of total T cell responses were stronger in *HIV*- men than in *HIV*+ men**

RESULTS

Fig. 1. Numbers of CMV ORFs That Elicited CD4 and CD8 Responses in Individual HIV- And HIV+ Men

Upper Row: All ORFs; Lower Row: ORFs stratified by Time of Expression

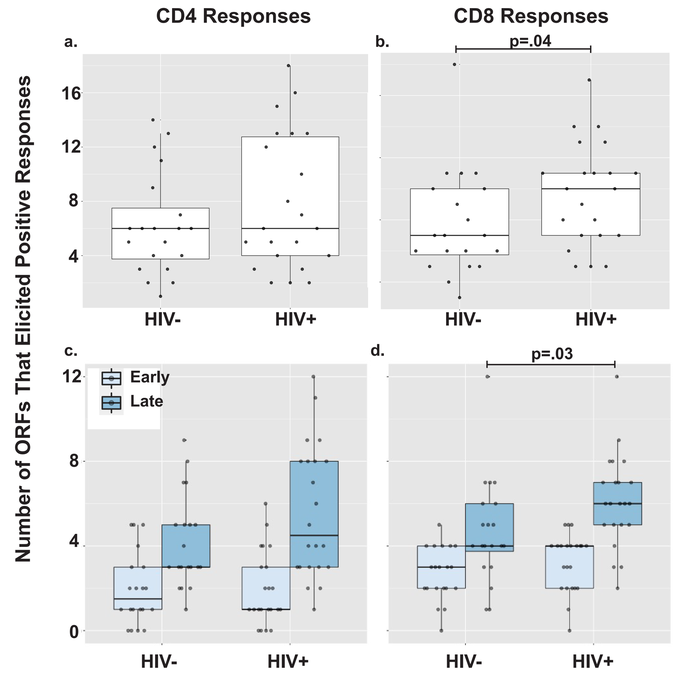


Table 2. Polyfunctional Index of All Positive CD4 and CD8 Responses, Stratified by HIV Status

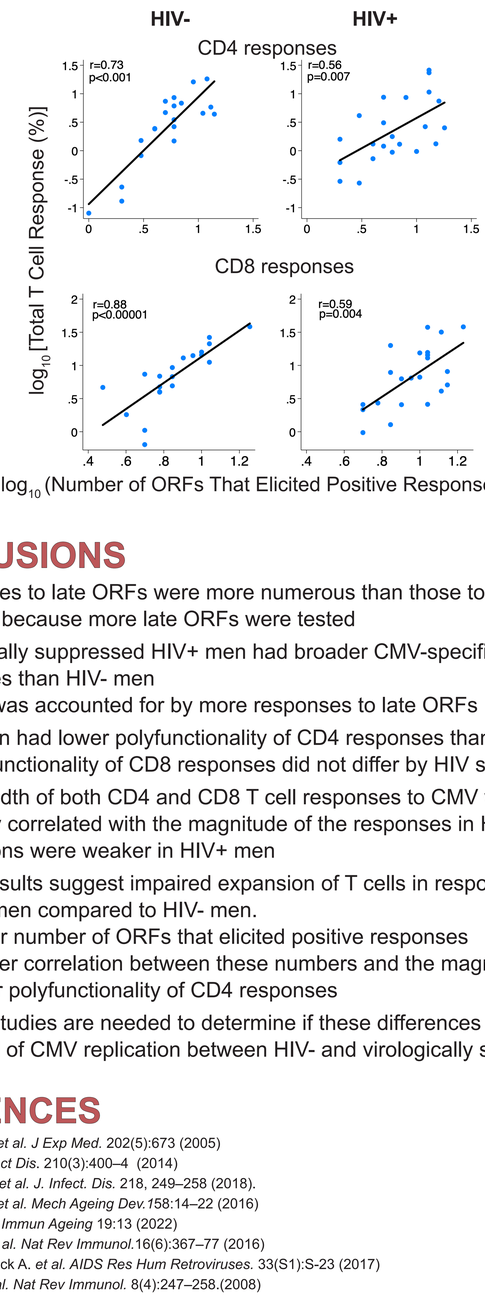
Polyfunctional Index	HIV-	HIV+	p-value
CD4 Responses	**.59 (.34)***	**.33 (.30)**	**0.00005**
CD8 Responses	.49 (.25)	.47 (.27)	1

*** Median (Interquatile range);**

Bold indicates significant difference between HIV- and HIV+ men

Fig. 2. Correlations Between Numbers of ORFs That Elicited Positive Responses And Total T cell Response

(Stratified by HIV Status and T cell Subset)



CONCLUSIONS

- Responses to late ORFs were more numerous than those to early ORFs, probably because more late ORFs were tested
- Virologically suppressed HIV+ men had broader CMV-specific CD8 T cell responses than HIV- men
  - This was accounted for by more responses to late ORFs
- HIV+ men had lower polyfunctionality of CD4 responses than HIV- men
  - Polyfunctionality of CD8 responses did not differ by HIV status
- The breadth of both CD4 and CD8 T cell responses to CMV was strongly and positively correlated with the magnitude of the responses in HIV- men, but the correlations were weaker in HIV+ men
- These results suggest impaired expansion of T cells in response to CMV ORFs in HIV+ men compared to HIV- men.
  - Higher number of ORFs that elicited positive responses
  - Weaker correlation between these numbers and the magnitude of responses
  - Lower polyfunctionality of CD4 responses
- Further studies are needed to determine if these differences reflect differences in control of CMV replication between HIV- and virologically suppressed HIV+ men

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