Viral Replicative Capacity Displays a Non-Linear Association to Cytokine Profiles Observed in Acutely Infected Volunteers in Contrast to Viral Load and CD4 T-cell Counts

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ABSTRACT

Using the Meso Scale Discovery (MSD) V-PLEX 40 biomarker cytokine multiplex kit, the cytokine profiles of 108 HIV+ Zambian volunteers were investigated for their associations to virus replicative capacity, CD4 count and viral load. A direct correlation was observed for CD4 count and viral load associated to IP-10 and VCAM-1. Association to virus replicative capacity (vRC) was non-linear for 6 distinct cytokines and subsequent analysis revealed that this relationship was being driven by a small subset of volunteers. Further characterization of these volunteers is ongoing.

BACKGROUND

Acute HIV infection is characterized by a rapid and robust expression of type I interferons (IFN-I), IFN-I–stimulated genes, and inflammatory cytokines. This inflammatory response, particularly during chronic infection, contributes to disease progression. Levels of 38 inflammatory cytokines, chemokines, and markers of gut damage and microbial translocation were assessed in individuals around the time of sero-conversion to assess the effect of different patient phenotypes on the early inflammatory milieu (Table 1). It has been previously observed that individuals infected with a high viral replicative capacity virus can be characterized through a distinct inflammatory cytokine profile characterized by a heightened type I and type II IFN response and elevated levels of key inflammatory cytokines such as IL-6 and IL-1β (1).

Additional studies have previously identified associations with viremia and increasing levels of IP-10, TNF-α, MCP-1, IL-6, IL-8, IL-10, IL-15 and IL-18 (2). The identification of key markers of HIV infection through a comprehensive assessment of cytokine profiles in individuals with a broad range of disease phenotypes will be used to inform vaccine design in future HIV clinical trials.

Table 1. Volunteer phenotypes. *SP vL calculated as the log10 of the average viral load scores for all visits in 12M post sero-conversion

Samples (N)0	CD4/µL	vL/mL	SP vL*	Days Post EDI	vRC Score
108	172 - 1759	49 - 1555504	2.152 – 6.239	17 - 273	0.133 – 3.320

METHODS

Multiplex cytokine analysis was performed on Zambian HIV+ volunteers infected with Clade C isolate using the MSD V-Plex 40-Plex Biomarker cytokine Kite (Cat# K15209D-1). Sample preparation was as manufacturer's instructions except that for all kits except Vascular Injury Panel samples were prepared as undiluted plasma. Data acquisition was with a Quickplex SQ120 Instrument and Discovery WORKBENCH 4.0 software. Data analysis was performed using log transformed data with Spearman Rank correlations performed for continuous outcomes and t-student test for comparison between low and high vRC. Thresholding of volunteer's vRC separating in to low and high vRC was as described(1). This resulted in a vRC threshold of 1.397. P Values have been corrected for test multiplicity (FDR) as described previously (3).

RESULTS

Statistical analysis of vRC demonstrated that there was a non-linear relationship observed between the vRC score observed for each volunteer and the measured cytokine concentration. Using the same distribution as previously described (1), The distribution of the vRC scores for the sample was separated in to the lowest tercile (low vRC) and the upper two terciles (high vRC (Figure1). Separation of volunteers by the vRC score enabled analysis of the relationships between vRC and cytokines to become apparent.

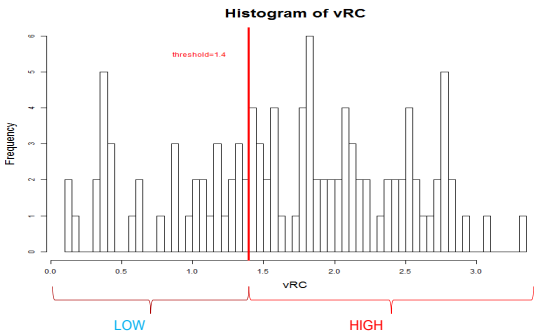

Figure 1. Distribution of vRC among the 108 patients included in the analysis. Threshold for high vRC determined at the first tercile.

The initial analysis was to assess whether evaluation of 6 core cytokines using the V-PLEX platform was able to observed the same trends as had been assessed by Luminex assessment (Table 2).

Table 2. Cytokine profiles of 6 cytokines when determined by Luminex assessment and MSD V-PLEX. Luminex[1] values (mean pg/mL) transcribed from Claiborne et al 2015. V-PLEX[2] values (mean pg/mL)

Cytokine	Low vRC[1]	High vRC[1]	P Value[1]	Low vRC[2]	High vRC[2]	P Value[2]
IL-6	1.88	3.94	0.004	0.68	1.14	0.024
IL-10	5.5	10.74	0.004	0.72	1.21	0.001
IFN-γ	4.38	10.26	0.014	6.33	16.61	0.001
IP-10	639.56	1108.43	0.018	490.46	1022.43	2.00E-04
TNF-α	10.76	13.87	0.028	3.344	3.93	0.075
IL-7	1.81	2.65	0.046	1.20	1.61	0.019

The segregation of volunteers in those with low vRC and those with high vRC enabled the identification of key cytokines associated with replicative capacity. Statistically significant associations observed between IL-6, IL-10, IFN-γ and IP-10 and vRC (Table 2) were delineated using log transformed scatter plots (Figure 2). Analysis of these plots revealed that a key subset of individuals are responsible for the differences in the mean concentrations of these cytokines. Further analysis is ongoing to characterize the individuals that are driving these responses.

RESULTS

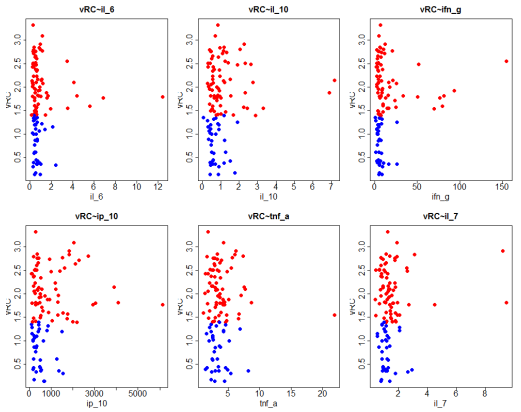

Figure 2. Distribution of vRC among the 108 patients included in the analysis. Threshold for high vRC determined at the first tercile.

In addition to replicative capacity assessment, Spearman Rank correlations were performed for volunteer visit CD4 count and viral load and Set Point viral load. Through this analysis correlations were observed for IP-10 to CD4 count (negative) and viral load (positive). A positive association was also observed for VCAM-1 to both volunteer visit viral load and set point viral load (data not shown – $p<0.05$).

CONCLUSION

- Cytokine multiplexing using MSD V-PLEX 40-ples biomarker kits demonstrated comparable assessment of core cytokines compared to Luminex evaluation (1)
- High viral Replicative capacity associates in a non-linear relationship to IL-6, Il-10, IFN-γ, IP-10 and IL-7
- Direct correlation observed between IP-10 and viral load (positive) and CD4 count (negative).

REFERENCES

1. Claiborne et al. 2015. Proceedings of National Academy of Science.
2. Stacey et al. 2009. Journal of Virology. **88** (8) 8719-8733
3. Benjamini, Y. and Hochberg, Y. (1995). JRSS B 57, 289–300.

ABBREVIATIONS

vL – Viral Load

EDI – Estimated Date of Infection

SP vL – Set Point Viral Load.

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J2 Somewher you need to define abbreviations SP, EDI and
Jo; 04/10/2016

JH3 Done
Jonathan Hare; 10/10/2016

J5 Were you to add reference here?
Jo; 04/10/2016