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Allison Ross Eckard, MD
eckarda@musc.edu

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Vitamin D Supplementation Attenuates Immune Activation in HIV+ Aviremic Youth

Allison Ross Eckard[1,2], Mary Ann O'Riordan[3], Vin Tangpricha[2], Danielle Labbato[3], Ann Chahroudi[2], Grace A. McComsey[3]

[1]Medical University of South Carolina, Charleston, South Carolina, USA; [2]Emory University School of Medicine Atlanta, Georgia, USA; [3]Case Western Reserve University and Rainbow Babies & Children's Hospital, Cleveland, Ohio, USA

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ABSTRACT

Background: Heightened immune activation and global immune dysfunction drive HIV disease progression and co-morbidities. Vitamin D has pleiotropic effects on the immune system, but little is known about the long-term effects of supplementation in HIV-infected youth.
Methods: This is a 24-month randomized, active-control, double-blind trial investigating 3 different monthly vitamin D_3 doses [18,000 (standard), 60,000 (moderate) or 120,000 (high) IU/month] in 8-26 year old HIV+ youth on antiretroviral therapy with baseline 25-hydroxyvitamin D (25(OH)D) ≤30 ng/mL and HIV-1 RNA <1000 copies/mL. Soluble and cellular markers of immune activation were measured by ELISA and flow cytometry, respectively.
Results: 68 subjects completed the 24-month study period: 66% male, 88% black with median (Q1, Q3) age of 20 (15, 23) years and CD4 count of 654 (430, 894) cells/mm^3. Baseline 25(OH)D was 17 (14, 22) ng/mL and increased within each dosing group (standard: +11 (9, 19); moderate: +20 (8, 25); high: +25 (19, 38) ng/mL; all P<0.001). Overall, all markers of monocyte activation decreased significantly [sCD14 (-0.8 ng/mL, P<0.0001); CD14+CD16+ (-10.4%, P<0.0001); CD14^{dim}CD16+ (-4.4%, P=0.04)], but CD4+ and CD8+ T-cell activation/exhaustion markers did not. There were no significant differences between marker changes based on randomized arm or correlations between changes in 25(OH)D and changes in markers for all subjects considered together. However, when the analysis included only those with undetectable HIV-1 RNA throughout the study period (N=28), significant correlations were seen between changes in 25(OH)D and changes in proportion of patrolling monocytes (CD14^{dim}CD16+; R=0.44, P=0.03) and activated CD8+ T-cells expressing PD-1 (CD8+CD38+HLA-DR+PD-1+; R=-0.43, P=0.02) with a trend toward significance for activated CD8+ T-cells (CD8+CD38+HLA-DR+; R=-0.33, P=0.09). None of these correlations were seen among the subjects who had detectable HIV-1 RNA at any point during the study (CD14^{dim}CD16+: R=0.03, P=0.86; CD8+CD38+HLA-DR+PD-1+: R=0.04, P=0.82; CD8+CD38+HLA-DR+: R=-0.03, P=0.88) (Figure).
Conclusions: Changes in serum 25(OH)D concentrations were strongly associated with changes in proportions of patrolling monocytes, activated CD8+ T-cells and exhausted CD8+ T-cells, but only in the context of sustained viral suppression. These data suggest an important immunomodulatory role of vitamin D in HIV and should be further investigated as an adjuvant treatment to antiretroviral therapy.

BACKGROUND

- HIV+ patients have increased levels of immune activation and exhaustion.
- This phenomenon is associated with HIV disease progression and mortality and an increased risk of HIV-related co-morbidities.
- Vitamin D has pleotropic immunomodulatory effects, and we have shown some decreases in immune activation and exhaustion after 12 months of high dose vitamin D supplementation.
- Here, we present data on changes in immune activation and exhaustion in HIV after 24 months of vitamin D supplementation.

METHODS

- **STUDY DESIGN**
  - Randomized, active-controlled, double-blind trial investigating two doses of oral vitamin D_3: 60,000 IU (moderate dose) and 120,000 IU (high dose) vs. a standard dose of 18,000 IU (control dose) given monthly for 24 months
  - Randomization stratified by EFV use at entry
- **SELECTION OF SUBJECTS**
  - **Inclusion criteria:** HIV infection, age between 8-25 years, cumulative ARV duration ≥6 months, current ARV regimen consistently for ≥3 months prior to enrollment, HIV-1 RNA level ≤1,000 copies/mL
  - **Exclusion criteria:** Acute illness ≤30 days prior to enrollment, inflammatory condition, malignancy, medication use which could lead to changes in immune activation markers, pregnancy/breastfeeding
- **STUDY ASSESSMENTS**
  - **Clinical and laboratory evaluation:** Questionnaires, extensive chart review, weight, height, HIV-1 RNA, CD4 count, serum 25(OH)D concentration
  - **Immune markers:** cryopreserved PBMCs were assessed by flow cytometry and categorized based on size, granularity and the expression of specific phenotypes on the cell surface
    - **CD4 T-cell activation:** CD4+CD38+HLA-DR+
    - **CD8 T-cell activation:** CD8+CD38+HLA-DR+
    - **CD4 T-cell exhaustion:** CD4+CD38+HLA-DR+PD-1+
    - **CD8 T-cell exhaustion:** CD8+CD38+HLA-DR+PD-1+
    - **Inflammatory monocytes:** CD14+CD16+
    - **Patrolling monocytes:** CD14dimCD16+
    - **sCD14:** shed from activated monocytes and measured in plasma by ELISA
- **STATISTICAL ANALYSIS**
  - All analyses were conducted as intent-to-treat
  - ***After initial results were analyzed showing no difference in marker changes based on vitamin D dosing group, all HIV+ subjects were analyzed based on HIV-1 RNA status: undetectable at baseline and throughout the 24-month study period vs. detectable at any time point during study period***
  - **Group comparisons:** appropriate two-sample and three-sample tests
  - **Within-group:** appropriate one-pair tests
  - **Bivariate correlations:** Spearman correlation coefficients
  - **Multivariable regressions:** Used stepwise selection process with variables selected for inclusion based on clinical significance. P≤0.15 remained in final models.
  - P<0.05 considered significant for all analyses.

RESULTS

Changes over 24 Months for All Subjects Combined by Vitamin D Dosing Group

Median (Q1, Q3)	Standard Dose (N=26)	P†	Moderate Dose (N=22)	P†	High Dose (N=20)	P†	P*
25(OH)D, ng/mL	+11.2 (+8.6, +19.0)	**<0.0001**	+19.5 (+8.0, +24.5)	**<0.0001**	+24.5 (+19.4, +38.0)	**<0.0001**	**0.0002**
% CD4+CD38+HLA-DR+[a]	-0.1 (-0.5, +0.6)	0.79	-1.1 (-1.4, -0.8)	0.18	+0.1 (-1.2, +1.4)	0.64	0.91
% CD8+CD38+HLA-DR+[a]	-0.3 (-2.3, +2.2)	0.86	+0.4 (-0.3, +0.9)	0.61	-1.2 (-4.5, +1.3)	0.65	0.52
% CD4+CD38+HLA-DR+PD1+[a]	-0.2 (-0.4, +0.2)	0.17	-0.1 (-0.2, +0.3)	0.89	-0.1 (-0.6, +0.2)	0.39	0.95
% CD8+CD38+HLA-DR+PD1+[a]	-0.2 (-1.0, +0.3)	0.40	-0.3 (-0.6, +0.4)	0.60	-0.4 (-1.3, +0.5)	0.42	0.64
% CD14+CD16+[a]	-6.5 (-18.4, -3.5)	**<0.0001**	-7.1 (-10.1, -0.4)	**0.002**	-4.3 (-13.3, -1.4)	**0.01**	0.42
% CD14dimCD16+[a]	-2.1 (-10.8, +2.1)	0.07	-1.1 (-11.4, +3.0)	0.30	-1.8 (-5.2, +3.2)	0.84	0.41
sCD14, ng/mL	-1.1 (-1.7, -0.7)	**<0.0001**	-1.1 (-1.4, -0.8)	**<0.0001**	-1.0 (-1.3, -0.6)	**<0.0001**	0.20

[a]Data represent the % of that cell type expressing the given phenotype; †Within-group changes; *Standard dose vs. high dose

Baseline Characteristics

Median (Q1, Q3) or no. (%)	All Subjects with Known HIV-1 RNA Status (N=62)	HIV+ with Undetectable HIV-1 RNA (N=29)	HIV+ with Detectable HIV-1 RNA (N=33)	P*
Clinical and Sociodemographics				
Age, years	19.5 (14.5, 21.8)	19.6 (13.9, 21.3)	19.1 (16.8, 23.0)	0.55
Male sex	41 (66%)	17 (59%)	24 (73%)	0.24
Black race	54 (87%)	22 (76%)	32 (97%	**0.01**
BMI, kg/m^2	21.6 (19.1, 25.0)	21.8 (19.2, 24.8)	20.9 (19.1, 25.9)	0.88
Current smoking	9 (15%)	1 (3.5%)	8 (24%)	**0.02**
Current alcohol use	25 (41%)	7 (25%)	18 (55%)	**0.02**
25(OH)D, ng/mL	16.7 (13.5, 20.8)	15.1 (13.0, 20.7)	17.0 (14.5, 23.0)	0.36
HIV Variables				
Current CD4, cells/mm^3	654 (441, 872)	659 (465, 872)	652 (419, 867)	0.67
Nadir CD4, cells/mm^3	287 (114, 407)	255 (109, 389)	317 (119, 475)	0.35
Perinatal transmission	36 (58%)	20 (69%)	16 (49%)	0.10
Previous AIDS diagnosis	25 (41%)	15% (54%)	10 (30%)	0.07
HIV duration, years	10.1 (2.9, 15.8)	12.4 (5.0, 15.1)	8.1 (2.1, 16.5)	0.10
ARV duration, years	6.6 (1.6, 10.2)	9.2 (2.6, 12.9)	5.4 (1.3, 7.7)	**0.03**
NRTI duration, years	6.1 (1.6, 10.2)	8.5 (2.4, 11.7)	5.4 (1.3, 7.7)	0.09
PI duration, years	3.4 (0.9, 8.4)	5.1 (1.2, 9.7)	2.6 (0.7, 6.6)	0.33
EFV duration, years	0.5 (0.0, 1.6)	0.73 (0.0, 2.6)	0.5 (0.0, 1.3)	0.38
TDF duration, years	1.6 (0.6, 2.6)	1.9 (1.2, 3.2)	1.5 (0.6, 2.3)	0.17
Current PI use	45 (73%)	12 (42%)	14 (42%)	0.93
Current EFV use	17 (27%)	10 (35%)	7 (79%)	0.24
Current TDF use	26 (42%)	18 (62%)	27 (82%)	0.08
Immune Activation Markers				
% CD4+CD38+HLA-DR+[a]	1.7 (1.2, 2.7)	1.4 (1.1, 2.2)	1.9 (1.3, 3.2)	0.07
% CD8+CD38+HLA-DR+[a]	3.7 (2.5, 6.4)	3.4 (2.2, 4.4)	4.1 (2.6, 9.4)	0.10
% CD4+CD38+HLA-DR+PD1+[a]	0.7 (0.5, 1.1)	0.7 (0.5, 1.1)	0.9 (0.5, 1.6)	0.27
% CD8+CD38+HLA-DR+PD1+[a]	1.2 (0.6, 2.2)	1.2 (0.6, 1.8)	1.3 (0.6, 2.7)	0.51
% CD14+CD16+[a]	11.9 (7.5, 19.9)	12.7 (8.5, 19.9)	11.3 (6.5, 20.5)	0.36
% CD14dimCD16+[a]	8.7 (5.3, 19.1)	12.4 (5.9, 20.3)	7.2 (4.9, 16.2)	0.13
sCD14, ng/mL	1.7 (1.4, 2.1)	1.7 (1.4, 2.1)	1.6 (1.4, 2.1)	0.75

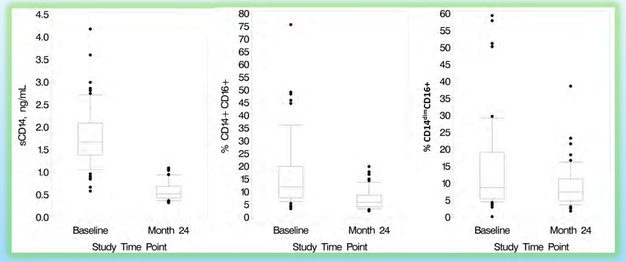
[a]Data represent the % of that cell type expressing the given phenotype; *P value between HIV+ with undetectable and detectable HIV-1 RNA

Changes over 24 Months in Relevant Variables by HIV-1 RNA Designation

Median (Q1, Q3)	Subjects Combined (N=62)	P†	Subjects with Undetectable HIV-1 RNA (N=29)	P†	Subjects with Detectable HIV-1 RNA (N=33)	P†	P*
25(OH)D, ng/mL	+19.4 (+9.7, +26.8)	**<0.0001**	+18 (+10, +24)	**<0.0001**	+21 (+9, +28)	**<0.0001**	0.49
% CD4+CD38+HLA-DR+[a]	+0.0 (-0.6, +1.0)	0.39	-0.04 (-0.8, 0.80)	0.85	0.2 (-0.5, +1.1)	0.34	0.51
% CD8+CD38+HLA-DR+[a]	-0.5 (-2.5, +1.5)	0.56	-0.3 (-1.8, 1.5)	0.85	-0.1 (-3.8, +2.0)	0.63	0.62
% CD4+CD38+HLA-DR+PD1+[a]	-0.1 (-0.4, +0.2)	0.17	-0.1 (-0.5, 0.01)	**0.04**	-0.04 (-0.4, +0.3)	0.87	0.19
% CD8+CD38+HLA-DR+PD1+[a]	-0.2 (-0.8, +0.4)	0.26	-0.3 (-0.9, 0.2)	0.25	-0.2 (-0.8, +0.9)	0.87	0.40
% CD14+CD16+[a]	-6.2 (-13.3, -2.5)	**<0.0001**	-7.7 (-15, -4.6)	**<0.0001**	-4.2 (-11.1), -0.9)	**0.002**	0.11
% CD14dimCD16+[a]	-2.1 (-11.4, +3.0)	**0.03**	-3.4 (-11.4, 1.9)	**0.03**	-0.5 (-9.8, +3.3)	0.56	0.21
sCD14, ng/mL	-1.1, (-1.5, -0.8)	**<0.0001**	-1.1 (-1.4, -0.8)	**<0.0001**	-1.1 (-1.6, -0.89)	**<0.0001**	0.68

[a]Data represent the % of that cell type expressing the given phenotype; †P value within group; ‡P value between the two HIV+ groups

Significant Decreases in Immune Markers for Subjects Combined

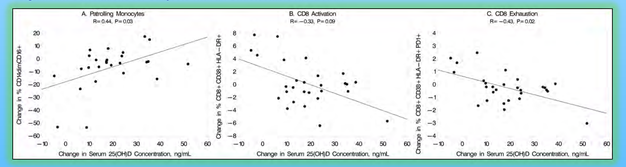


Correlations between change in 25(OH)D concentration and changes in immune activation markers

Immune Activation Marker	Subjects Combined (N=62)	Subjects with Undetectable HIV-1 RNA (N=29)	Subjects with Detectable HIV-1 RNA (N=33)
% CD4+CD38+HLA-DR+	R=0.08; P=0.53	R=-0.03; P=0.90	R=0.15; P=0.43
% CD8+CD38+HLA-DR+	R=-0.15; P=0.26	**R=-0.33; P=0.09**	R=-0.03; P=0.88
% CD4+CD38+HLA-DR+PD1+	R=-0.02; P=0.89	R=-0.15; P=0.46	R=0.04; P=0.82
% CD8+CD38+HLA-DR+PD1+	R=-0.19; P=0.16	**R=-0.43; P=0.02**	R=-0.02; P=0.90
% CD14+CD16+	R=-0.08; P=0.57	R=-0.14; P=0.49	R=-0.06; P=0.74
% CD14dimCD16+	R=0.20; P=0.14	**R=0.44; P=0.03**	R=-0.74; P=0.86
sCD14, ng/mL	R=0.16; P=0.23	R=0.20; P=0.30	R=0.11; P=0.55

R=Spearman Correlation Coefficient

Significant Correlations for Subjects with an Undetectable HIV-1 RNA



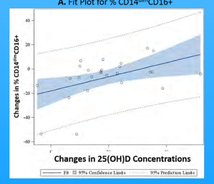
Regressions with Stepwise Selection Process for Subjects with Undetectable HIV-1 RNA (N=29)

	Δ in 25 (OH)D	Age	HIV Duration	ARV Duration	Current CD4	R²
% CD4+CD38+HLA-DR+	--	--	--	--	P=0.06	0.14
% CD8+CD38+HLA-DR+	**P=0.01**	--	--	--	--	0.23
% CD4+CD38+HLA-DR+PD1+	**P=0.04**		**P=0.008**		P=0.14	0.43
% CD8+CD38+HLA-DR+PD1+	**P=0.009**	--	--	--	--	0.24
% CD14+CD16+	**P=0.03**	**P=0.04**	--	**P=0.08**		0.42
% CD14dimCD16+	**P=0.02**	--	--	--	--	0.22
sCD14, ng/mL	--	--	--	--	**P=0.046**	0.15

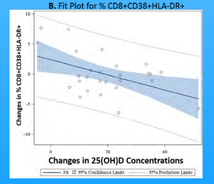
Regressions with Stepwise Selection Process with Subjects Regardless of HIV-1 RNA (N=62)

	Δ in 25 (OH)D	Age	HIV Duration	ARV Duration	Current CD4	R²
% CD4+CD38+HLA-DR+	**0.004**				**0.0002**	0.34
% CD8+CD38+HLA-DR+					**0.003**	0.15
% CD4+CD38+HLA-DR+PD1+	**0.049**				**0.008**	0.19
% CD8+CD38+HLA-DR+PD1+					**0.003**	0.16
% CD14+CD16+	0.06	0.13		0.05		0.17
% CD14dimCD16+	0.07					0.06
sCD14, ng/mL	0.14					0.04

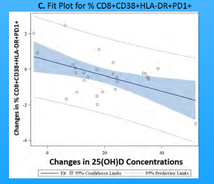
A. Fit Plot for % CD14^{dim}CD16+



B. Fit Plot for % CD8+CD38+HLA-DR+



C. Fit Plot for % CD8+CD38+HLA-DR+PD1+



SUMMARY OF RESULTS

- All subjects had significant increases in 25(OH)D concentrations regardless of dose, and there were no differences in immune activation changes based on vitamin D supplementation arm.
- All subjects had significant decreases in % of cells expressing phenotypes representative of monocyte activation, but no changes in T-cell activation.
- There were no significant differences in immune activation changes based on HIV-1 RNA status; however, only subjects who maintained an undetectable HIV-1 RNA level throughout the study period showed significant correlations between changes in 25(OH)D and changes in CD8 activation, CD8 exhaustion and patrolling monocytes.
- In multivariable regression, 25(OH)D changes were independently associated with changes in CD8 activation, CD4/CD8 exhaustion and inflammatory & patrolling monocytes in the undetectable HIV-1 RNA group, a relationship that was not seen as consistently in the group who did not maintain an undetectable HIV-1 RNA.

CONCLUSIONS

The results suggest that vitamin D supplementation affects levels of immune activation, particularly monocyte activation, but effects appear to be more profound in the context of viral suppression. Further studies are indicated investigating the mechanisms related to these changes and the potential implications on co-morbidities that are associated with immune activation.

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