Reduced Immune Activation during TDF-FTC Therapy in HIV-negative Individuals

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ABSTRACT

BACKGROUND: Higher levels of immune activation are associated with an increased risk of HIV acquisition. Previous research *in vitro* has identified immunomodulatory properties of tenofovir (TFV) in various cell lines. However, it is unknown whether TFV influences immune activation *in vivo*. We aimed to evaluate the effects of TFV therapy on cellular immune activation in HIV-negative individuals.

METHODOLOGY: HIV-negative adults received daily co-formulated tenofovir disoproxil fumarate (TDF) 300 mg-emtricitabine (FTC) 200 mg for 30 days followed by a 30 day washout period. Whole blood for flow cytometry was collected at baseline and on days 30 and 60. Immune activation was determined by measuring % HLA-DR and CD38 expression on $CD4^+$ and $CD8^+$ T-cells. Additionally, intracellular TFV-diphosphate (TFV-DP) was quantified in purified $CD4^+$ and $CD8^+$ cells, which were collected at one study visit in each participant. Immune activation data were $\log_e$ transformed prior to analysis. Data were analyzed with one-way ANOVA for repeated measures and pairwise comparison for significant results between baseline, day 30 and day 60. The relationship between immune activation and intracellular $CD4^+$ and $CD8^+$ TFV-DP was assessed using the Spearman correlation coefficient. A p value ≤0.05 was considered significant. Data are presented as geometric mean (95% CI).

RESULTS: Data were available from 19 participants (10 female; 1 Hispanic; 9 African American). Co-expression of CD38/HLA-DR on $CD4^+$ cells was reduced, but not significantly (ANOVA p=0.25); baseline 2.61% (2.1 to 3.2), versus 2.11% (1.64 to 2.70) at day 30 (30 days of daily TDF-FTC), and 2.13% (1.64 to 2.76) at day 60 (30 days off drug). Significant differences for immune activation markers on $CD8^+$ cells were demonstrated (ANOVA p=0.02). Co-expression of CD38/HLA-DR on $CD8^+$ cells was 3.97% (2.98 to 5.29) at baseline, 2.71% (2.01 to 3.66) at day 30, p=0.03, and 2.41% (1.65 to 3.53) at day 60, p=0.008. Differences between day 30 and day 60 were not significant (p=0.58). No significant correlation was identified between CD38/HLA-DR expression and intracellular levels of TFV-DP in $CD4^+$ (r= -0.18, 95% CI, -0.61 to 0.34; p=0.51) or $CD8^+$ (r= -0.01, 95% CI, -0.53 to 0.52; p=0.97).

CONCLUSIONS: Exposure to TDF-FTC was associated with decreased immune activation in HIV-negative individuals. The effect endured for 30 days after drug discontinuation, which parallels the long half-life of intracellular TFV moieties. A decrease in immune activation could contribute to the known antiviral effect of TDF-FTC as pre-exposure prophylaxis (PrEP) in HIV-negative patients.

BACKGROUND

- High levels of immune activation have been associated with an increased risk of HIV acquisition[1,2]. Promoting immune quiescence could be targeted as a modifiable HIV risk factor to reduce the risk of HIV infection.
- TFV induces the secretion of various cytokines *in vitro*[3,4], but the immunomodulatory effects of TFV *in vivo* are unknown. Currently, no proven interventions to induce immune quiescence *in vivo* are currently available.

OBJECTIVE

- To evaluate the effects of TFV therapy on the cellular immune activation of HIV-negative individuals.

METHODS

- HIV-negative individuals participating in "Cell Prep" (NCT01040091), a 60-day pharmacokinetic study of daily co-formulated oral tenofovir disoproxil fumarate/emtricitabine (TDF-FTC), were enrolled. Participants received TDF-FTC for a period of 30 days followed by a 30 day "wash out" period.

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METHODS (cont'd)

- Whole blood for flow cytometry was collected at baseline (before initiation of TDF-FTC), day 30 and day 60 (30 days after drug discontinuation). Plasma for cytokines and soluble markers was obtained at same time intervals and stored frozen at -80ºC for further analysis.
- Immune activation in $CD4^+$ and $CD8^+$ T cells was determined using flow cytometry by measuring the percentage of HLA-DR and CD38 expression. Cytokine levels (IL-1a, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-15, IL-17, IL-23, IFN-γ, TNF-α,TNF-β) were measured using the 15-plex high-sensitivity Quansys Multiplex cytokine array ELISA (Quansys Biosciences, Logan, UT). sCD14, sCD27 and hs-CRP were measured using assay specific ELISA. All wells were analyzed at 450nm on a Coulter Microplate Reader.
- Purified $CD4^+$ and $CD8^+$ T cells were collected at one study visit for each participant and intracellular quantification of TFV-diphosphate (TFV-DP) in T cell subsets was performed using a validated LC-MS/MS method[5]. Intracellular levels of TFV-DP were adjusted for time on therapy by using a one-phase association model.
- Data were $\log_e$ transformed and one-way ANOVA for repeated measures and pairwise comparison for significant results between baseline, day 30 and day 60 were utilized.
- The relationship between $CD4^+$ and $CD8^+$ immune activation and intracellular TFV-DP was evaluated using the Spearman correlation coefficient. Data are presented as geometric mean (95% CI). A p value ≤ 0.05 was considered to be statistically significant.

RESULTS

Table 1. Demographic characteristics of the study population (N=19).

Characteristic	Number
Males	9
Females	10
African American	9 (5 Female)
Non-African American	
White	9 (5 Female)
Hispanic	1 (Male)
Median age (range) years	30 (20-47)

RESULTS (cont'd)

Table 2. Immune changes after 30 days of daily TDF/FTC therapy and after 30 days in "wash out" period (day 60).

	Baseline	ANOVA P value	Day 30	P value*	Day 60	P value*
%HLA-DR+/CD38+ CD4+	2.61 (2.10 to 3.24)	0.25	2.11 (1.64-2.70)	0.14	2.13 (1.64 to 2.76)	0.16
%HLA-DR+/CD38+ CD8+	3.97 (2.98 to 5.29)	0.02	2.71 (2.01 to 3.66)	0.03	2.41 (1.65 to 3.53)	0.008
sCD14 (mcg/ml)	1.63 (1.51 to 1.74)	0.06	1.51 (1.38 to 1.64)	0.03	1.51 (1.42 to 1.61)	0.04
sCD27 (pg/ml)	184.1 (163.4 to 207.6)	0.001	184.1 (165.9 to 204.2)	0.98	168.4 (149.3 to 190)	0.001
hs-CRP (mcg/ml)	1.91 (1.25 to 2.92)	0.16	1.24 (0.76 to 2.00)	0.06	1.59 (0.95 to 2.66)	0.42
IL-10 (pg/ml)	1.88 (1.55 to 2.29)	0.37	2.00 (1.65 to 2.44)	0.54	1.74 (1.40 to 2.15)	0.42
TNF-alpha (pg/ml)	8.21 (6.74 to 10.02)	0.26	8.32 (6.84 to 10.13)	0.86	7.38 (5.77 to 9.43)	0.18
IFN-gamma (pg/ml)	0.76 (0.48 to 1.20)	0.93	0.73 (0.49 to 1.09)	0.89	0.81 (0.50 to 1.32)	0.81

Data are geometric mean (95% CI). *Compared to baseline. Cytokine and soluble markers measured in N=18 participants.

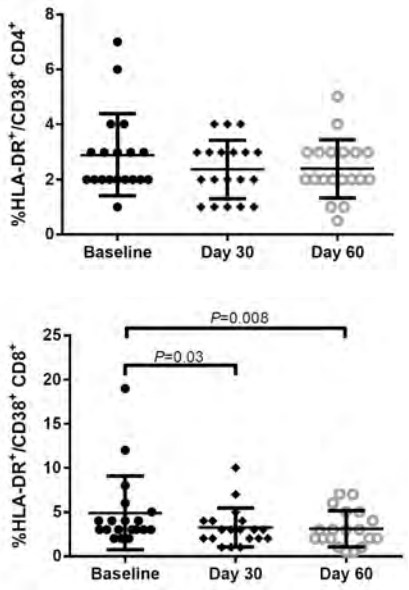

Figure 1. $CD4^+$ T and $CD8^+$ T cell activation after 30 days of TDF-FTC (line is mean ± SD).

- No correlation between intracellular levels of TFV-DP and immune activation in $CD4^+$ (r= -0.18, 95% CI, -0.61 to 0.34; p=0.51) and $CD8^+$ (r= -0.01, 95% CI, -0.53 to 0.52; p=0.97) was observed

CONCLUSIONS

- Short-term daily TDF-FTC was associated with reduced immune activation as measured by CD38 and HLA-DR expression on $CD8^+$ T cells and, to a lesser extent, on $CD4^+$ T cells in HIV-negative subjects. This effect was paralleled by an initial decrease in monocyte activation soluble markers followed by a decrease in lymphocyte activation soluble markers.
- The mechanism by which the immune activation was reduced was not elucidated in this study, but the results are consistent with *in vitro* studies that demonstrate immunomodulatory effects for TFV.
- The potential clinical implications of the reduced immune activation observed in this study require additional evaluation, but could include enhanced chemoprophylactic effect for PrEP and/or slowing the progression of end organ complications from overactive immune activation in HIV-infected individuals.

Acknowledgments and References

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