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Elevated IFN-γ in HIV patients using a novel assay for adaptive and innate immunity

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Background

HIV infection is characterised by immune activation involving both the adaptive and innate immune responses

Immune activation persists in patients following antiretroviral therapy (ART)

It is associated with poor CD4+ T cell recover and serious non-AIDS events (SNAE)

Aim

To evaluate adaptive and innate immunity using a novel assay in HIV-infected patients and normal controls

To compare the responses in HIV infected patients prior to and on ART in order to determine whether age, sex, duration of infection, CD4 count, CD4 nadir or viral load is associated with increased responses

Methods

Study design
A cross-sectional study in HIV and non-HIV infected individuals

Study population
79 HIV infected participants were recruited from The Alfred Hospital and Melbourne Sexual Health Centre (MSHC), Melbourne Australia

- 59 study participants were on suppressive ART defined as receiving ART for at least 12 months and a HIV viral load < 50 copies/ml on at least 2 occasions 6 months apart.
- 20 of the HIV infected participants were naïve to ART

228 healthy controls

Exclusion criteria
< 18 years old, immunosuppressive medications including prednisolone and/or history of transplantation

Study assay
Whole blood was assessed using the Quantiferon Monitor® (QFM) assay according to the manufacturer's instructions.

Antigen coated tubes contained either 1:1 IU/ml or 0.1:0.1 IU/ml (QFM 1:10) of anti-CD3 (T-cell receptor ligand) and R848 (TLR7 ligand). Mitogen (PHA) was used as a positive control.

One ml of whole blood is taken into an antigen coated tube. They are then incubated for up to 24 hours at 37oC without humidification before the plasma is harvested.

Production of IFN-γ (IU/mL) were measured in supernatant using a standard ELISA. Results are then calculated using Analysis Software (Cellestis Ltd).

Predictors of IFN-γ production were determined using univariable and multivariable quantile median regression analysis.

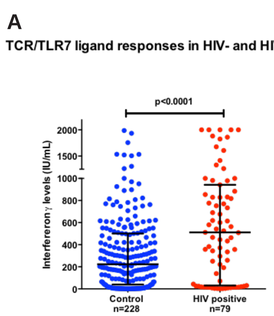
Results

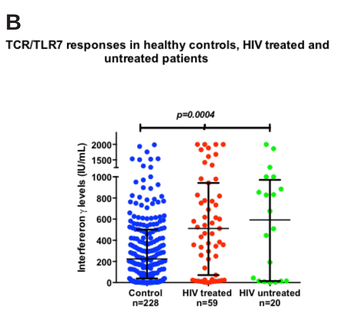
Table 1: Baseline Characteristics of the HIV study population

Characteristic	HIV-infected (n=79)		
	Treated (n=59)	Untreated (n=20)	P value
Age, median (IQR, years)	47 (41-56)	31 (26-41)	<0.001
Male sex (%)	56 (95)	19 (95)	1.0
Current CD4 T cell count (median, cells/ul)	600	518	0.18
Nadir CD4 T cell count (median, cells/ul)	210		
Current HIV RNA (median, copies/ml)	<50	25,903	<0.001
Duration of known HIV infection (median, years)	12.3	1.4	0.004

HIV- infected patients on suppressive ART were more likely to be older and been infected with HIV for a longer period than untreated HIV-infected patients.

Figure 1: A. Comparison of adaptive and innate immune responses in HIV infected patients and normal healthy controls B. Adaptive and immune responses in healthy controls compared to HIV treated and non treated patients.



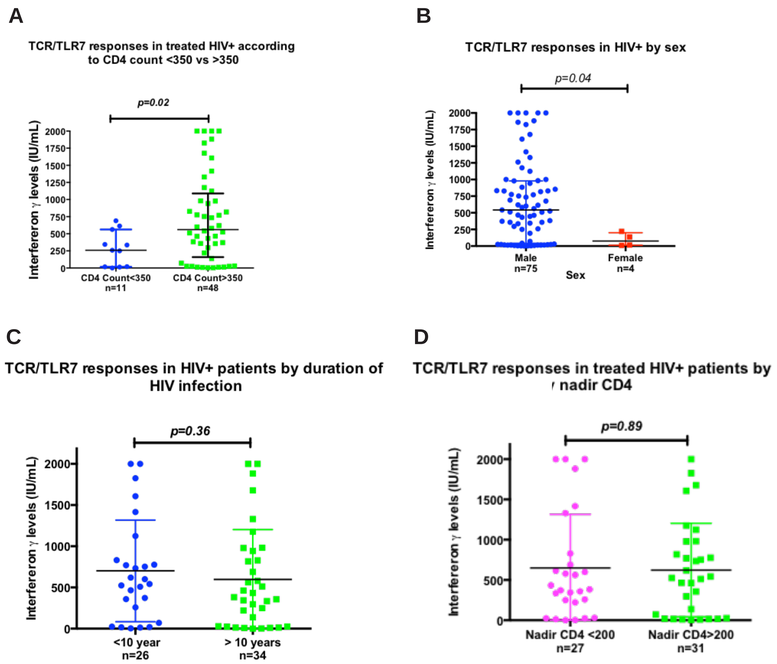


IFN-γ production with QFM 1:10 was significantly higher in HIV infected patients compared to healthy controls (median IFN-γ 512 vs 223 IU/ml, p<0.0001)

Significant differences remained whether in participants both on or off ART (median IFN-g 512 vs 593 vs 223 IU/ml respectively, K-Wallis test of difference p=0.0004)

Figure B: Predictors of adaptive and innate immune IFN responses in HIV infected patients according to CD4 count, sex and duration of HIV infection.

Results – continued



IFN-γ production in patients on ART was significantly higher in participants with CD4 count >350 cells/μL compared to a CD4 count <350 cells/μL (561 vs 259 IFN IU/ml p=0.02).

IFN-γ production was significantly higher in male HIV-infected patients (median IFN 542 vs 77 IU/ml p=0.04).

There were no known associations between IFN-γ responses and age (p=1.0), viral load (p=0.56), nadir CD4 count (p=0.89) or duration of HIV infection (p=0.36).

Using a multivariable analysis with 6 variables, neither CD4 nor sex were independently predictive of IFN-γ production.

Conclusion

Using a high throughput assay which assesses both adaptive and innate immunity, we show marked increases in IFN-γ production in HIV-infected patients both on and off ART

HIV infected patients had significantly higher IFN-γ responses compared to normal healthy controls

Further research is warranted to determine if changes in Quantiferon Monitor are also associated with SNAEs.