

Preservation HIV-1–Specific IFN γ + CD4+ T-Cell Responses in Breakthrough Infections After Exposure to Tenofovir Gel in the CAPRISA 004 Microbicide Trial

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Abstract: The Centre for the AIDS Program of Research in South Africa 004 trial demonstrated reduction of sexual HIV-1 acquisition in women using a vaginal microbicide containing tenofovir. A better understanding of the consequences of antiretroviral-containing microbicides for immune responses in individuals with intercurrent HIV-1 infection is needed for future trials combining the use of microbicides with HIV-1 vaccines. Investigation of immune responses in women who acquired HIV-1 although using tenofovir

gel showed significantly higher ($P = 0.01$) Gag-specific IFN γ + CD4+ T-cell responses. The use of tenofovir-containing gel around the time of infection can modulate HIV-1 immunity, and these immunological changes need to be considered in future trials combining vaccines and microbicides.

Key Words: HIV-1, vaginal microbicide, tenofovir, HIV-1–specific CD4+ T-cell help

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INTRODUCTION

A recent randomized controlled trial undertaken by the Centre for the AIDS Program of Research in South Africa (CAPRISA) reported that a vaginal microbicide gel containing 1% tenofovir reduced the risk of HIV-1 infection in women by 39%.¹ Although confirmation of these findings in additional studies are needed and similar studies are currently underway, these results will have significant implications for the design of future prevention trials, including vaccine trials in which both vaccinees and placebo recipients might receive prophylactic antiretroviral drugs in gel or oral formulation.² A better understanding of the consequences of antiretroviral-containing microbicides for immune responses in individuals with intercurrent HIV-1 infection is therefore critical to take these immunological changes into consideration in the design of future trials combining the use of microbicides with HIV-1 vaccines.² In the present study, we investigated innate and adaptive immune responses during primary HIV-1 infection in women who acquired HIV-1 although using either tenofovir gel or placebo in the CAPRISA004 trial.

MATERIALS AND METHODS

Study Population

Cryopreserved peripheral blood mononuclear cell (PBMCs) were collected from sexually active HIV-1 clade C–infected 18-year-old to 40-year-old women in urban and rural KwaZulu-Natal, South Africa, enrolled in CAPRISA 004.¹ The eligibility and exclusion criteria for the parent trial have been previously reported.¹ Participants who

acquired HIV stopped using study gel on confirmation of HIV infection, as per the trial protocol.¹ From the total of 98 intercurrent HIV infections that occurred, 36 randomly selected HIV-1–infected female adults exposed either to tenofovir gel (n = 17) or placebo (n = 19) were selected for this substudy. The study was approved by the University of KwaZulu-Natal and Massachusetts General Hospital Biomedical Research Ethics Committee, Family Health International Protection of Human Subjects Committee, and the South African Medicines Control Council with each subject giving informed consent for participation.

Characterization of Phenotype and Function of Innate and Adaptive Immune Cells Using Multiparameter Flow Cytometry

The phenotypic characteristics of natural killer (NK) cells and myeloid dendritic cells (mDCs) were assessed by multiparameter flow cytometry using cryopreserved PBMC collected within 3 months of HIV-1 infection. After gating on lineage negative (CD3neg, CD19neg, CD56neg) lymphocytes, anti-CD11c was used to identify mDCs, as described.^{3,4} Antibodies directed against Human Leukocyte Antigen (HLA)-DR, CD83, and CD86 were used to study the activation and maturation stage of mDCs directly ex-vivo. NK cells were defined as CD3negCD56/CD16+ cells, and antibodies directed against HLA-DR, CD38, and CD69 were used to study the activation status of NK cells, as described.⁵

Intracellular measurement of interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukin 2 (IL-2) production by CD4+ and CD8+ T cells was undertaken as described.^{6,7} Cryopreserved PBMCs were stimulated for 6 hours at 37°C; 5% CO₂ with Gag and Nef peptide pools spanning the HIV-1 clade C consensus sequence (final concentration 2 μg/ml per peptide) in the presence of brefeldin A. Negative controls with PBMCs and media alone and positive controls stimulated with phytohemagglutinin were included in the assays. Between 500,000–1,000,000 events were acquired on a LSRII flow cytometer, and data analysis was performed using FlowJo version 8.8.2 (TreeStar, Inc). Boolean gating was used to create a full array of the 8 possible response patterns when testing 3 functions, and data were further analyzed using SPICE 5 and PESTLE software programs (kindly provided by Drs Roederer and Nozzi, National Institute of Allergy and Infectious Diseases, National Institutes of Health).

Statistical Analyses

Statistical analyses and graphical presentation was done using Graphpad Prism 5 (Graphpad). Results are given as averages with standard deviations. Paired 2-tailed Student *t* tests were used to test statistical significance. Differences after comparisons were considered statistically significant if *P* < 0.05.

RESULTS

Characteristics of Study Subjects

Cryopreserved PBMCs collected from 36 sexually active HIV-1–infected women in urban and rural KwaZulu-

TABLE 1. Characteristics of Study Subjects

	Tenofovir	Placebo	<i>P</i>
n	17	19	—
Age	24 (19–37)	23 (19–31)	0.69
Days post infection	85 (20–345)	64 (15–481)	0.30
Initial CD4+ T-cell count	454 (182–955)	556 (240–1036)	0.17
CD4+ T-cell count post infection	464 (197–955)	510 (240–1036)	0.11
Initial viral log (log)	4.66 (2.60–6.53)	4.42 (2.60–5.85)	0.72
Viral load post infection (log)	4.96 (2.60–5.86)	4.51 (2.60–5.51)	0.58

Average and range is shown.

Natal enrolled into the CAPRISA 004 trial¹ were randomly selected and studied. Investigators were blinded regarding the study group for the immunological analysis. Seventeen of these women were part of the tenofovir gel arm and 19 of the placebo arm. The demographic, immunological, and virological characteristics of the study cohort are presented in Table 1. Women in the 2 arms did not differ in the days since infection, and HIV-1 viral loads and CD4+ T-cell counts at presentation or analysis, and did also not differ in the distribution of low, intermediate, and high adherers.

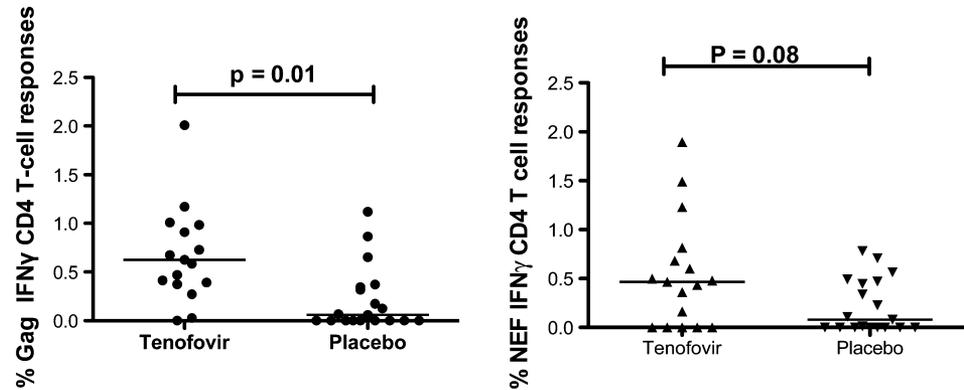
Lack of Differences in Innate Immune Cells Between the Study Arms at 3 Months Post Infection

We initially examined the frequencies and activation status of NK cells and mDCs between women in the tenofovir or placebo arms. The average frequencies of NK cells did not differ between the 2 arms (average NK cells 4.7% ± 3.8% vs. 5.5% ± 4.6%). Furthermore, the average percentage of NK cells expressing HLA-DR (12% ± 6.3% vs. 12.9% ± 9.2%), CD38 (68.9% ± 25.3% vs. 67.1% ± 28.6%) or CD69 (10.3% ± 10.9% vs. 10.7% ± 7.1%) did not differ between the women who received tenofovir compared with those who received placebo (*P* > 0.05 for all comparisons). Similarly, the average percentage of mDCs (5.7% ± 3.2% vs. 5.9% ± 1.9%) and their activation status (HLA-DR: 19.8% ± 19.5% vs. 17.5% ± 11.3%; CD83: 6.8% ± 9.5% vs. 6.7% ± 4.2%, and CD86: 19.3% ± 21.4% vs. 11.5% ± 11.7%; *p* > 0.05 for all comparisons) did not differ between tenofovir and placebo gel recipients. Taken together, these data demonstrate that frequencies and activation status of cells of the innate immune system were not significantly impacted by the use of tenofovir gel in women with breakthrough HIV-1 infection approximately 3 months after infection.

Preservation of HIV-1–Specific IFNγ CD4+ T Cells During Primary Infection

Previous studies have shown that HIV-1 Gag and Nef are targeted by HIV-1–specific T cells during primary infection, and that virus-specific CD4+ T-cell responses are rapidly lost after infections.⁸ We compared HIV-1 clade C Gag-specific and Nef-specific CD4+ and CD8+ T-cell activity

FIGURE 1. Higher HIV-1 Gag-specific and Nef-specific CD4+ T-cell responses in tenofovir recipients. The dot plot represents the median % IFN γ + CD4+ T cells in response to HIV-1 Gag (left panel) and Nef (right panel) in women enrolled in the tenofovir arm (n = 17) compared with women who received placebo (n = 19).



between women who received tenofovir gel or placebo recipients. As shown in Figure 1, Gag-specific IFN- γ + CD4+ T-cell responses were significantly higher in the women in the tenofovir gel arm compared with placebo ($P = 0.01$). No correlation was observed between days postinfection and Gag-specific IFN- γ + CD4+ T-cell responses ($R = -0.2$; $P = 0.3$). HIV-1 Nef-specific CD4+ T-cell responses also tended to be higher in women in the tenofovir gel arm compared with placebo recipients ($P = 0.08$, Fig. 1). No significant differences were observed in the percentage of Gag-specific and Nef-specific CD4+ T-cells producing TNF- α or IL-2 between the 2 groups (data not shown).

Gag-specific and Nef-specific IFN- γ + CD8+ T-cell responses were also higher in the tenofovir arm compared with placebo though those differences did not reach statistical significance (Gag: average 1.1% IFN- γ + CD8+ T cells \pm 1.0% in tenofovir arm vs. 0.7% \pm 0.7% in placebo, $P = 0.33$; Nef: average 1.1% IFN- γ + CD4+ T cells \pm 1.4% in tenofovir arm vs. 0.35% \pm 0.5% in placebo, $P = 0.35$). Furthermore, Gag and Nef-specific CD8+ T cells IL-2 and TNF- α production did not differ significantly between the 2 groups (data not shown). We also assessed the polyfunctionality of the CD4+ and CD8+ T cells by the simultaneous quantification of three functions (IL-2, IFN- γ , and TNF- α production) in response to Gag and Nef stimulation and observed no significant differences in the polyfunctionality of the CD4+ and CD8+ T cells in the tenofovir arm compared with placebo ($P > 0.05$). Overall, a vast majority of HIV-1-specific T cells were monofunctional (>90%) in both the tenofovir gel and placebo group. Taken together, these data demonstrate that the HIV-1-specific CD8+ T-cell responses in women with breakthrough infection during tenofovir microbicide use did not significantly differ from those that used placebo, but that Gag-specific IFN γ + CD4+ T cells responses were significantly higher in HIV-1-infected women randomized to the tenofovir gel arm.

DISCUSSION

The use of a tenofovir-containing vaginal microbicide gel for the prevention of HIV-1 infection in sexually active, HIV-1-uninfected adult women in KwaZulu-Natal, South Africa, showed a significant reduction of HIV-1 infection rates by

39% in the primary intent-to-treat analysis.¹ Here, we examined the innate and adaptive immune responses in women with breakthrough HIV-1 infection that either used tenofovir microbicide gel or were part of the placebo arm. The frequencies and activation status of principal effector cells of the innate immune response, including NK cells and mDCs, assessed within 3 months of HIV-1 infection were not affected by the use of the tenofovir gel. Previous studies have demonstrated a significant expansion of NK cells in infected individuals during the early phase of HIV-1 infection and a subsequent contraction of NK cell and mDC populations.^{9–11} We can therefore not exclude that NK cell and mDC frequencies in the initial 3 months of infection might have been affected by the use of the tenofovir gel, and that these changes in innate effector cells might have subsequently contributed to the observed functional differences in HIV-1-specific CD4+ T cells.¹²

HIV-1-specific T cells are considered critical for the control of HIV-1 replication and disease progression.^{8,13–15} Although HIV-1-specific CD8+ T-cell responses to both Gag and Nef peptides did not significantly differ between the groups, HIV-1 Gag-specific IFN- γ + CD4+ T-cell responses were significantly higher in women in the tenofovir gel arm compared with the placebo arm. HIV-1-specific CD4+ T cell responses are rapidly lost after acute infection in the presence of continuing HIV-1 replication, and this is in contrast to HIV-1-specific CD8+ T-cell frequencies which tend to increase over at least the first year of HIV-1 infection with continues exposure to antigen.^{16–20} Previous studies have shown that CD4+ T cells and in particular gut-associated CD4+ T cells are severely depleted within the first few days/weeks of infection²¹ and that HIV-1 preferentially infects HIV-1-specific CD4+ T cells.²² The presence of an antiretroviral agent, such as tenofovir, during this crucial period might protect CD4+ T cells from deletion, allowing for a preservation of HIV-1-specific IFN- γ CD4+ T cells. The observation that women receiving tenofovir gel maintained higher Gag-specific CD4+ T-cell responses despite the reported lack of differences in viral load set-point between tenofovir gel and placebo recipients¹ was however unexpected, and the long-term clinical benefit of this preservation, and its consequences for HIV-1-specific immune function, will require further investigation.

Taken together, our studies in a subset of women who experienced breakthrough HIV-1 infection despite randomization to tenofovir gel usage demonstrate no significant alteration in the frequencies and activation status of key innate immune cells and HIV-1-specific CD8+ T-cell responses 3 months after infection. However, tenofovir gel applied vaginally around the time of HIV-1 transmission might protect HIV-1-specific CD4+ T-cell responses in infected individuals. This study demonstrates for the first time that the use of vaginal microbicides containing antiretroviral drugs can modulate HIV-1-specific immunity in individuals with breakthrough infection. These consequences of microbicide use for immune responses in individuals that acquire HIV-1 have to be considered in the design of future trials that will combine microbicides with HIV-1 vaccines.

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