Vitamin D significantly alters the immune transcriptome and augments CD4 recall responses in HIV

Raskit Lachmann [1]; Margaret-Anne Bevan[1]; Sangmi Kim[1],Nishma Patel [1];Raddhima Banga [1]; Satya Baarathi[2]; Prashanthi Karyala[2]; Narayan Rao[3], Nagasuma Chandra [2]; Catherine Hawrylowicz [4]; Anna Vyakarnam [1,5]* & Barry Peters[1]*

* Joint senior authors

[1]Dept of Infectious Diseases, King's College London, [2]Dept of Biochemistry & [5]Centre for Infectious Diseases, Indian Institute of Science, Bangalore [3]Genotypic Ltd, Bangalore [4]Division of Asthma & Allergy, King's College London

INTRODUCTION

Vitamin D deficiency in HIV patients is associated with more rapid disease progression, AIDS events and higher mortality (1),

The vitamin D receptor (VDR) is ubiquitously expressed, including immune cells, e.g. monocytes, DC, activated T cells and Tregs and vitamin D-deficiency associated with conditions such as cancer, autoimmune diseases, diabetes, cardiovascular disease and infections (2). Vitamin D regulates directly or indirectly more than 200 genes (>1% of the human genome) (3).

Vitamin D supplementation caused changes among others in the expression of genes involved in apoptosis, immune function, transcriptional regulation, epigenetic modification and proliferation (3). Among the regulated immune function genes were genes for immunoreceptors, chemokines and chemokine receptors and growth factors/cytokines (4).

These considerations promoted us to conduct a detailed longitudinal study to assess benefits of administering a single high oral dose of 200,000 IU cholecalciferol in HIV-infected subjects. Specifically, we examined the impact of vitamin D supplementation on CD4 T-cell recall responses, which are dramatically impaired early on in HIV-infection and precipitate rus-induced immunosuppression.

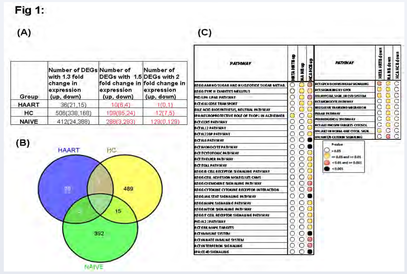
METHODOLOGY

This prospective controlled study enrolled 28 subjects with low plasma vitamin D (Vit D) (<20ng/l, 50micromol/l) comprising 17 HIV+ patients (11 on HAART, 6 treatment naive) and 11 healthy controls. A single dose of 200,000 IU oral cholecalciferol was administered. Blood samples were analysed at baseline and one month. Advanced multi-colour flow cytometry was used to assess T-cell recall responses and correlated with key immunomodulatory plasma factors. In addition the whole blood transcriptome was analysed to identify key immune pathways impacted by vitamin D.

REFERENCES

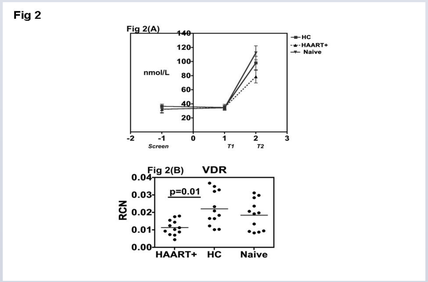
1. Viard JP, *et al.* (2011) Vitamin D and clinical disease progression in HIV infection: results from the EuroSIDA study. *AIDS* 25(10):1305-1315.
2. Pludowski P, *et al.* (2013) Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality-A review of recent evidence. *Autoimmunity reviews*.
3. Hossein-Nezhad A, Spira A, & Holick MF (2013) Influence of vitamin d status and vitamin d3 supplementation on genome wide expression of white blood cells: a randomized double-blind clinical trial. *PloS one* 8(3):e58725.
4. Griffin MD, Xing N, & Kumar R (2004) Gene expression profiles in dendritic cells conditioned by 1alpha,25-dihydroxyvitamin D3 analog. *The Journal of steroid biochemistry and molecular biology* 89-90(1-5):443-448.

Fig 1: Unique transcriptome changes induced by Vitamin D



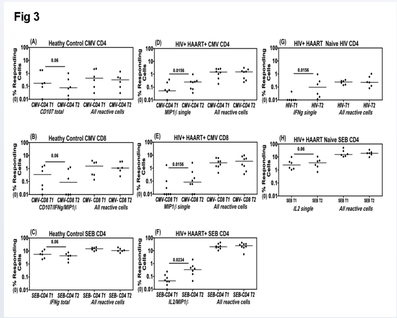
(A) Table shows the mean number of differentially expressed genes (DEGs) at 4 weeks post Vit D relative to pre-Vit D in HAART, HC and Naïve subjects at different fold change (FC) cut offs (FC: 1.319, 1.5 and 2). (B) Venn diagram illustrates the similarities and dissimilarities of DEGs (FC of 1.319) across HAART, HC and Naïve groups, with DEGs expressed in more than one condition shown in overlapping areas. (C) Table summarizing DEG enriched pathways impacting key immune functions identified in HAART, Naïve and HC is listed, with coloured circles representing statistical significance of data set.

Figure 2:Vitamin D levels and VDR expression.



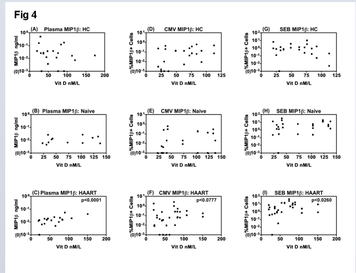
A) Mean (+SE) Vitamin D levels in plasma from HC (square), HAART (triangle, dotted line) and Naive subjects (inverse triangle) are shown at screening (-1), before supplementation (T1) and 4 week (T2) after supplementation. (B) Median RCN (relative copy number) of VDR mRNA levels relative to GAPDH is shown. P-value determined by one –way annova and Mann-Whitney U test.

Figure 3: Vitamin D induces functional changes to CD4 T cell recall responses



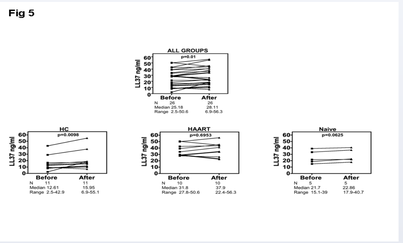
Dot plots show median frequencies of T cell subsets identified to statistically differ at 4 weeks after Vit D (T2) relative to prior supplementation (T1). Changes to CMV CD4, CMV-CD8 and SEB CD4 were noted in HC (A,B,C) and HAART (D,E,F). G and H show changes in Naïve group, to CD4 HIV-specific and CD4 SEB responses respectively. Each dot represents a single donor. Each dot plot shows differences in specific T-cell subsets at T2 v T1 relative to all reactive T cells in the same sample as measured by the ICS assay expressing IFNg, IL-2, MIP1b and CD107a. P-values determined by Wilcoxin-matched t-test is shown.

Fig 4: Vitamin D levels correlate with plasma MIP1b & MIP1b+CD4 T-cell responses in HAART+ subjects



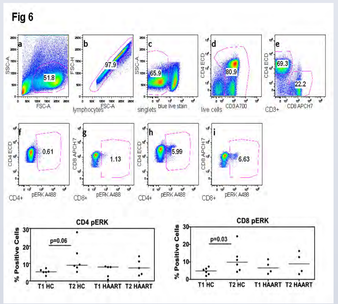
Scatter graphs show correlation between VitD levels and plasma MIP1b concentration (A,B,C) and frequency of CD4+ cells secreting *only* MIP1b specific to CMV (D,E,F) or following SEB stimulation (G,H,I). Data from each clinical group was separately analysed and shown. P-value determined by Spearman r.

Figure 5: Vitamin D enhances soluble cathelicidin



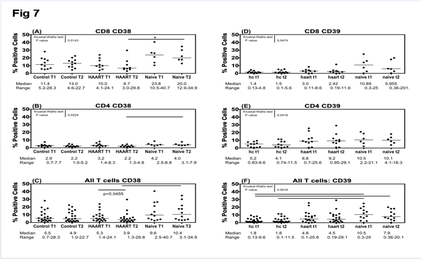
ELISA measurement of plasma Cathelecidin (LL37) before and after vitamin D is shown in (a) all samples tested across groups and samples from each of the three groups tested (b,c,d). P-values determined by Wilcoxin-matched t-test is shown.

Figure 6: Vitamin D enhances pERK mobilization



A sequential gating strategy for the definition of CD4+ CD3+ T cells expressing pERK is shown, based on: (A) FSC versus SSC (B) FSC-A versus FSC-H to identify singlets (C) exclusion of dead cells by blue viability staining (D) identifying CD3+ CD4 T cells and (E) CD3+ CD8 T-cells. Phosphorylation of ERK is shown for (F) unstimulated CD4, (G) CD8 T cells and for (H) CD4 and (I) CD8 T cells stimulated with 1ng/ml PMA for 10 min. Dot plots show median frequencies of p-ERK+ CD4 (J) and CD8 (K) T cells at 4 weeks after Vit D (T2) relative to prior supplementation (T1) in HC and HAART subjects. P-values determined by Wilcoxin-matched t-test is shown.

Figure 7: Vitamin D dampens T cell CD38 expression



Scatter plots show the median frequencies of CD38+ (A,B,C) and CD39+ (D,E,F) in CD8 (A,D), CD4 (B,E) and both CD4 and CD8 (C,F) T cells. P-value of overall differences was determined by one-way Annova. Wilcoxon matched paired test or Mann-Whitney U-test was used to confirm differences between two time points within a group or between group differences, respectively.

DISCUSSION

Vit D therapy may be a useful adjunct to HAART therapy in HIV infection by improving the quality of the T-cell response. And the innate immune response. This study provides the biological rationale for further studies designed and powered to determine the effect of Vitamin D on clinical endpoints in HIV

CONTRIBUTIONS

BP conceived the study, and designed the clinic trial, was the principle investigator for the clinical trial and contributed to all aspects of the trial design and co-wrote the manuscript; AV was the major contributor to the laboratory aspects of the trial design, contributed to the clinical aspects of trial design, supervised all laboratory aspects and co-wrote the manuscript; CH, contributed to all aspects of the trial design and co-wrote the manuscript ; RL was a contributor to the laboratory aspects of the trial design, performed most of the laboratory experiments, and co-wrote the manuscript; KS performed some of the laboratory experiments; M B helped recruit and manage the patients within the study and helped manage the data, FM helped with aspects of the clinical study design, recruit and manage the patients within the study and helped manage the data:As helped with the clinical trial design and data handling; SS, PK, NC, SN, MA, CB all contributed to performing and analyzing the microarray experiments.

ACKNOWLEDGEMENTS

The research was funded by the following grants: MRC (UK) Confidence in Concept, an unrestricted grant contribution from GlaxoSmithKline and ViiV pharmaceuticals. Department of Biotechnology, Government of India, Ramalingaswamy Fellowship. The Infectious Diseases Biobank, within the National Institute for Health Research (NIHR) Biomedical Research Centre at Kings Health Partners, London., provided laboratory support & biobanking. Guys and St Thomas' Hospital trust provided clinical space and facilities. The volunteers, both people with HIV attending Guys and St Thomas' Hospital Trust (GSTT), and healthy volunteers from amongst staff at Kings College London and GSTT volunteers their time and efforts as participants.

RESEARCH POSTER PRESENTATION DESIGN © 2012
www.PosterPresentations.com