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MAJOR ARTICLE

Initiating Intramuscular Depot Medroxyprogesterone Acetate Increases Frequencies of Th17-like Human Immunodeficiency Virus Target Cells in the Genital Tract of Women in South Africa: A Randomized Trial

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Background. Cervicovaginal CD4+ T cells are preferential targets for human immunodeficiency virus (HIV) infection and have consequently been used as a proxy measure for HIV susceptibility. The ECHO randomized trial offered a unique opportunity to consider the association between contraceptives and Th17-like cells within a trial designed to evaluate HIV risk. In a mucosal substudy of the ECHO trial, we compared the impact of initiating intramuscular depot medroxyprogesterone acetate (DMPA-IM), copper-IUD, and the levonorgestrel (LNG) implant on cervical T cells.

Methods. Cervical cytobrushes from 58 women enrolled in the ECHO trial were collected at baseline and 1 month after contraceptive initiation. We phenotyped cervical T cells using multiparameter flow cytometry, characterized the vaginal microbiome using 16s sequencing, and determined proteomic signatures associated with Th17-like cells using mass spectrometry.

Results. Unlike the LNG implant or copper-IUD, DMPA-IM was associated with higher frequencies of cervical Th17-like cells within 1 month of initiation ($P = .012$), including a highly susceptible, activated population co-expressing CD38, CCR5, and α4β7 ($P = .003$). After 1 month, women using DMPA-IM also had more Th17-like cells than women using the Cu-IUD ($P = .0002$) or LNG implant ($P = .04$). Importantly, in women using DMPA-IM, proteomic signatures signifying enhanced mucosal barrier function were associated with the increased abundance of Th17-like cells. We also found that a non–*Lactobacillus*-dominant microbiome at baseline was associated with more Th17-like cells post–DMPA-IM ($P = .03$), although this did not influence barrier function.

Conclusions. Our data suggest that DMPA-IM–driven accumulation of HIV-susceptible Th17-like cells might be counteracted by their role in maintaining mucosal barrier integrity.

Clinical Trials Registration. NCT02550067.

Keywords. hormonal contraception; HIV risk; Th17 cells; mucosal barrier integrity.

The randomized Evidence for Contraceptive Options and HIV Outcomes (ECHO) trial found no differences in human immunodeficiency virus (HIV) incidence between women assigned to intramuscular depot medroxyprogesterone acetate (DMPA-IM), a copper T intrauterine device (Cu-IUD), or 2-rod levonorgestrel (LNG) implant [1], adding to our understanding of contraceptive use and HIV susceptibility [2–5]. Importantly, ECHO offered a unique opportunity to explore contraceptive-induced biological changes, particularly those pertinent to HIV susceptibility. Cervical CD4+ T cells, especially T-helper 17 (Th17) cells, are preferential targets for HIV due to their cell-surface markers [6–10], and have been used as a proxy for HIV susceptibility [11]. An increase in these HIV target cells, whether a result of contraceptive use, or other factors, would consequently increase HIV risk. However, Th17 cells may also play a protective role in the context of HIV acquisition, by

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mediating mucosal immunity and barrier repair [12, 13]. It remains vital to understand the relationship between contraceptive use and Th17 target cells, particularly within the ECHO trial where HIV seroconversion was an endpoint. Therefore, we conducted a nested substudy evaluating the impact of contraceptive initiation on cervical Th17-like cells.

METHODS

Ethics Statement

This study was approved by the Human Research Ethics Committees of the Universities of Cape Town (HREC 371/2015) and Washington (STUDY00000261) and FHI360 (523201). All women provided informed written consent.

ECHO Trial

The ECHO trial (Clinicaltrials.gov ID NCT02550067) enrolled 7829 women to 3 contraceptive arms: the Cu-IUD, DMPA-IM, and LNG-implant [1]. These women were sexually active, HIV seronegative, and seeking contraception. Women were excluded if they received DMPA, norethisterone enanthate (Net-EN), an implant, or IUD in the previous 6 months; were pregnant; had a hysterectomy; or had known sexually transmitted infections (STIs). At enrollment, participants were screened for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). Treatment was provided for curable STIs and bacterial vaginosis diagnosed syndromically, according to national guidelines or, for CT/NG, diagnosed etiologically.

Substudy of Biological Mechanisms

Cervical cytobrushes from 80 Cape Town women were collected and processed [14]. Cervicovaginal secretions were collected by a disposable menstrual cup inserted for 45 minutes, then removed and placed in a 50-mL sterile tube. A lateral vaginal wall swab was collected through use of a speculum and placed into Digene transport media. Samples were transported to the laboratory within 4 hours of collection, at 4°C. Menstrual cup secretions were processed [14], diluted 4:1 in phosphate-buffered saline, and stored at −80°C. The swab was stored at −80°C. Researchers were blinded to the assigned contraceptive arm until the analyses were complete.

Multiparameter Flow Cytometry

Cytobrush cells were phenotyped ex vivo at enrollment and post–contraception initiation from the same 80 women, of whom 63 women completed both study visits (Cu-IUD, n = 24; DMPA-IM, n = 22; LNG implant, n = 17). Cytobrushes were flushed approximately 30 times with transport media using a disposable Pasteur pipette before transferring to a fresh tube and flushing with an additional 6 mL of media. Cells were centrifuged at 300 × *g* for 10 minutes at 4°C. The cell pellet was stained with CD3, CD4, CD8, CD38, HLA-DR, CCR6, CCR10, CCR5, and α4β7 (Supplementary Table 1), then acquired on a BD LSRII using FACSDiva. Post-compensation analysis was performed using FlowJo (TreeStar), Pestle and Spice [15]. Gating was based on fluorescence-minus-one controls. Samples with less than 100 live CD3+ events were excluded from further analyses [16], resulting in n = 58 participants with matched samples at 2 time points (Cu-IUD, n = 23; DMPA-IM, n = 20; LNG implant, n = 15). Gating and analysis were independently checked.

Microbial 16S rRNA Gene Sequencing

Bacterial gDNA was extracted using the DNeasy Powersoil HTP 96 kit (Qiagen) from thawed lateral wall swabs following the manufacturer's protocol. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified using modified universal primers. Dual-index barcodes and Illumina sequencing adaptors were attached using polymerase chain reaction (PCR) primers [17], and amplicons were pooled in equimolar amounts for sequencing with Illumina MiSeq platform (300-bp paired-end). Following demultiplexing, raw reads were processed using DADA2 and samples with less than 2000 reads were excluded. Taxonomic annotation was carried out using the Ribosomal Database Project's Naïve Bayesian classifier. Sequence classification was trained against the Silva training set version 132 (https://github.com/itsmisterbrown/updated_16S_dbs). Run-specific contamination filtering was performed using microfiltR (https://github.com/itsmisterbrown/microfiltR). Sequencing runs were merged using custom scripts (https://github.com/itsmisterbrown/marker_gene_dataset_merging_functions).

Classification of Microbial Communities

Partition around medoids (PAM) clustering of community profiles using Bray-Curtis distance on relative abundance transformed taxonomic profiles resulted in the identification of 5 community state types (CSTs) [18]. CSTs III-A and I-B were dominated by *Lactobacillus* taxa. CST IV-B was primarily comprised *Gardnerella vaginalis*. CSTs IV-A and IV-D were the most diverse and associated with clinical bacterial vaginosis diagnosis.

Proteomic Analysis of Cervicovaginal Secretions

Cervicovaginal secretions were analyzed by label-free tandem mass spectrometry as described previously [19]. Samples were centrifuged at 14 000 × *g* for 20 minutes at 4°C, followed by protein determination by bicinchoninic acid (BCA) assay (Millipore). Protein from each sample (25 g) was denatured using urea exchange buffer (8 M, 50 mM HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], pH 8.0) then filtered using Nanosep filter cartridges (10 kDa). Samples were reduced and alkylated with 25 mM DTT (dithiothreitol) and 50 mM iodoacetamide (IAA), respectively, before overnight trypsin digestion (2 µg/100 µg protein). Desalting of peptides was pe

rformed by reverse-phase liquid chromatography (Agilent 1200). Equal amounts of peptide were then analyzed using a nano-flow Easy 1000 system connected in-line to an Orbitrap Q Exactive mass spectrometer (ThermoFisher Scientific). Database searches were carried out using the Mascot search engine (version 2.4; Matrix Science) using the SwisProtKB Human database (UniProt, 2018). Results were analyzed using Scaffold Q+ software (version 4.9.0; Proteome Software), and protein identifications were restricted to those that passed a ≤1% false discovery rate (FDR) at the protein level, ≤0.1% FDR at the peptide level, and had more than 2 unique peptides/protein. Label-free protein abundance was calculated using Progenesis QI software (Nonlinear Dynamics).

Statistical Analysis

Prism 9 (GraphPad) was used for Wilcoxon matched pairs, Mann–Whitney *U* tests, and Kruskal–Wallis to compare frequencies of cells. Regression analyses between Th17-like cells and the microbiome were performed in SPSS Statistics version 27 (IBM Corporation). Post–DMPA-IM protein levels and Th17-like cells were normalized by baseline to calculate a $\log_2$ fold-change (L2FC) value. Protein L2FCs were correlated to their matched Th17 L2FC value using the Pearson's method ($P < .05$). Pathway analysis was performed on proteins that passed a critical value of $\alpha = 0.05$ using both Ingenuity Pathway Analysis (QIAGEN) and DAVID Knowledgebase (Bioinformatics). The Benjamini–Hochberg method was used to adjust for multiple testing. A *P* value of <0.05 was considered statistically significant.

Table 1. Baseline Characteristics According to Randomized Arm

	Cu-IUD (n = 23)	DMPA-IM (n = 20)	LNG Implant (n = 15)	*P*
Age,[a] years	22 (20–28)	24 (22–25)	22 (20–25)	0.80
BMI,[a] kg/m^2	29 (26–38)	27 (23–32)	26 (22–32)	0.14
Days since last menstrual period[a]	17 (11–27)	20.5 (10.3–29)	21 (12.3–30.5)	0.51
Genital infections, n (%)				
Chlamydia trachomatis	4 (17.4%)	6 (30%)	4 (26.7%)	0.63
Neisseria gonorrhoeae	2 (8.7%)	3 (15%)	1 (6.7%)	0.75
Bacterial vaginosis (BV),[b] n (%)				
Positive	10 (43.5%)	10 (50%)	7 (46.7%)	0.73
Intermediate	3 (13%)	2 (10%)	0 (0%)	
Negative	10 (43.5%)	8 (40%)	8 (53.3%)	
Demographics, n (%)				
Never married	23 (100%)	20 (100%)	14 (93.3)	0.26
Married	0 (0%)	0 (0%)	1 (6.7%)	
Primary school education	1 (4.3%)	0 (0%)	0 (0%)	0.71
Secondary education	22 (95.7%)	20 (100%)	14 (93.3%)	
Post-secondary education	0 (0%)	0 (0%)	1 (6.7%)	
Sexual risk behavior in the last 3 months, n (%)				
>1 partner	2 (8.7%)	4 (20%)	1 (6.7%)	0.46
1 partner	21 (91.3%)	16 (80%)	14 (93.3%)	
New partner	0 (0%)	4 (20%)	0 (0%)	0.01
Condomless sex	19 (82.6%)	13 (65%)	9 (60%)	0.31
Condom use (always)	4 (17.4%)	7 (35%)	6 (40%)	0.14
Condom use (often)	0 (0%)	0 (0%)	1 (6.7%)	
Condom use (sometimes)	13 (56.5%)	11 (55%)	8 (53.3%)	
Condom use (never)	6 (26.1%)	2 (10%)	0 (0%)	

Abbreviations: BMI, body mass index; Cu-IUD, copper T intrauterine device; DMPA-IM, intramuscular depot medroxyprogesterone acetate; LNG, 2-rod levonorgestrel.

[a]Values are median and interquartile range.

[b]By Nugent scoring: score of 0–3 is negative, score of 4–6 is intermediate, score of 7–10 is positive.

RESULTS

Baseline and 1-month cytobrushes were collected from 80 Cape Town women, a median of 4.3 weeks apart (interquartile range [IQR], 4–9.4 weeks). Participants were similar between arms, with no differences in age, body mass index, or STI prevalence (Table 1).

We studied cervical CD4+ T cells before and after contraceptive initiation using flow cytometry. The gating strategy is shown in Supplementary Figure 1. At baseline, the frequency of CD4+ T cells was similar across study arms (Supplementary Figure 2*A*), and contraception did not alter this (Figure 1*A* and 1*B*). However, DMPA-IM use was associated with a reduced frequency of CD4+ HLA-DR+ T cells compared with baseline ($P = .008$) and with Cu-IUD ($P = .002$; Figure 1*C*). Contraceptives did not alter CCR5 (Figure 1*D*) or α4β7 (Figure 1*E*) expression.

We next examined the impact of contraceptives on Th17-like HIV target cells, identified by expression of CCR6 and CCR10 (CCR6+ CCR10−) [20–22]. There were no differences in the frequency of cervical Th17-like cells between contraceptive arms at baseline (Supplementary Figure 2*B*). Of note, only the initiation of DMPA-IM resulted in a significant increase in the frequency of Th17-like cells, compared with baseline ($P = .012$; median change, 12.8%; Figure 2*A*). At 1 month, women using DMPA-IM had 1.7-fold higher frequencies of Th17-like cells compared with women using the Cu-IUD ($P = .0002$) and 1.2-fold more than women using an LNG implant ($P = .04$; Figure 2*B*). Furthermore, DMPA-IM induced a significant increase in the frequency of activated, CD38+ Th17-like cells compared with baseline ($P = .044$; median difference, 9.2%; Figure 2*C*), such that women using DMPA-IM had significantly higher frequencies of activated Th17-like cells compared with Cu-IUD ($P = .026$; median, 32.6%) but not the LNG implant ($P = .215$; median, 39.3%) at 1 month (Figure 2*D*). DMPA-IM use was also associated with a decrease in the frequency of HLA-DR+ Th17-like cells ($P = .004$; median difference, −4.2%; Figure 2*E*), although the levels of these cells at 1 month was similar across study arms (medians: 10.4%, 8.28%, and 9.18% for Cu-IUD, DMPA-IM, and LNG implant, respectively; Figure 2*F*). The activation profile of Th17-like cells was significantly skewed 1 month after DMPA-IM initiation compared with baseline ($P = .044$), and this was also

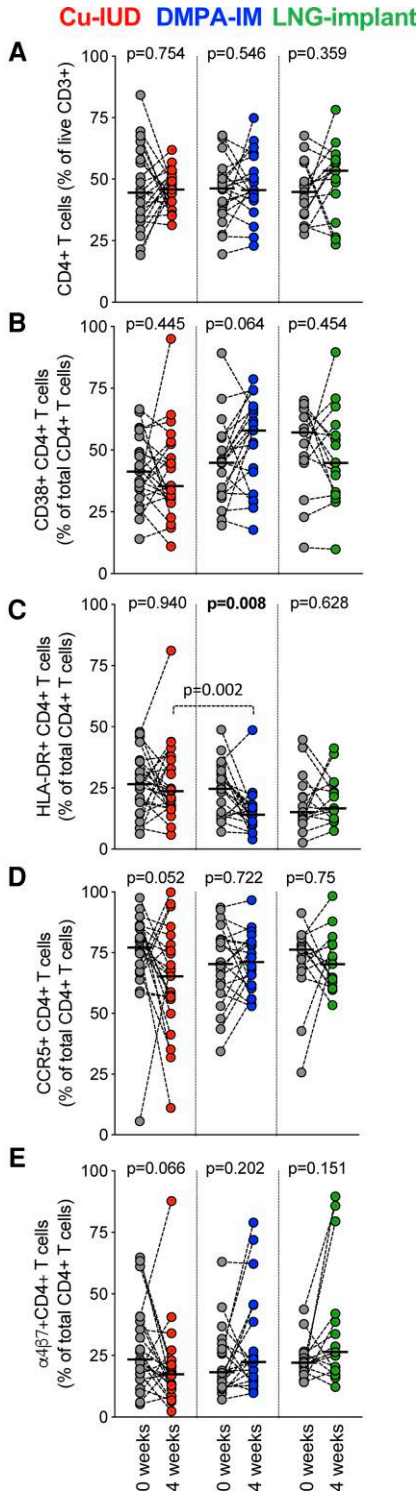

Figure 1. Frequencies of cervical CD4+ T cells in women randomized to the Cu-IUD (n = 23; red), DMPA-IM (n = 20; blue), or LNG implant (n = 15; green) at baseline and 1 month after initiating contraception. Graphs show the differences pre-post contraceptive initiation for the frequencies of cervical (*A*) CD4+ T cells, (*B*) CD38+ CD4+ T cells, (*C*) HLA-DR+ CD4+ T cells, (*D*) CCR5+ CD4+ T cells, and (*E*) α4β7+ CD4+ T cells. The horizontal bars indicate the median. Statistical comparisons were performed using the Wilcoxon matched-pairs tests for matched data or the Kruskal-Wallis test with false discovery rate correction for cross-sectional data. Abbreviations: Cu-IUD, copper T intrauterine device; DMPA-IM, intramuscular depot medroxyprogesterone acetate; LNG, 2-rod levonorgestrel.

significantly different compared with the Cu-IUD ($P = .024$; Figure 2*G*).

Next, the expression of the HIV co-receptors CCR5 and α4β7 was measured. Generally, CCR5 was expressed on the majority of cervical Th17-like cells (median, 74%; IQR, 63.75–86.5%; Figure 3*A*), with no differences at baseline (Supplementary Figure 2*B*). Neither DMPA-IM nor the LNG implant induced changes in the frequency of CCR5+ Th17-like cells (Figure 3*A*). However, women using the Cu-IUD had a substantial decrease in the frequency of CCR5+ Th17-like cells compared with baseline ($P = .038$; median difference, −13.3%). Nonetheless, there were no differences in the frequency of CCR5+ Th17-like cells between study arms after 1 month of contraceptive use (Figure 3*B*). The density of CCR5 per cell (measured by the median fluorescent intensity [MFI]) was significantly increased in women using the Cu-IUD ($P = .004$; median difference, 800 units; Figure 3*C*), suggesting there are fewer CCR5+ cells for HIV to infect, but these may be more susceptible to HIV on an individual cell basis. Despite this, the MFI of CCR5 was similar between contraceptive arms post-initiation (Figure 3*D*). Unlike CCR5, fewer than one-quarter of cervical Th17-like cells expressed α4β7 (median, 22.3%; IQR, 15.15–35%; Figure 3*E*). Although not statistically significant, 12 of 58 women (n = 5, n = 5, and n = 2 in DMPA-IM, LNG-implant, or CU-IUD arms, respectively) had an approximately 3-fold increase in the frequency of α4β7, with post-contraceptive expression exceeding 75% in 6 of 12 of these women. There were no differences in α4β7+ Th17-like cells between study arms at 1 month (Figure 3*F*), and none of the contraceptives induced MFI changes (Figure 3*G* and 3*H*). Thus, DMPA-IM use was associated with an increase in activated Th17-like cells, but unexpectedly had little effect on the overall expression of HIV co-receptors [11, 23].

Since HIV entry into cells may depend on the co-expression of cell-surface markers [6], we assessed co-expression of these markers across study arms. Overall, Th17-like cells expressing CCR5 alone were the most highly abundant, irrespective of study arm or time point (median, 28.5%; IQR, 17.73–39.01%; Figure 4). Compared with baseline, only DMPA-IM initiation resulted in an increased frequency of CD38+ CCR5+ α4β7+ Th17-like cells, a population likely to be highly HIV susceptible ($P = .003$), while both the Cu-IUD and LNG implant induced decreases in other Th17-like populations (Figure 4*A*). We next compared the most abundant subsets (median frequency >5%) between contraceptive arms at 1 month (Figure 4*B*). The frequency of CD38+ CCR5+ α4β7+ Th17-like cells was higher in women using progestin-only contraceptives compared with those using the Cu-IUD (DMPA-IM [median, 10.05%] vs Cu-IUD [median, 3.9%]; $P = .005$; and LNG implant [median, 9.82%] vs Cu-IUD; $P = .014$; Figure 4*B*) but similar between DMPA-IM and LNG implant ($P = .247$). Therefore, DMPA-IM initiation led to a significant increase

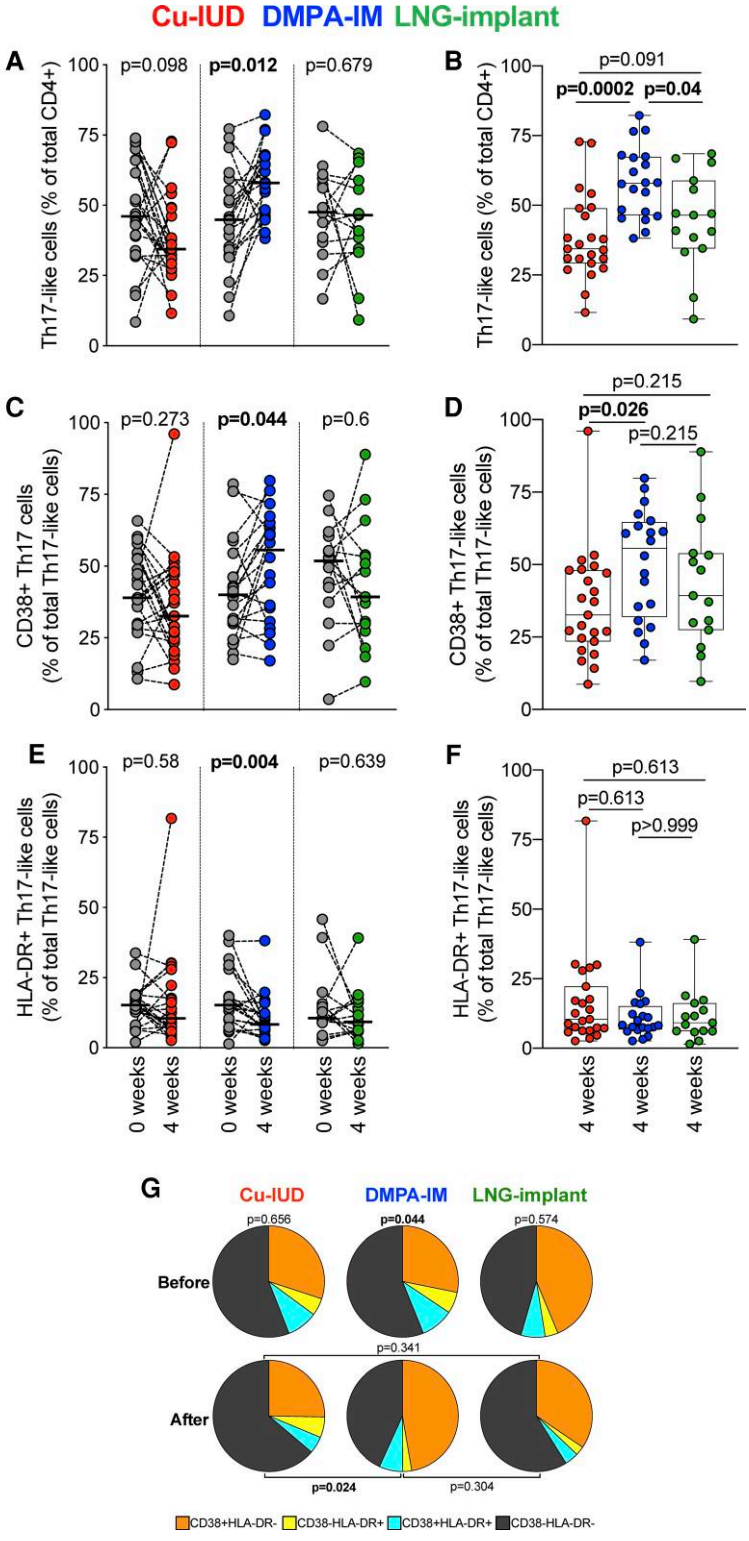

Figure 2. The frequency of activated Th17 HIV target cells in women randomized to the Cu-IUD (n = 23; red), DMPA-IM (n = 20; blue), or LNG implant (n = 15; green). (*A*) The frequency of Th17-like cells (CCR6+ CCR10–) before and after initiation of contraception. (*B*) The cross-sectional comparison of Th17 frequency post-contraceptive initiation across study arms. (*C*) The frequency of CD38+ Th17 cells before and after initiation of contraception. (*D*) The cross-sectional comparison of CD38+ Th17 frequency post-contraceptive initiation across study arms. (*E*) The frequency of HLA-DR+ Th17 cells before and after initiation of contraception. (*F*) The cross-sectional comparison of HLA-DR+ Th17 frequency post-contraceptive initiation across study arms. The horizontal bars indicate the median. (*G*) Proportions of activated Th17 cells expressing different combinations of CD38 and HLA-DR. Statistical comparisons were performed using the Wilcoxon matched-pairs tests for matched data or the Kruskal-Wallis with false discovery rate correction for cross-sectional comparisons. Abbreviations: Cu-IUD, copper T intrauterine device; DMPA-IM, intramuscular depot medroxyprogesterone acetate; HIV, human immunodeficiency virus; LNG, 2-rod levonorgestrel; Th17, T-helper 17.

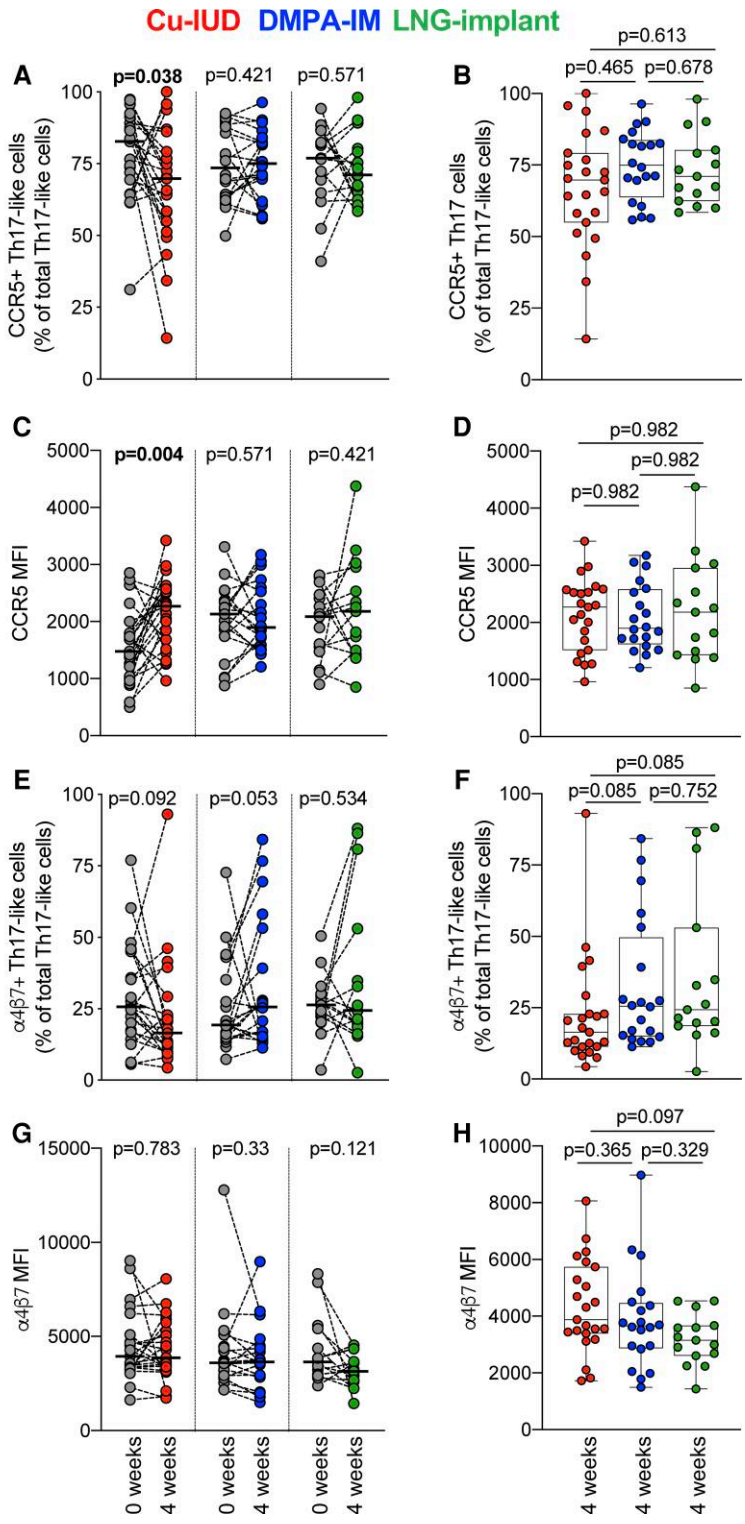

Figure 3. The expression of HIV receptors CCR5 and α4β7 on Th17 target cells in women randomized to the Cu-IUD (n = 23; red), DMPA-IM (n = 20; blue), or LNG implant (n = 15; green). (*A*) The frequency of CCR5+ Th17 before and after initiation of contraception. (*B*) The cross-sectional comparison of CCR5+ Th17 frequency post-contraceptive initiation across study arms. (*C*) The MFI of CCR5 on Th17 cells before and after initiation of contraception. (*D*) The cross-sectional comparison of CCR5 MFI post-contraceptive initiation across study arms. (*E*) The frequency of α4β7+ Th17 cells before and after initiation of contraception. (*F*) The cross-sectional comparison of α4β7+ Th17 frequency post-contraceptive initiation across study arms. (*G*) The MFI of α4β7 on Th17 cells before and after initiation of contraception. (*H*) The cross-sectional comparison of α4β7 MFI post-contraceptive initiation across study arms. Th17 cells were defined as CD4+ CCR6+ CCR10−. The horizontal bars indicate the median. Statistical comparisons were performed using the Wilcoxon matched-pairs tests for matched data or the Kruskal-Wallis with false discovery rate correction for cross-sectional comparisons. Abbreviations: Cu-IUD, copper T intrauterine device; DMPA-IM, intramuscular depot medroxyprogesterone acetate; LNG, 2-rod levonorgestrel; MRI, median fluorescent intensity; Th17, T-helper 17.

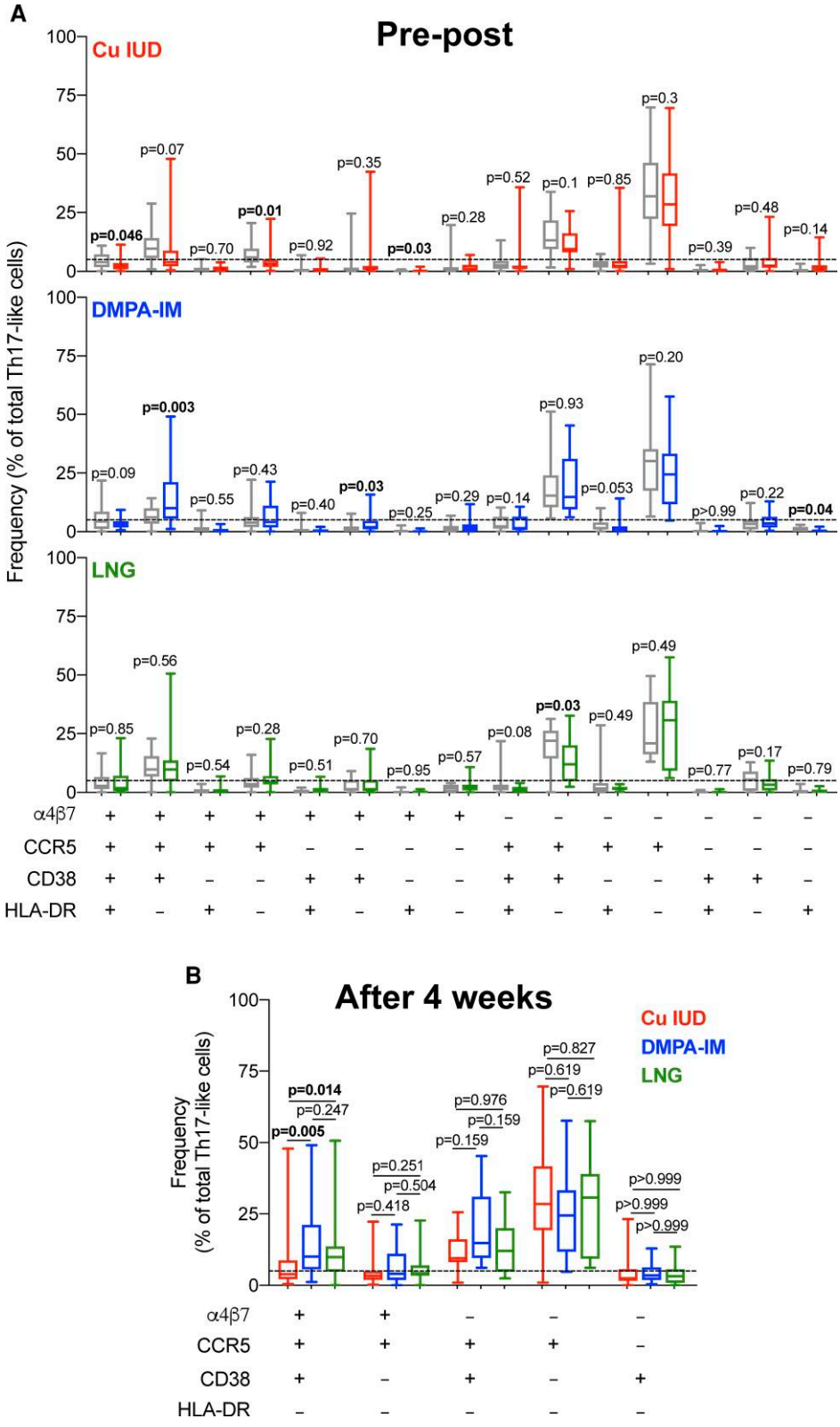

Figure 4. Changes in the co-expression of HIV target cell markers on Th17 cells from women randomized to the Cu-IUD (n = 23; red), DMPA-IM (n = 20; blue), or LNG implant (n = 15; green). The cross-sectional comparison of Th17 populations expressing CD38, HLA-DR, α4β7, and CCR5 in different combinations: (*A*) before and after the start of contraception and (*B*) between study arms post-contraceptive initiation with the 5 most abundant populations (medians >5% for at least 1 contraceptive arm at either time point). The dashed line indicates the 5% cutoff. Th17 cells were defined as CD4+ CCR6+ CCR10−. Statistical comparisons were performed using the Wilcoxon matched-pairs tests for matched data or the Kruskal-Wallis with false discovery rate correction for cross-sectional comparisons. Abbreviations: Cu-IUD, copper T intrauterine device; DMPA-IM, intramuscular depot medroxyprogesterone acetate; HIV, human immunodeficiency virus; LNG, 2-rod levonorgestrel; Th17, T-helper 17.

in susceptible Th17-like cells from baseline and, in comparison, to women using Cu-IUD.

The vaginal microbiome may also influence HIV susceptibility, and since Th17 cells mediate antibacterial immunity, we next evaluated whether the vaginal microbiome influenced the abundance of Th17-like cells in the context of contraceptive use. Using available sequencing data (n = 47), 5 CSTs were identified at baseline, with no clear separation by contraceptive arm (Figure 5*A*). Overall, CSTs III-A, and IV-D were the most common, comprising 27.7% and 29.8% of women, respectively. Women were grouped according to whether they had baseline *Lactobacillus* spp.–dominant microbiota (CSTs III-A or I-B) or not (CSTs IV-A, IV-B, or IV-D). Only 15 of 47 women (32%) had a *Lactobacillus*-dominant (LD) microbiome at baseline, with nearly half of them randomized to DMPA-IM (7/15; 46%). Cervical Th17-like cell frequencies were similar between baseline and 1 month in women with an LD vaginal microbiome and those without (Figure 5*B*). Interestingly, the initiation of DMPA-IM resulted in an increase in Th17-like cell abundance in women with non-LD microbiomes ($P = .03$) but not in women with LD microbiomes. Women using DMPA-IM also had higher frequencies of Th17-like cells at 1 month, compared with those using the Cu-IUD (Figure 5*C*), in both the LD ($P = .025$) and non-LD groups ($P = .008$). Similarly, women with non-LD microbiomes using DMPA-IM had elevated Th17-like cells compared with those using the LNG implant ($P = .043$), but not in the subgroup of LD women. Finally, the microbiome at baseline or after 4 weeks of contraceptive use was not associated with Th17-like cell abundance post–DMPA-IM (Figure 5*D*). These data suggest that the microbiome could play a role in the contraceptive-induced accumulation of Th17-like cells and should be investigated further.

To better understand potential pathways accompanying elevated Th17-like cells in women using DMPA-IM, we interrogated cervicovaginal proteome signatures related to Th17-like cells. We identified 634 proteins by mass spectrometry in samples collected at baseline and post–DMPA-IM initiation. Of these, 87 of 634 (13.7%) proteins were significantly correlated with increased Th17-like cells post–DMPA initiation. Pathway analysis proteins negatively associated with Th17-like cell numbers included inflammatory pathways, including the acute phase response, the liver X receptor (LXR)/retinoid receptor (RXR) activation, and movement of neutrophils ($P < .001$; Figure 6*A*). In contrast, increases in Th17-like cells positively correlated with proteins involved in mucosal barrier integrity, including epithelial cell–cell adhesion junction proteins ($P < .0001$), protease inhibition ($P < .0001$), actin cytoskeletal components ($P = .0006$), and cell-matrix adhesion ($P = .0094$; Figure 6*A*). Hierarchical clustering showed a well-defined association between increased abundance of Th17 cells with tight junction and structural proteins, while antiproteases, known to play a role in wound healing and tissue maintenance, were found to decrease (Figure 6*B*). Although the microbiome did not significantly impact Th17-associated proteins in women using DMPA-IM, an LD microbiome was more prevalent with increased Th17-associated proteins involved in cell–cell adhesion (Supplementary Figure 3), suggesting a relationship between mucosal barrier disruption, Th17 cells, and the microbiome. Overall, our important findings suggest that Th17-like cells in the genital tract maintain mucosal barrier function in the context of DMPA-IM use. Thus, any increased risk of HIV infection in women using DMPA-IM due to increased HIV target cell availability is likely abrogated by the same Th17-like cells promoting mucosal barrier integrity.

DISCUSSION

Mucosal Th17 cells make efficient HIV targets [8, 24, 25], evidenced by their profound depletion in early infection [20, 26]. We compared the consequences of contraceptives on local HIV target cells in the ECHO study, the only randomized contraceptive trial conducted to date.

DMPA-IM had the most pronounced effect on cervical Th17-like cells. Women assigned to DMPA-IM had significantly increased frequencies of Th17-like cells compared with baseline and compared with women assigned to Cu-IUD or LNG implant after 1 month. We also observed an increase in an activated, HIV-susceptible Th17-like cell population co-expressing CCR5 and α4β7 (CD38+ CCR5+ α4β7+) after 1 month of DMPA-IM use. However, there was no increased activation or abundance in the total CD4+ T-cell population, consistent with some previous findings [23] and highlighting the need for deeper examination of susceptible subsets.

We initially hypothesized that women using DMPA-IM would experience higher HIV incidence rates related to an increased abundance or activation of cervical Th17-like cells [3, 26–28]. Although we could not directly measure associations between Th17-like cells and HIV acquisition, the ECHO trial reported no increased risk of HIV in DMPA-IM users compared with others. However, we still observed an increased abundance of cervical Th17-like cells post–DMPA-IM initiation, in direct contrast to the prediction that increased HIV target cell infiltration would translate to higher HIV risk.

Our key finding was the Th17-associated increase in mucosal barrier proteins in women using DMPA-IM. Although we ascribe Th17 cells to the role of HIV target cells, their primary role is in maintenance of barrier integrity [12, 13]. Our data suggest that the DMPA-IM–driven accumulation of HIV-susceptible Th17-like cells might be counteracted by their role in maintaining the epithelial barrier of the female genital tract. Previously, DMPA-IM has been associated with compromised barrier function, including a reduction in epithelial repair proteins, which may be a mechanism by which HIV risk is increased [29, 30]. Consequently, our data showing

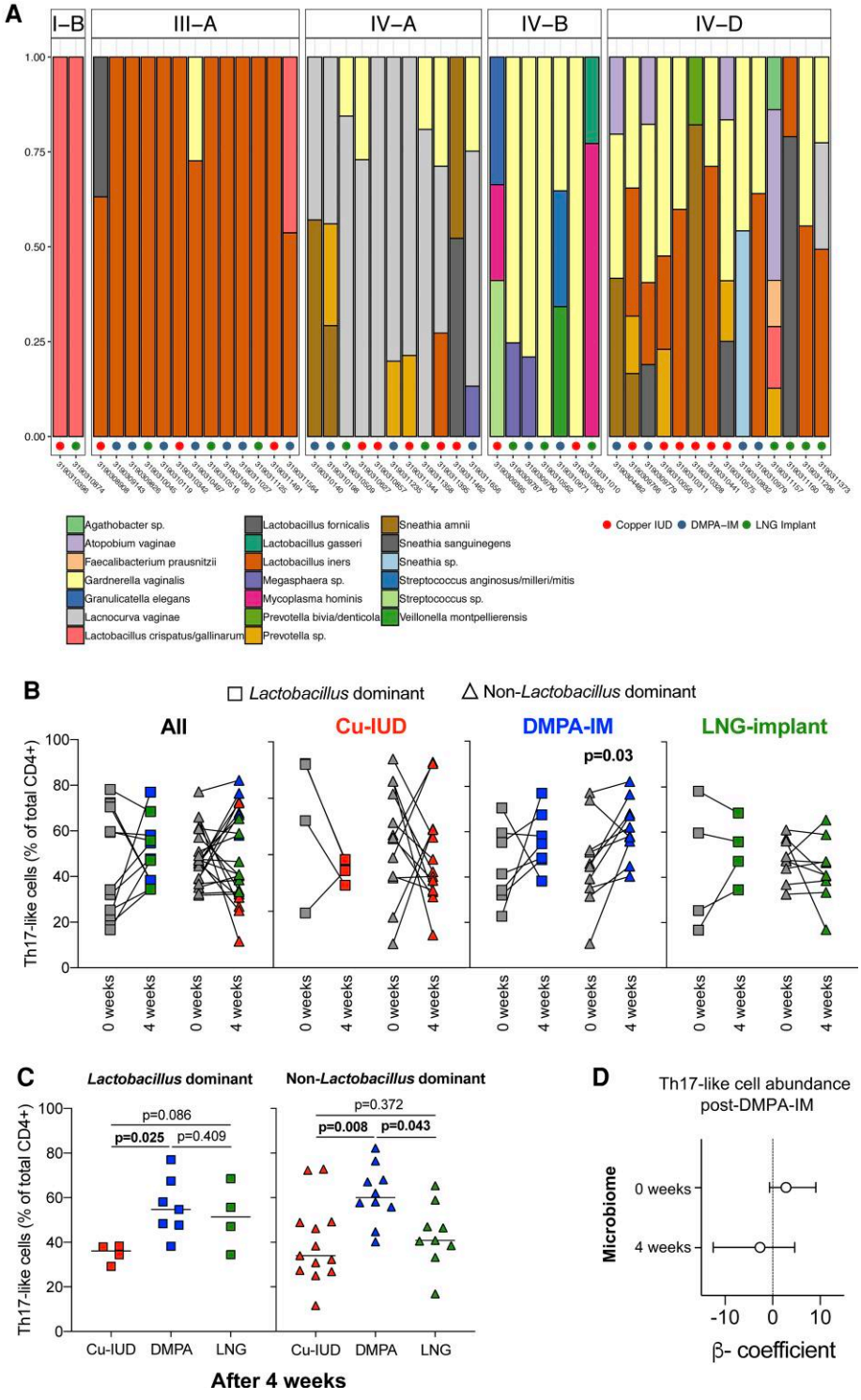

Figure 5. The effect of the vaginal microbiome at baseline on the abundance of Th17-like cells in women randomized to the Cu-IUD (n = 17; red), DMPA-IM (n = 17; blue), or LNG implant (n = 13; green). (*A*) Relative abundance of the different vaginal bacterial species in each participant at baseline, where data were available (n = 47). The CSTs are shown above the panel. The color of the dots at the bottom of the panel indicates which contraceptive arm the participant was randomized to (red for CU-IUD, blue for DMPA-IM, and green for LNG implant). (*B*) Th17 cell frequency before and after initiation of contraception in women with a *Lactobacillus*-dominant (CSTs III-A or I-B; n = 15) or non–*Lactobacillus*-dominant microbiome (CSTs IV-A, IV-B, or IV-D; n = 32). Statistical comparisons were performed using the Wilcoxon matched-pairs tests. (*C*) The cross-sectional comparison of Th17 frequency post-contraceptive initiation across study arms in women with a *Lactobacillus*-dominant or non–*Lactobacillus*-dominant microbiome. Statistical comparisons were performed using Kruskal–Wallis with false discovery rate correction. (*D*) Linear associations between women with *Lactobacillus*-dominant microbiomes at baseline (pre) and at month 1 post–DMPA-IM (post) and Th17-like cell abundance post–DMPA-IM initiation. The associations are shown as a β-coefficient with error bars representing the 95% confidence interval. Abbreviations: CST, community state type; Cu-IUD, copper T intrauterine device; DMPA-IM, intramuscular depot medroxyprogesterone acetate; HIV, human immunodeficiency virus; LNG, 2-rod levonorgestrel; Th17, T-helper 17.

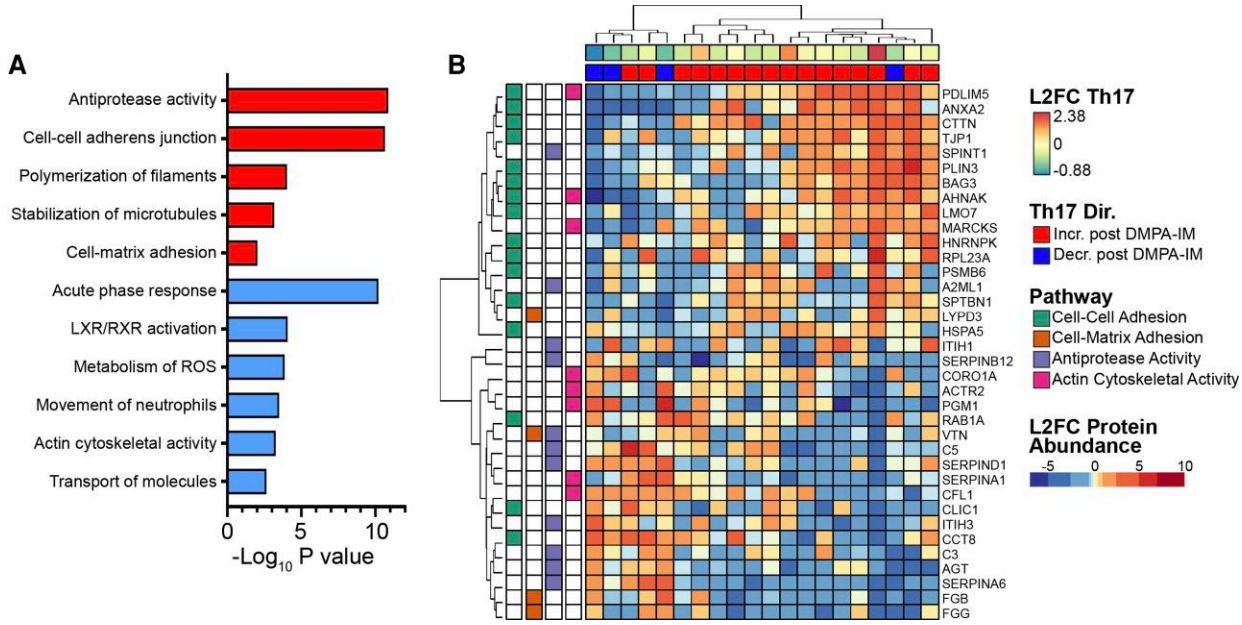

Figure 6. Proteome signatures associated with Th17 cell levels in women randomized to DMPA-IM. Cervicovaginal samples taken at baseline and post–DMPA-IM initiation (n = 20) were analyzed by label-free mass spectrometry. Longitudinal differences in Th17-like cells and proteins were correlated using Pearson's *R* test, which found 13.7% of proteins to be significantly correlated ($P = .05$). (*A*) Pathway annotation using IPA and DAVID bioinformatics identified 5 unique biological pathways increased and 6 decreased with the increase in Th17-like cells. (*B*) Hierarchical clustering showing the associations between the increase in Th17 cells and corresponding increase in mucosal barrier integrity proteins. Abbreviations: DMPA-IM, intramuscular depot medroxyprogesterone acetate; Th17, T-helper 17.

maintained mucosal barrier function are in agreement with the lack of greater HIV incidence in women using DMPA-IM in the ECHO trial. We found no link between the microbiome and Th17-associated mucosal barrier proteins, although others have reported detrimental changes in barrier function–associated protein signatures during dysbiosis [31].

Previous reports suggest that DMPA-IM impacts Th17 cells by influencing the vaginal microbiome [32]. Here, women with non-LD microbiomes at baseline had more Th17-like cells post–DMPA-IM. However, the sample size for our analysis was small, and these data need to be interpreted cautiously. Studies also show that women with moderate dysbiosis using hormonal contraception had mucosal barrier protein profiles similar to women with LD microbiomes [31]. Based on our findings, we may consider that hormonal contraception may promote mucosal barrier function through the accumulation of Th17-like cells, despite vaginal dysbiosis.

The differences in Th17-like cell abundance between contraceptives may reflect differences in pharmacodynamic properties [3]. Indeed, the increase in Th17-like cells post–DMPA-IM coincides with peak concentrations of medroxyprogesterone acetate (MPA), suggesting a transient effect [3, 33, 34], which may be too short-lived to substantially increase HIV risk [32, 35]. In line with our proteomic analyses, Th17-like cells may also be accumulating in response to transient MPA-induced epithelial thinning [29, 36, 37]. Th17-like cells may return to baseline levels once MPA concentrations plateau.

Our findings have implications for the use of cervical Th17-like cells as a proxy for HIV risk. If we consider a minimum threshold of target cells required to impact HIV acquisition risk, the sampling method used to collect cervical cells is particularly relevant. Cytobrushes collect relatively superficial cervical intraepithelial cells, which are easily accessible to luminal HIV. However, the abundance of cervical cells is unlikely to correspond to that of vaginal, endometrial, or submucosal HIV-susceptible cells, which have the potential to be infected, either by cell migration or contact with HIV in the instance of reduced barrier function [38, 39].

In conclusion, we report an increase in the abundance of highly susceptible, cervical Th17-like HIV target cells in women using DMPA-IM, evident within 1 month of initiation. Importantly, proteomic signatures related to enhanced mucosal barrier function corresponded to the abundance of Th17-like cells in these women. Thus, the DMPA-IM–driven accumulation of HIV-susceptible Th17-like cells might be counteracted by their role in maintaining mucosal barrier integrity.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. Conceived and designed the study: J.-A. S. P, H. J., R. Heffron, S. E. B., A. B., and R. B. Cohort management and sample collection: G. N., M. O., T. P.-P., C. S., K. B. H., J. M. B, H. J., and R. Heffron. Performed the experiments: R. B., T. F. R, S. Z. J., H. G., R. Harryprasad, B. P. B., L. N.-R., and H.A. Analyzed the data: R. B., T. F. R, S. D., K. B. H, B. P. B., L. N.-R., and H. A. Wrote the manuscript: R. B., B. P. B., L. N.-R., H. A., J.-A. S. P, H. J., and R. Heffron.

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References

1. ECHO. HIV incidence among women using intramuscular depot medroxyprogesterone acetate, a copper intrauterine device, or a levonorgestrel implant for contraception: a randomised, multicentre, open-label trial. Lancet **2019**; 394: 303–13.
2. Polis CB, Curtis KM, Hannaford PC, et al. An updated systematic review of epidemiological evidence on hormonal contraceptive methods and HIV acquisition in women. AIDS **2016**; 30:2665–83.
3. Hapgood JP, Kaushic C, Hel Z. Hormonal contraception and HIV-1 acquisition: biological mechanisms. Endocr Rev **2018**; 39:36–78.
4. Heffron R, Achilles SL, Dorflinger LJ, et al. Pharmacokinetic, biologic and epidemiologic differences in MPA- and NET-based progestin-only injectable contraceptives relative to the potential impact on HIV acquisition in women. Contraception **2019**; 99:199–204.
5. Mwatelah R, McKinnon LR, Baxter C, Abdool Karim Q, Abdool Karim SS. Mechanisms of sexually transmitted infection-induced inflammation in women: implications for HIV risk. J Int AIDS Soc **2019**; 22:e25346.
6. Joag VR, McKinnon LR, Liu J, et al. Identification of preferential CD4+ T-cell targets for HIV infection in the cervix. Mucosal Immunol **2015**; 9:1–12.
7. Monteiro P, Gosselin A, Wacleche VS, et al. Memory CCR6$^+$ CD4$^+$ T cells are preferential targets for productive HIV type 1 infection regardless of their expression of integrin 7. J Immunol **2011**; 186:4618–30.
8. Alvarez Y, Tuen M, Shen G, et al. Preferential HIV infection of CCR6$^+$ Th17 cells is associated with higher levels of virus receptor expression and lack of CCR5 ligands. J Virol **2013**; 87:10843–54.
9. Cicala C, Martinelli E, McNally JP, et al. The integrin $\alpha_4\beta_7$ forms a complex with cell-surface CD4 and defines a T-cell subset that is highly susceptible to infection by HIV-1. Proc Natl Acad Sci USA **2009**; 106:20877–82.
10. Ding J, Tasker C, Lespinasse P, et al. Integrin α4β7 expression increases HIV susceptibility in activated cervical CD4+ T cells by an HIV attachment-independent mechanism. J Acquir Immune Defic Syndr **2015**; 69:509–18.
11. Byrne EH, Anahtar MN, Cohen KE, et al. Association between injectable progestin-only contraceptives and HIV acquisition and HIV target cell frequency in the female genital tract in South African women: a prospective cohort study. Lancet Infect Dis **2016**; 16:441–8.
12. Khader SA, Gaffen SL, Kolls JK. Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. Mucosal Immunol **2009**; 2:403–11.
13. Bettelli E, Korn T, Oukka M, Kuchroo VK. Induction and effector functions of TH17 cells. Nature **2008**; 453:1051–7.

14. Dabee S, Barnabas SL, Lennard KS, et al. Defining characteristics of genital health in South African adolescent girls and young women at high risk for HIV infection. PLoS One **2019**; 14:e0213975.
15. Roederer M, Nozzi JL, Nason MC. SPICE: exploration and analysis of post-cytometric complex multivariate datasets. Cytometry A **2011**; 79:167–74.
16. Liebenberg LJ, Gamieldien H, Mkhize NN, et al. Stability and transport of cervical cytobrushes for isolation of mononuclear cells from the female genital tract. J Immunol Methods **2011**; 367:47–55.
17. Gohl DM, Vangay P, Garbe J, et al. Systematic improvement of amplicon marker gene methods for increased accuracy in microbiome studies. Nat Biotechnol **2016**; 34:942–9.
18. DiGiulio DB, Callahan BJ, McMurdie PJ, et al. Temporal and spatial variation of the human microbiota during pregnancy. Proc Natl Acad Sci USA **2015**; 112: 11060–5.
19. Noël-Romas L, Perner M, Molatlhegi R, et al. Vaginal microbiome-hormonal contraceptive interactions associate with the mucosal proteome and HIV acquisition. PLoS Pathog **2020**; 16:e1009097.
20. McKinnon LR, Nyanga B, Kim CJ, et al. Early HIV-1 infection is associated with reduced frequencies of cervical Th17 cells. J Acquir Immune Defic Syndr **2015**; 68: 6–12.
21. Acosta-Rodriguez EV, Rivino L, Geginat J, et al. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. Nat Immunol **2007**; 8:639–46.
22. Duhen T, Geiger R, Jarrossay D, Lanzavecchia A, Sallusto F. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. Nat. Immunol **2009**; 10:857–63.
23. Tasker C, Pizutelli V, Lo Y, Ramratnam B, Roche NE, Chang TL. Depot medroxyprogesterone acetate administration increases cervical CCR5 + CD4 + T cells and induces immunosuppressive milieu at the cervicovaginal mucosa. AIDS **2020**; 34:729–35.
24. Gosselin A, Monteiro P, Chomont N, et al. Peripheral blood $CCR4^+$ $CCR6^+$ and $CXCR3^+$ $CCR6^+$ $CD4^+$ T cells are highly permissive to HIV-1 infection. J Immunol **2010**; 184:1604–16.
25. Kader M, Wang X, Piatak M, et al. α4+β7hiCD4 + memory T cells harbor most Th-17 cells and are preferentially infected during acute SIV infection. Mucosal Immunol **2009**; 2:439–49.
26. Stieh DJ, Matias E, Xu H, et al. Th17 cells are preferentially infected very early after vaginal transmission of SIV in macaques. Cell Host Microbe **2016**; 19: 529–40.
27. McKinnon LR, Nyanga Billy, Chege Duncan, et al. Characterization of a human cervical $CD4^+$ T cell subset coexpressing multiple markers of HIV susceptibility. J Immunol **2011**; 187:6032–42.
28. Masson L, Passmore J-AS, Liebenberg LJ, et al. Genital inflammation and the risk of HIV acquisition in women. Clin Infect Dis **2015**; 61:260–9.
29. Ferreira VH, Dizzell S, Nazli A, et al. Medroxyprogesterone acetate regulates HIV-1 uptake and transcytosis but not replication in primary genital epithelial cells, resulting in enhanced T-cell infection. J Infect Dis **2015**; 211:1745–56.
30. Birse KD, Romas LM, Guthrie BL, et al. Genital injury signatures and microbiome alterations associated with depot medroxyprogesterone acetate usage and intravaginal drying practices. J Infect Dis **2016**; 215:590–8.
31. Borgdorff H, Gautam R, Armstrong SD, et al. Cervicovaginal microbiome dysbiosis is associated with proteome changes related to alterations of the cervicovaginal mucosal barrier. Mucosal Immunol **2016**; 9:621–33.
32. Mitchell CM, McLemore L, Westerberg K, et al. Long-term effect of depot medroxyprogesterone acetate on vaginal microbiota, epithelial thickness and HIV target cells. J Infect Dis **2014**; 210:651–5.
33. Jeppsson S, Johansson EDB. Medroxyprogesterone acetate, estradiol, FSH and LH in peripheral blood after intramuscular administration of Depo-provera® to women. Contraception **1976**; 14:461–9.
34. Fotherby K, Saxena BN, Shrimanker K, et al. A preliminary pharmacokinetic and pharmacodynamic evaluation of depot-medroxyprogesterone acetate and norethisterone oenanthate. Fertil Steril **1980**; 34:131–9.
35. Li L, Zhou J, Wang W, et al. Effects of three long-acting reversible contraceptive methods on HIV target cells in the human uterine cervix and peripheral blood. Reprod Biol Endocrinol **2019**; 17:26.
36. Miller L, Patton DL, Meier A, Thwin SS, Hooton TM, Eschenbach DA. Depomedroxyprogesterone-induced hypoestrogenism and changes in vaginal flora and epithelium. Mech Dev **2000**; 96:431–9.
37. Calla NEQ, Vicetti Miguel RD, Boyaka PN, et al. Medroxyprogesterone acetate and levonorgestrel increase genital mucosal permeability and enhance susceptibility to genital herpes simplex virus type 2 infection. Mucosal Immunol **2016**; 9:1571–3.
38. Davis ASW, Vick SC, Pattacini L, et al. The human memory T cell compartment changes across tissues of the female reproductive tract. Mucosal Immunol **2021**; 14:862–72.
39. Shanmugasundaram U, Critchfield JW, Pannell J, et al. Phenotype and functionality of $CD4^+$ and $CD8^+$ T cells in the upper reproductive tract of healthy premenopausal women. Am J Reprod Immunol **2014**; 71:95–108.