

https://dspace.org/

PreHuman Immunodeficiency Virus HIV 47hi CD4 T Cells and HIV Risk Among Heterosexual Individuals in Africa

T Omole; Huong Mai Nguyen; Agata Marcinow; Myo Minn Oo; Glenda Gray ...

OXFORD UNIV PRESS INC
https://hdl.handle.net/10539/48938
SCOPUS

Downloaded from a DSpace repository. https://dspace.org/

The Journal of Infectious Diseases

MAJOR ARTICLE

Pre–Human Immunodeficiency Virus (HIV) α4β7^{hi} CD4^{+} T Cells and HIV Risk Among Heterosexual Individuals in Africa

Tosin E. Omole,[1] Huong Mai Nguyen,[1] Agata Marcinow,[1] Myo Minn Oo,[1] Naima Jahan,[1] Aloysious Ssemaganda,[1] Giulia Severini,[1] Katherine K. Thomas,[2] Connie Celum,[2,3] Nelly Mugo,[2,4] Andrew Mujugira,[2,5] James Kublin,[6,7] Lawrence Corey,[6,7] Aida Sivro,[1,8,9,10] Jairam R. Lingappa,[2,11] Glenda Gray,[6,12] and Lyle R. McKinnon[1,8,13]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Canada; [2]Department of Global Health; [3]Departments of Medicine and Epidemiology, University of Washington, Seattle; [4]Sexual Reproductive and Adolescent Child Health Research Program, Kenya Medical Research Institute, Nairobi; [5]Infectious Diseases Institute, Makerere University, Kampala, Uganda; [6]HIV Vaccine Trials Network; [7]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Center, Seattle, Washington; [8]Mucosal Immunology Laboratory, Centre for the AIDS Program of Research in South Africa (CAPRISA), Durban; [9]JC Wilt Infectious Disease Research Centre, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba; [10]Department of Medical Microbiology, University of KwaZulu-Natal, Durban, South Africa; [11]Departments of Medicine and Pediatrics, University of Washington, Seattle; [12]Office of the President, South African Medical Research Council, Cape Town; and [13]Department of Medical Microbiology and Immunology, University of Nairobi, Kenya

Background. CD4^{+} T cells expressing α4β7 are optimal targets for human immunodeficiency virus (HIV) infections, with higher pre-HIV α4β7^{hi} expression linked to increased HIV acquisition and progression in South African women. However, similar associations were not observed in men who have sex with men or people who inject drugs in the Americas, indicating need for further research.

Methods. This retrospective case-control study enrolled heterosexual men and women from South Africa (HIV Vaccine Trials Network [HVTN] 503) and East Africa (Partners Preexposure Prophylaxis/Couples' Observational Study [PP/COS]), quantifying α4β7 expression on CD4^{+} T cells as a predictor of subsequent HIV risk using flow cytometry analyses.

Results. Associations between α4β7^{hi} expression and HIV acquisition varied across cohorts. In HVTN 503, women had a higher risk estimate compared to men, but this was not significant. In PP/COS, α4β7^{hi} expression was generally protective, particularly in Ugandans. Additionally, α4β7^{hi} expression inversely correlated with peak viral load in PP/COS but not in HVTN 503; in the latter cohort, α4β7^{hi} expression was inversely correlated with the CD4/CD8 ratio and predicted rapid CD4^{+} T-cell decline, similar to what was observed previously in South Africa.

Conclusions. These findings suggest that α4β7^{hi} expression on CD4^{+} T cells may not predict HIV acquisition and progression in all contexts, which may be due to cohort effects, modes of transmission, viral clade, or other factors.

Keywords. α4β7; HIV acquisition; HIV progression; Africa; CD4^{+} T cells.

The integrin α4β7 is expressed on the surface of immune cells including CD4^{+} T cells where it mediates homing to gut-associated lymphoid tissues [1, 2]. Several studies have suggested that α4β7 is involved in human immunodeficiency virus (HIV) pathogenesis. During acute infection, α4β7-expressing cells are highly susceptible to HIV/simian immunodeficiency virus (SIV) and preferentially depleted; these cells highly coexpress other HIV-susceptible markers such as the main HIV coreceptor, CCR5, and α4β7 is preferentially expressed on Th17 cells [3–5]. Some data suggest that α4β7 may act as a nonessential receptor that aids viral attachment via interaction with gp120 of some HIV type 1 (HIV-1) strains [1, 6, 7]. As a potential cure strategy, 1 study combined blocking α4β7 using a monoclonal antibody with antiretroviral therapy (ART) and reported significant virological control in rhesus macaques infected with SIVmac239-nef-stop virus [8]. Two subsequent studies that tested this α4β7-blockade strategy in a similar infection model did not replicate this effect [9, 10]. While targeting α4β7 may not be a viable cure approach, CD4^{+} T cells expressing this molecule are nonetheless important targets of lentiviral infection.

Cohort studies examining the role of integrin α4β7 as a predictor of HIV acquisition or progression have also generated

Received 20 September 2024; editorial decision 20 December 2024; accepted 20 December 2024; published online 25 December 2024

Presented in part: 18th International Congress of Immunology, organized by the International Union of Immunological Societies (IUIS), Cape Town, South Africa, November 2023. The abstract is published in *Frontiers* (poster 2291).

Correspondence: Lyle McKinnon, PhD, Department of Medical Microbiology and Infectious Diseases, 504-745 Bannatyne Ave, Basic Medical Sciences Building, University of Manitoba, Winnipeg, MB R3E 0J9, Canada (lyle.mckinnon@umanitoba.ca).

The Journal of Infectious Diseases® 2025;231:e770–80

© The Author(s) 2024. Published by Oxford University Press on behalf of Infectious Diseases Society of America.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.

https://doi.org/10.1093/infdis/jiae638

Downloaded from https://academic.oup.com/jid/article/231/4/e770/7932397 by UNIV OF WITWATERSRAND user on 01 August 2025

mixed results. In heterosexual South African women, we showed that women with higher pre-HIV $\alpha4\beta7^{hi}$ $CD4^+$ T cells had higher risk of HIV infection and progressed faster compared to women with lower $\alpha4\beta7^{hi}$ $CD4^+$ T cells [11]. This was not replicated in a United States–based study among men who have sex with men (MSM) and people who inject drugs (PWID) [12]. Despite similar study design and methods, the characteristics of the populations differed, which may contribute to the discrepancies between the studies. Therefore, gaps still exist regarding understanding the role of α4β7 and HIV outcomes across populations, clades, and geographies.

MATERIALS AND METHODS

Study Design and Population

We carried out a nested, retrospective, matched case-control study to evaluate the association between pre-HIV $\alpha4\beta7^{hi}$ $CD4^+$ T cells and HIV outcomes in HVTN 503 and Partners PrEP (preexposure prophylaxis) combined with Couples' Observational Study (COS) cohorts (hereafter PP/COS) [13–17]. All participants in the parent cohorts provided informed written consent to have specimens stored for future immunological research, and the substudies reported here were approved by the institutional review board (IRB) at the University of Manitoba and at local IRBs where the studies were conducted. We analyzed samples from participants who were later diagnosed with HIV (PLDH) and compared these to those who remained HIV uninfected (nonseroconverters [NSCs]). PLDH were sampled at the last available pre-HIV infection visit and matched to controls within the same cohort based on prespecified criteria (Supplementary Methods).

Flow Cytometry Analyses

The expression of α4β7 was quantified on $CD4^+$ T cells using flow cytometry of peripheral blood mononuclear cells (PBMCs). In brief, cryopreserved PBMCs were thawed and rested for 3 hours in media (RPMI 1640 + 10% fetal bovine serum + 1% penicillin-streptomycin) at 37°C and 5% carbon dioxide and stained with panels of fluorescently conjugated antibodies (Supplementary Methods).

Statistical Methods

Medians with interquartile range (IQR) and percentages were used to summarize continuous and categorical variables, respectively. Mann-Whitney test was used to compare $\alpha4\beta7^+$ $CD4^+$ T cells between groups. Conditional logistic regression (CLR) was used to measure HIV acquisition associations, with 3 $\alpha4\beta7^+$ subsets as primary continuous explanatory variables. Each integrin subset was modeled separately in unadjusted and multivariable analyses, which adjusted for potential confounders. Stratified CLR was used to compare the α4β7–HIV associations based on sex, country, and treatment arms within each cohort.

Spearman rank correlation test was used to measure associations between α4β7 and HIV peak and set point viral load (VL), and CD4/CD8 ratio post–HIV infection. Cox regression was used to predict time to $CD4^+$ T-cell decline to <500 cells/μL prior to ART initiation. $CD4^+$ T-cell counts measured during the first 180 days of follow-up were censored to exclude the short-lived CD4 drops during the acute infection [11]. In Cox regression analyses, α4β7 expression was dichotomized as above/below the median. CLR was performed using SAS version 9.4. Other statistical analyses and data visualizations were computed in RStudio 4.2.2. All statistics are 2-tailed and P values ≤.05 were considered statistically significant. P values were adjusted for multiple testing using the Benjamini-Hochberg method with p.adjust.method in R software.

RESULTS

Study Participants

Our study included males and females at risk of HIV infection. The median age was 32 years (IQR, 27–40 years) for PP/COS and 22 years (IQR, 20–26 years) for HVTN 503. Of the 180 samples processed for PP/COS, we excluded 6 (3.3%) due to <50% cell viability. For HVTN 503, we excluded 19 (7.9%) samples due to low viability. In PP/COS, 75% of participants had a baseline risk score ≥5, reflecting seronegative partners with greater exposure to HIV acquisition. Due to the early close of HVTN 503, 52 (24%) and 157 (71%) of participants received only 1 or 2 doses of the vaccine regimen, respectively, while 12 (~5%) completed 3 vaccine doses as per protocol (Table 1).

Effect of Pre-HIV Frequencies of Integrin $\beta7^{hi}$ $CD4^+$ T-Cell Subsets on HIV Acquisition

We characterized subsets of $CD4^+$ T cells based on their expression of integrin β7 and CD45RA: $\beta7^{hi}$ $CD45RA^-$, $\beta7^{int}$ $CD45RA^+$, and $\beta7^{lo}$ $CD45RA^-$ (Figure 1*A*). Previous reports showed that most blood $\beta7^{hi}$ $CD4^+$ T cells coexpress integrin α4 [3, 4, 11, 18] and are mainly memory cells [4, 11, 18]. We considered $\beta7^{hi}$ $CD45RA^-CD4^+$ T cells to be $\alpha4\beta7^{hi}$. Relatively consistent measurements of $\beta7^{hi}$ expression on $CD4^+$ T cells from the same healthy volunteer PBMCs were obtained across multiple experimental batches (Supplementary Figure 1). We compared proportions of $\beta7^{hi}$ $CD4^+$ T-cell subsets by treatment arms, HIV acquisition status, sex, and country (Figure 1*B* and 1*C*). The frequency of $\beta7^{hi}$ $CD4^+$ T cells was only significantly different with treatment arms, with higher expression in the vaccine arm in HVTN 503 ($P = .014$; Figure 1*B*) and in participants who received oral PrEP in PP/COS ($P = .026$; Figure 1*C*) compared to the placebo groups. Similarly, the expression levels of $\beta7^{int}$ and $\beta7^{lo}$ cells were significantly different between PLDH and NSC, and between the PrEP and placebo arms, but only in PP/COS (Supplementary Figure 2).

Downloaded from https://academic.oup.com/jid/article/231/4/e770/7932397 by UNIV OF WITWATERSRAND user on 01 August 2025

Table 1. Baseline Characteristics of Participants

Characteristics	HVTN 503		Partners PrEP/COS	
	PLDH (n = 47)	NSC (n = 174)	PLDH (n = 35)	NSC (n = 139)
Age[a], y	23 (21–27)	22 (20–26)	30.3 (24.6–39.7)	32.2 (28.1–40.1)
CD4 count[a], cells/μL	415.5 (290–496.8)	NA	491 (422.2–632.5)	NA
Time from sampling to infection[a], d	177 (141–218.5)	NA	234 (147–321.5)	NA
Sex				
Female	26 (55.3)	72 (41.4)	19 (54.3)	61 (43.9)
Male	21 (44.7)	102 (58.6)	16 (45.7)	78 (56.1)
Treatment arm				
Placebo	24 (51.1)	86 (49.4)	15 (53.6)	60 (53.6)
Vaccine	23 (48.9)	88 (50.6)	NA	NA
PrEP	NA	NA	13 (46.4)	52 (46.4)
Cohort				
COS	NA	NA	7 (20.0)	27 (19.4)
Partners PrEP	NA	NA	28 (80.0)	112 (80.6)
HSV-2 positive	22 (46.8)	50 (28.7)	18 (72.0)	66 (63.5)
Treatment status				
Completed	3 (6.4)	9 (5.2)	NA	NA
Off treatment early	44 (93.6)	165 (94.8)	NA	NA
Ad5 titer				
≤200	19 (40.4)	69 (39.7)	NA	NA
>200	28 (59.6)	105 (60.3)	NA	NA
Baseline risk score[b]				
<5	NA	NA	9 (25.7)	34 (24.5)
≥5	NA	NA	26 (74.3)	105 (75.5)
Sites: South Africa				
Cape Town	10 (21.3)	40 (23.0)	NA	NA
eThekwini	11 (23.4)	14 (8.0)	NA	NA
Klerksdorp	8 (17.0)	32 (18.4)	NA	NA
Medunsa	5 (10.6)	22 (12.6)	NA	NA
Soweto	13 (27.7)	66 (37.9)	NA	NA
PHRU	NA	NA	2 (5.7)	4 (2.9)
Sites: Uganda				
Jinja	NA	NA	7 (20.0)	29 (20.9)
Kampala	NA	NA	14 (40.0)	55 (39.6)
Sites: Kenya				
Kisumu	NA	NA	4 (11.4)	32 (23.0)
Thika	NA	NA	2 (5.7)	8 (5.8)
Nairobi	NA	NA	6 (17.1)	11 (7.9)

All data are presented as sample sizes (No.) and corresponding column percentages (%) unless otherwise stated.

[a]Data are presented as median (interquartile range).

[b]Part of the matching criteria.

Abbreviations: Ad5, adenovirus type 5; COS, Couples' Observational Study; HSV-2, herpes simplex virus 2; HVTN, HIV Vaccine Trials Network; NA, not applicable; NSC, human immunodeficiency virus nonseroconverters; PHRU, Perinatal HIV Research Unit (Soweto, South Africa); PLDH, people later diagnosed with human immunodeficiency virus.

Pre-HIV $\beta7^{hi}$ $CD4^+$ T-cell expression was not significantly associated with HIV acquisition in either cohort. In HVTN 503, the pre-HIV $\beta7^{hi}$ odds ratio (OR) was >1 (OR, 1.06 [95% confidence interval {CI}, .96–1.18]; adjusted OR [aOR], 1.06 [95% CI, .93–1.19]), while PP/COS trended toward a protective effect (OR, 0.92 [95% CI, .83–1.01]; aOR, 0.93 [95% CI, .84–1.03]) (Table 2). In sex-stratified CLR analyses, the odds of HIV acquisition among female participants (aOR, 1.15 [95% CI, .93–1.43) was higher compared to male participants (aOR, 1.01 [95% CI, .78–1.29) in HVTN 503 (Figure 2*A* and 2*B*). In PP/COS, preinfection $\beta7^{hi}$ $CD4^+$ T-cell frequency was significantly associated with reduced HIV acquisition among participants enrolled in Uganda (Figure 2*A*), but the association was diminished in adjusted analyses (aOR, 0.86 [95% CI, .69–1.06]; Figure 2*B*).

All results were confirmed with a second flow cytometry panel containing integrin β7 and CD45RA, which revealed consistent effect estimates, suggesting the reproducibility of these results (Supplementary Figure 3). Likewise, sensitivity analyses revealed consistent results (Supplementary Table 1 and 2). A summary of pre-HIV $\beta7^{hi}$ $CD4^+$ T-cell-HIV acquisition associations,

Downloaded from https://academic.oup.com/jid/article/231/4/e770/7932397 by UNIV OF WITWATERSRAND user on 01 August 2025

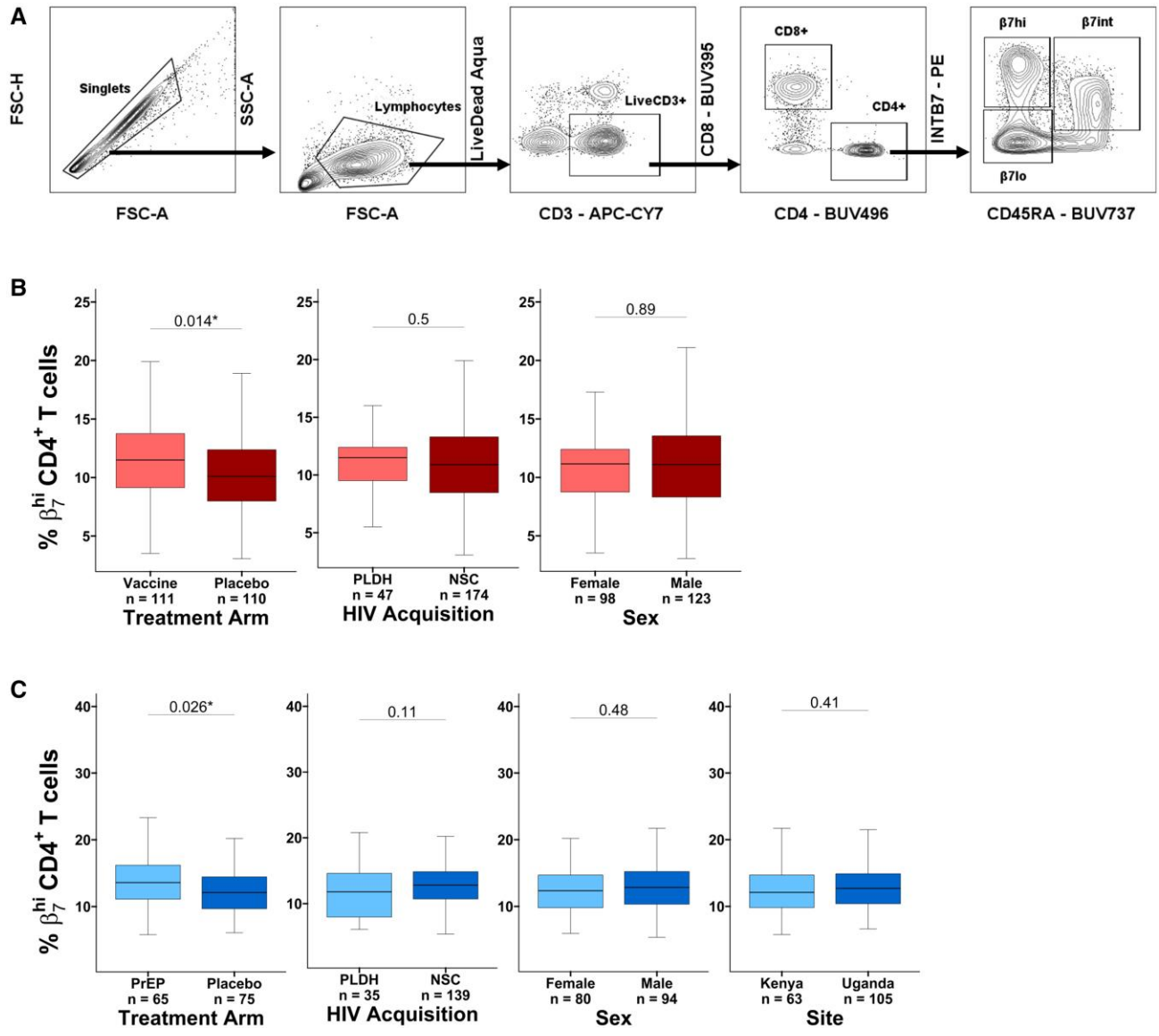


Figure 1. Pre-HIV integrin $\beta7^{hi}$ $CD4^+$ T-cell frequencies across subgroups. *A*, Representative gating strategy for flow cytometry analysis of integrin β7 subsets from frozen peripheral blood mononuclear cells. *B*, Frequencies of β7hi CD4+ T cells in HIV Vaccine Trials Network 503 cohort. and *C*, Frequencies of $\beta7^{hi}$ $CD4^+$ T cells in Partners PrEP/Couples' Observational Study cohort. Subgroups include treatment arm, HIV acquisition status, sex, and study site. Comparisons between 2 groups were analyzed using the Mann-Whitney test. Statistical significance is denoted as $*P < .05$. Abbreviations: FSC, forward scatter; HIV, human immunodeficiency virus; NSC, human immunodeficiency virus nonseroconverters; PLDH, people later diagnosed with human immunodeficiency virus; SSC, side scatter.

highlighting varying trends across studies, is provided in Supplementary Table 3.

Effect of Pre-HIV Frequencies of Integrin β7 $CD4^+$ T-Cell Subsets on HIV Progression

We next investigated pre-HIV frequencies of $\beta7^{hi}$ $CD4^+$ T cells as predictors of HIV disease progression, with viral loads (peak and set point VL; Figure 3*A* and 3*B*), CD4/CD8 ratio, and time to $CD4^+$ T-cell decline as the main outcome variables. In PP/COS, pre-HIV $\beta7^{hi}$ cells was inversely correlated with peak VL ($P = .024$; Figure 3*B*), while in HVTN 503, $\beta7^{hi}$ cells correlated inversely with mean CD4/CD8 ratio >180 days postinfection ($P = .042$; Figure 3*C*); a similar correlation was observed with CD4/CD8 ratio at last visit (Supplementary Figure 4). In unadjusted Cox regression, pre-HIV $\beta7^{hi}$ $CD4^+$ T cells predicted faster $CD4^+$ T-cell decline <500 cells/μL in HVTN 503 but not in PP/COS (Figure 3*D*). Specifically, participants with $\beta7^{hi}$ cells above the median experienced $CD4^+$ T-cell count <500 cells/μL at more than twice the rate compared to those with $\beta7^{hi}$ cells below the median (HVTN: hazard ratio [HR], 2.30 [95% CI, 1.10–5.00]; $P = 0.03$). Pre-HIV $\beta7^{hi}$ $CD4^+$ T cells remained a strong predictor of faster $CD4^+$ T-cell decline <500 cells/μL in HVTN 503

Downloaded from https://academic.oup.com/jid/article/231/4/e770/7932397 by UNIV OF WITWATERSRAND user on 01 August 2025

Table 2. Association of Pre–Human Immunodeficiency Virus (HIV) β7 CD4⁺ T Cells With HIV Acquisition

Integrin β7 Subsets	Cohorts	Unadjusted Models OR (95% CI)	*P* Value	Adjusted Models aOR (95% CI)	*P* Value
Hi	HVTN 503	1.06 (.96–1.18)	.254	1.06 (.93–1.19)	.388
	PP/COS	0.92 (.83–1.01)	.082	0.93 (.84–1.03)	.182
Int	HVTN 503	0.99 (.96–1.01)	.379	1.01 (.98–1.05)	.377
	PP/COS	1.05 (1.01–1.08)	.011*	1.05 (1.01–1.10)	.027*
Lo	HVTN 503	1.01 (.98–1.04)	.508	0.98 (.95–1.01)	.187
	PP/COS	0.96 (.93–.99)	.026*	0.96 (.91–1.00)	.061

The multivariable model for HVTN 503 was adjusted for age, sex, study site, adenovirus type 5 titer, and herpes simplex virus type 2 (HSV-2) status at baseline while PP/COS was adjusted for age, sex, study site, and HSV-2 status at baseline.

Odds ratios were estimated using conditional logistic regression models. Statistical significance is denoted as $*P < .05$.

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; HVTN, HIV Vaccine Trials Network; OR, odds ratio; PP/COS, Partners PrEP/Couples' Observational Study.

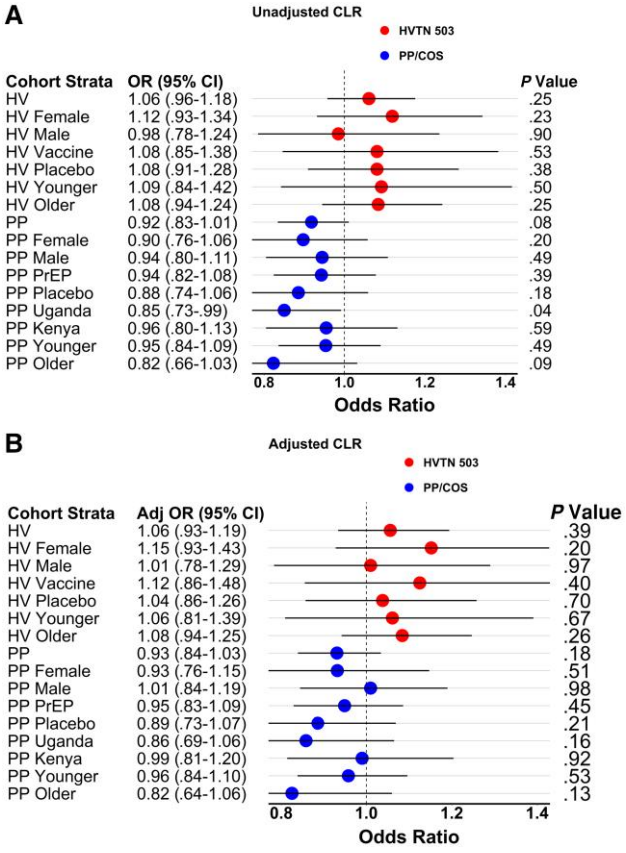


Figure 2. Odds ratios (ORs) for the association between pre-HIV integrin $\beta7^{hi}$ $CD4^+$ T cells and HIV acquisition. *A*, Unadjusted ORs. *B*, Adjusted ORs. Odds ratios were estimated using conditional logistic regression, with the analysis stratified by sex, age, treatment arm, and HIV prevention intervention where applicable. The multivariable model for HIV Vaccine Trials Network (HVTN) 503 was adjusted for age, adenovirus type 5 titer, and herpes simplex virus type 2 status at baseline, while the model for Partners PrEP/Couples' Observational Study (PP/COS) was adjusted for age and HSV-2 status at baseline. Abbreviations: CI, confidence interval; CLR, conditional logistic regression; COS, Couples' Observational Study; HV, HIV Vaccine Trials Network study 503; OR, odds ratio; PP, Partners PrEP; PrEP, preexposure prophylaxis.

after adjusting for VL, age, sex, study site, adenovirus type 5 (Ad5) titer, study arm, and herpes simplex virus type 2 (HSV-2) status at baseline (HVTN: adjusted HR [aHR], 2.72 [95% CI, 1.13–6.58]; $P = .03$; Figure 3*D*).

Associations Between Pre-HIV Frequencies of Naive and Central Memory $CD4^+$ T Cells Mirror the Associations of Integrin $\beta7^{int}$ and $\beta7^{hi}$ $CD4^+$ T-Cell Subsets on HIV Acquisition

We characterized $CD4^+$ T-cell memory phenotypes based on the expression of CCR7, CD45RA, and CD27 (Figure 4*A*, Supplementary Figure 5). Similar to the findings with $\beta7^{hi}$ $CD4^+$ T cells, central memory (T_{CM}) cells did not predict HIV acquisition in HVTN 503 but were associated with lower odds of HIV acquisition in PP/COS (PP: OR, 0.94 [95% CI, .90–.98]; $P = .005$; Figure 4*B*). As observed with $\beta7^{int}$ $CD4^+$ T cells, naive T cells (T_N) were associated with higher rates of HIV acquisition in PP/COS (PP: OR, 1.04 [95% CI, 1.01–1.07]; $P = .006$; Figure 4*B*). In HIV progression analyses, pre-HIV T_{CM} were associated with lower CD4/CD8 ratio post-infection (Figure 4*C*, Supplementary Figure 6). In contrast, T_N, like $\beta7^{int}$ $CD4^+$ T cells, positively correlated with CD4/CD8 ratio (Figure 4*D*, Supplementary Figure 6). Additionally, pre-HIV frequency of T_{CM} inversely correlated with peak VL ($P = .05$; Supplementary Figure 6) in PP/COS, in line with what was observed for $\beta7^{hi}$ $CD4^+$ T cells. Considering both T_{CM} and $\beta7^{hi}$ $CD4^+$ T cells are $CD45RA^-$ while T_N and $\beta7^{int}$ $CD4^+$ T cells are $CD45RA^+$, we estimated correlations between these subsets, observing positive correlations between T_{CM} and $\beta7^{hi}$ $CD4^+$ T cells ($P = <.001$), effector memory (T_{EM}) and $\beta7^{lo}$ $CD4^+$ T cells ($P = <.001$), and T_N and $\beta7^{int}$ $CD4^+$ T cells ($P = <.001$) (Supplementary Figure 6). In summary, the associations between pre-HIV frequencies of naive and central memory $CD4^+$ T-cell subsets mirror the patterns observed with integrin $\beta7^{int}$ and $\beta7^{hi}$ subsets, both in HIV acquisition and disease progression, emphasizing their interconnectedness.

Downloaded from https://academic.oup.com/jid/article/231/4/e770/7932397 by UNIV OF WITWATERSRAND user on 01 August 2025

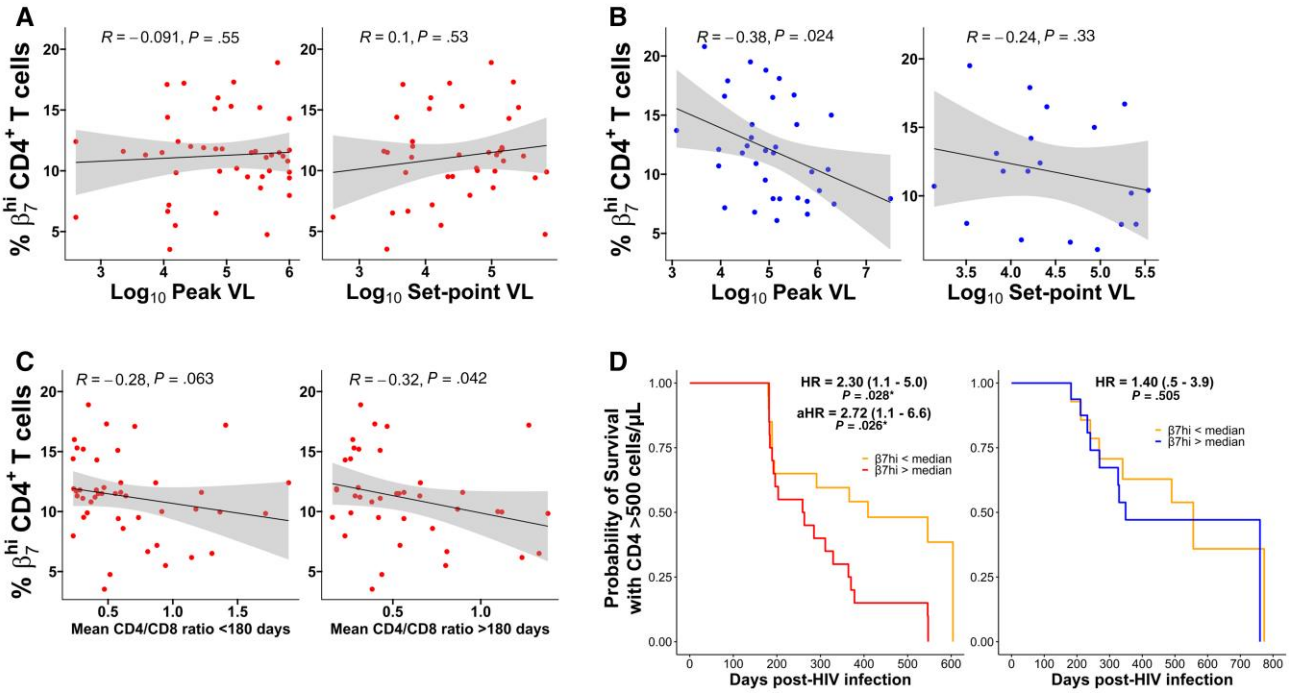


Figure 3. Association between pre-HIV integrin $\beta7^{hi}$ $CD4^+$ T cells and HIV progression. *A* and *B*, Correlation between integrin $\beta7^{hi}$ $CD4^+$ T cells and peak and set point viral load (VL) in HIV Vaccine Trials Network (HVTN) 503 (n = 45 and n = 40, respectively) and Partners PrEP/Couples' Observational Study (PP/COS) (n = 35 and n = 19, respectively). *C*, Correlation between integrin $\beta7^{hi}$ $CD4^+$ T cells and mean CD4/CD8 ratio at <180 d (n = 45) and >180 d (n = 40) postinfection in HVTN 503. *D*, Unadjusted hazard ratios (HRs) and adjusted HRs (aHRs) for $CD4^+$ T-cell decline <500 cells/μL in HVTN 503 (n = 40) and PP/COS (n = 30). Correlations were analyzed using Spearman rank correlation test and HRs were estimated using Cox regression.

Differential Phenotypic Characteristics of Integrin β7 Subsets in HVTN 503 Versus PP/COS Cohorts

Given the divergent point estimates for HIV acquisition risk observed between cohorts, we further investigated the phenotypic characteristics of each integrin β7 subset in both cohorts to elucidate the underlying factors that might contribute to these differences. Because of the overlap between α4β7 and Th17 cells, another HIV-susceptible immune subset, we examined the correlation between integrin β7 subsets with Th17 cells expressing interleukin 17 (IL-17), interleukin 22, interferon-γ, tumor necrosis factor–α, and granulocyte macrophage–colony-stimulating factor (gating strategy shown in Supplementary Figure 7). We observed a correlation between $\beta7^{hi}$ cells and IL-17–expressing $CD4^+$ T cells in HVTN 503 ($P < .001$; Figure 5*A*) while $\beta7^{hi}$ cells were not associated with IL-17^+ $CD4^+$ T cells in the PP/COS cohort ($P = .334$; Figure 5*B*). We next correlated integrin β7 subsets with demographic parameters, including age, baseline risk score, and HSV-2 status. We observed that age was the only demographic parameter that was associated with integrin β7 subsets. Specifically, in HVTN 503, integrin $\beta7^{hi}$ was correlated with age (HVTN: $r = 0.14$, $P = .042$; Figure 5*C*) while in PP/COS, integrin $\beta7^{int}$ was inversely correlated with age (PP: $r = -0.23$, $P = .002$; Figure 5*D*). We observed similar levels of $\beta7^{hi}$ expression between the 2 cohorts; however, the frequency of $\beta7^{int}$ cells was significantly lower and $\beta7^{lo}$ cells were higher in PP/COS compared to HVTN 503 (Supplementary Figure 8*A*). $CCR5^+$ cells were not significantly different on the $\beta7^{hi}$ subsets between the cohorts (gating strategy shown in Supplementary Figure 9). However, the frequency of activated ($CD38^+HLA\text{-}DR^+$), proliferating ($KI67^+$), and T_{CM} $CD4^+$ T cells were higher in PP/COS $\beta7^{hi}$ $CD4^+$ T cells compared to HVTN 503, while $\beta7^{hi}$ cells in HVTN 503 were higher in their frequency of T_{EM} and transitional memory (T_{TM}) phenotypes (Supplementary Figure 8*B* and 8*C*). The integrin $\beta7^{int}$ in PP/COS samples had significantly higher expression of naive cells than that in HVTN 503 (Supplementary Figure 8*D*). Detailed proportions of memory and activated cells within integrin β7 subsets are further characterized in Supplementary Figure 10).

DISCUSSION

Studies of α4β7 in HIV have generated conflicting findings. Here we further explored associations of $\alpha4\beta7^{hi}$ expression with HIV outcomes focusing on men and women exposed to HIV through heterosexual transmission. Through analyses of

Downloaded from https://academic.oup.com/jid/article/231/4/e770/7932397 by UNIV OF WITWATERSRAND user on 01 August 2025

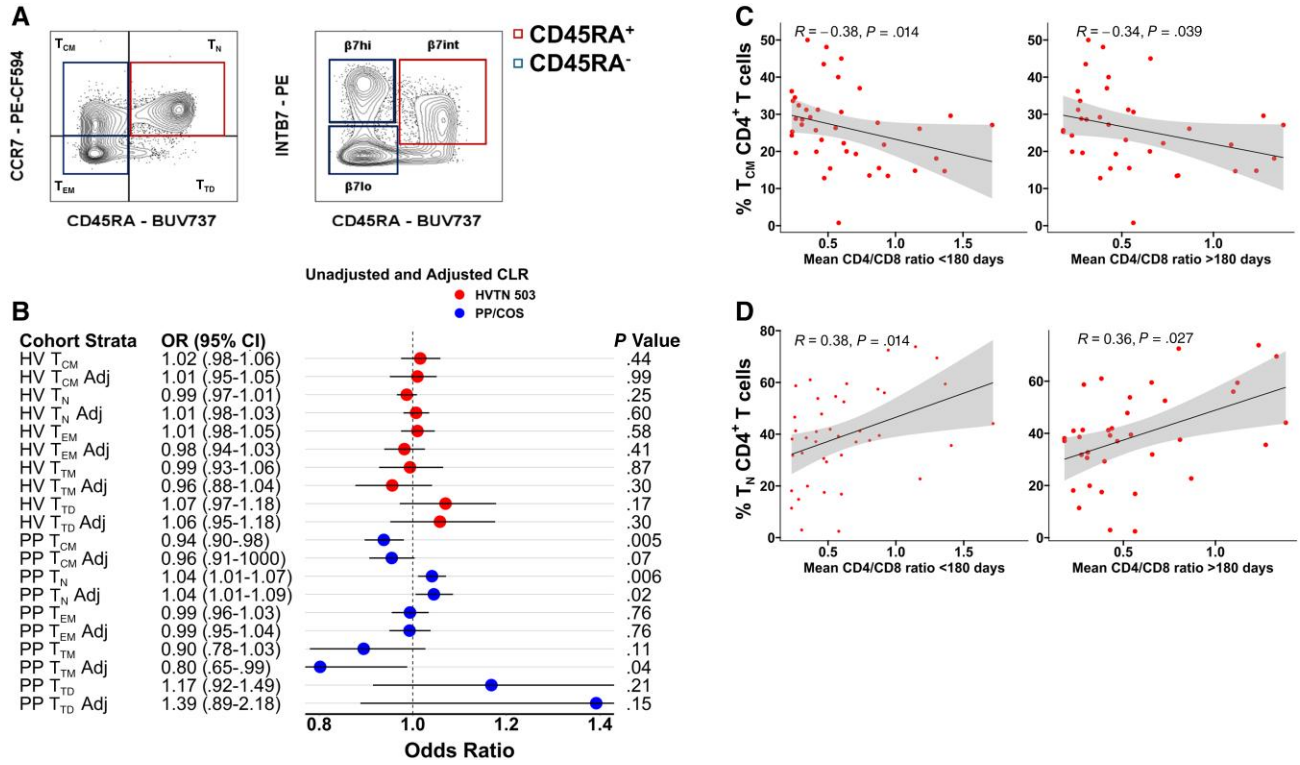


Figure 4. Association between pre-HIV memory CD4+ T-cell subsets and HIV outcomes. *A*, Representative plots illustrating the gating strategy overlap between T_{CM} and $\beta7^{hi}$ cells, as well as T_N and $\beta7^{int}$ cells. *B*, Unadjusted and adjusted odds ratios (ORs) for the association of memory CD4+ T-cell subsets with HIV acquisition. *C*, Correlation between T_{CM} and mean CD4/CD8 ratio in HIV Vaccine Trials Network (HVTN) study 503 at <180 d (n = 45) and >180 d (n = 40). *D*, Correlation between T_N and mean CD4/CD8 ratio in HVTN 503 at <180 d (n = 45) and >180 d (n = 40). ORs were estimated using conditional logistic regression. Multivariable models for HVTN 503 were adjusted for age, adenovirus type 5 titer, and herpes simplex virus type 2 (HSV-2) status at baseline, while models for the PP/COS cohort were adjusted for age and HSV-2 status at baseline. Correlations were assessed using Spearman rank correlation test. Abbreviations: CI, confidence interval; CLR, conditional logistic regression; COS, Couples' Observational Study; HV, HIV Vaccine Trials Network; HVTN, HIV Vaccine Trials Network; OR, odds ratio; PP, Partners PrEP; T_{CM}, central memory T cells; T_{EM}, effector memory T cells; T_N, naive T cells; T_{TD}, terminally differentiated T cells; T_{TM}, transitional memory T cells.

2 large cohorts from South and Eastern Africa, we did not observe statistically significant associations between integrin $\alpha4\beta7^{hi}$ expression and HIV susceptibility in either cohort. However, we noted divergent trends between cohorts, with varying estimates observed in subgroup analyses. Of note, we did replicate the association between $\alpha4\beta7^{hi}$ expression and disease progression in South Africans, confirming that $\alpha4\beta7^{hi}$ $CD4^+$ T cells were associated with faster disease progression, with a similar effect size as was observed in CAPRISA 004.

It is noteworthy that these cohorts exhibit important differences in the association of α4β7 with HIV acquisition relative to what has previously been reported. We have undertaken an exploration of potential reasons for these disparities, including factors such as age, sex, HIV clade, and the correlation of α4β7 with Th17 and memory phenotypes. Our previous study showed a modest association between systemic $\alpha4\beta7^{hi}$ expression and increased HIV acquisition in heterosexual South African females enrolled in CAPRISA 004 [11]. The present analyses are in general agreement with the acquisition finding in South Africa women in terms of observing a similar point estimate, for example, a 15% increase in HIV risk per 1% increase in $\alpha4\beta7^{hi}$ expression (albeit with 95% CIs that overlapped with 1). Since the sex-stratified sample sizes were not powered to find HIV acquisition associations in either cohort, these findings require further confirmation. Similar validation studies showed no association between $\alpha4\beta7^{hi}$ and HIV risk in MSM [12]. Males and females exhibit major differences in their sexual route of HIV transmission [19, 20], which may impact the interaction of target cells with HIV. Taken together, 1 interpretation of our data is that the association between $\alpha4\beta7^{hi}$ expression on $CD4^+$ T cells and HIV acquisition may be consistent in females from South Africa.

In addition to sex and route of exposure, the stronger associations observed between integrin $\alpha4\beta7^{hi}$ and HIV outcomes in South Africa may be partly due to differences in HIV clades. HIV clades exhibit varying geographic distribution patterns [21, 22]. While clade C is the predominant HIV clade in South Africa, clades A and D are mostly predominant in

Downloaded from https://academic.oup.com/jid/article/231/4/e770/7932397 by UNIV OF WITWATERSRAND user on 01 August 2025

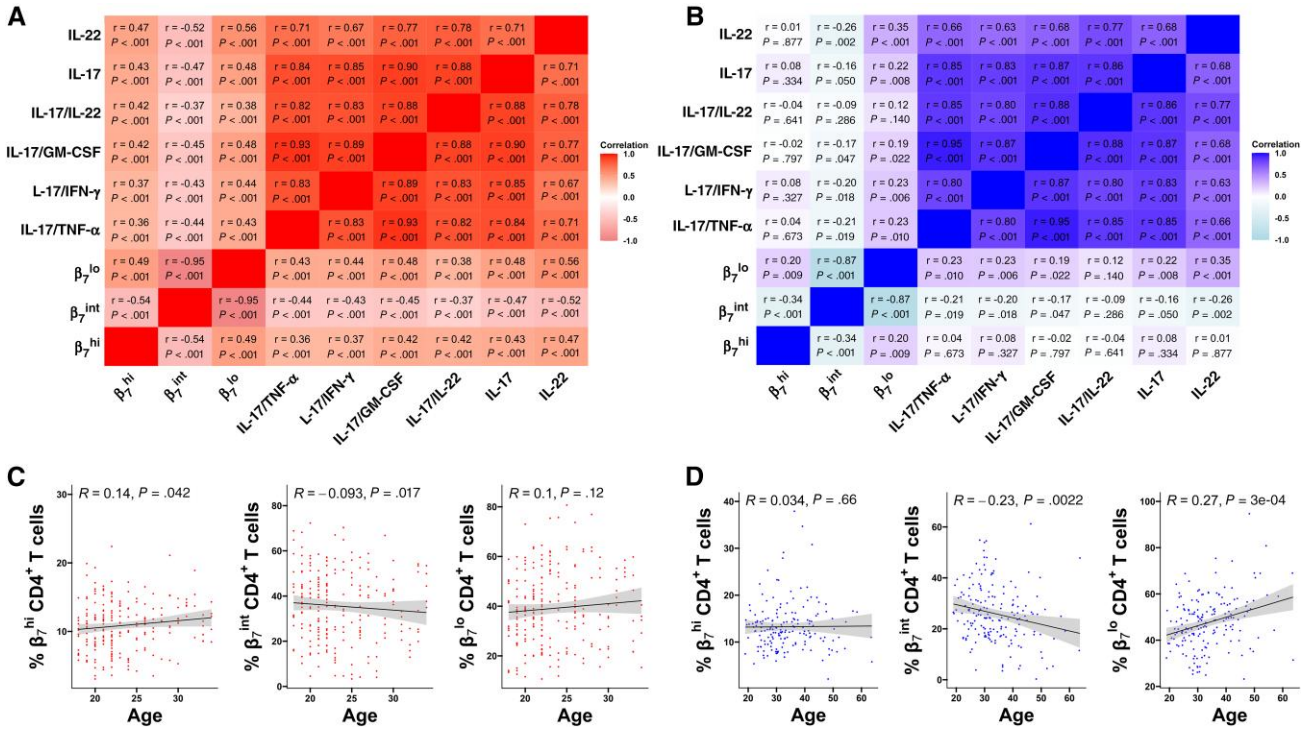


Figure 5. Correlation of integrin β7 CD4$^+$ T-cell subsets with Th17 subsets and age. *A* and *B*, Correlation matrices between integrin β7 subsets and Th17 CD4$^+$ T-cell subsets in HIV Vaccine Trials Network (HVTN) 503 (n = 130–221) and Partners PrEP/Couples' Observational Study (PP/COS) (n = 119–174). *C* and *D*, Correlation of integrin β7 subsets and participant age in HVTN 503 (n = 221) and PP/COS (n = 174). Sample sizes for the correlation matrices (*A* and *B*) ranged from 119 to 221, based on data availability for each parameter. Correlations were analyzed using Spearman rank correlation test. Abbreviations: GM-CSF, granulocyte macrophage–colony-stimulating factor; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

Kenya and Uganda, while subtype B is mostly found in the Americas [21, 23]. Some data suggest that HIV clades may interact differently with α4β7 [7, 24]. For example, α4β7 cells may bind directly to HIV gp120 second variable loop (V2) of some strains of HIV and act as a nonessential receptor for the virus [6, 7]. However, the strongest binding affinity of α4β7 with HIV was found with strains having P/SDI/V binding motifs, which is a motif most prevalent among HIV clade C from South Africa compared to other HIV clade C strains and other clades [7]. We have also previously reported that in South Africa, being infected by HIV strains with a V2 loop containing the P/SDI/V motif was associated with a higher frequency of pre-HIV α4β7 [11]. The type of infecting virus may also influence the rate of HIV progression after infection [22, 25]. Interestingly, our data support the hypothesis that the association between α4β7^{hi} and HIV acquisition may be influenced by how α4β7 interacts with different HIV clades, with stronger α4β7^{hi} effects in women from South Africa where clade C predominates.

We showed that individuals in the active arm in HVTN 503 upregulated α4β7^{hi} frequencies on their blood CD4$^+$ T cells compared to individuals who received placebo. The vaccine used in HVTN 503 was an Ad5-vectored trivalent vaccine designed to induce T-cell immunity [26, 27]. Unfortunately, the vaccines failed to offer protection against HIV infection and may have increased HIV susceptibility of vaccinated individuals seropositive for Ad5 [13, 26], possibly due to the expansion of activated Ad5-specific T cells, including those expressing α4β7^{hi}, thereby increasing the number of HIV target cells [13, 28, 29]. Similarly, in PP/COS, there was a higher expression of α4β7^{hi} CD4$^+$ T cells among participants in the PrEP intervention compared to the placebo arm of that trial. The impact of PrEP on immune cell frequency and distribution has been examined, particularly in mucosal sites where HIV transmission commonly occurs [30]. While some studies have shown that PrEP does not significantly alter the number or distribution of immune cells [31], others have shown transient changes in the frequency of immune cells after initiating PrEP [30, 32]. Overall, our data showed that HIV prevention interventions may influence the expression levels of α4β7^{hi} on CD4$^+$ T cells; however, in neither case did these appear to impact associations between α4β7^{hi} and HIV risk, which in both cohorts were similar between treatment arms.

Examination of phenotypic differences between integrin β7 subsets in HVTN 503 and PP/COS cohorts provides valuable insights into the factors contributing to variations in HIV

Downloaded from https://academic.oup.com/jid/article/231/4/e770/7932397 by UNIV OF WITWATERSRAND user on 01 August 2025

acquisition risk. These include correlations with IL-17–expressing $CD4^+$ T cells, demographic parameters, and distinctive memory T-cell phenotypes. T_{CM} were more strongly correlated with $\alpha4\beta7^{hi}$ cells in HVTN 503 compared to PP/COS. Interestingly, our data suggest that $\alpha4\beta7^{hi}$ cells are predominantly T_{CM}, while $\alpha4\beta7^{lo}$ cells tend to be enriched for T_{EM} [4, 18]. This observation prompts the intriguing possibility that $\alpha4\beta7^{hi}$ cells may exhibit a preference for migration to gut lymphoid tissue. Likewise, integrin $\beta7^{int}$ and naive $CD4^+$ T cells were associated with increased HIV acquisition in PP/COS but not in HVTN 503, despite strong correlations between these 2 subsets in both cohorts. Additionally, we observed an inverse correlation between integrin $\beta7^{int}$ and naive T-cell subsets with increasing age in PP/COS. Notably, participants in the PP/COS group were approximately 10 years older on average compared to HVTN 503 and included several individuals >40 years of age. Age could serve as a significant confounding factor due to decreases in naive cells and increases in memory cells as individuals age. Since the $\alpha4\beta7^{hi}$ $CD4^+$ T-cell variable is a proportion, its values may increase as the proportion of naive $\alpha4\beta7^{int}$ $CD4^+$ T cells decreases with age. This could potentially influence the associations between integrin β7 and HIV. Notably, $\alpha4\beta7^{hi}$ cells correlated with Th17 cell subsets in HVTN 503 but not in PP/COS. Th17, like α4β7 cells, are also highly susceptible to HIV and have been implicated in both HIV acquisition and disease progression [4, 18, 33, 34]. Taken together, the phenotypic differences in $\alpha4\beta7^{hi}$ cells between cohorts may contribute to the associations between α4β7 and HIV outcomes that have been observed.

A strength of our study is that we adopted similar designs and analyses as previous studies [11, 12]. In addition, we adopted multiple quality control procedures to ensure consistency of experiments over time and reproduced our main flow cytometry results using a second panel. One limitation of our study is that we did not have access to corresponding mucosal samples to measure $\alpha4\beta7^{hi}$ expression in the mucosa. However, frequencies of $\alpha4\beta7^{hi}$ $CD4^+$ T cells in the blood correlate with those in the genital tract [11, 35]. If feasible, future studies should include mucosal sampling to more accurately capture immune events at the mucosal level. A second limitation was our inability to match cases and controls on exposure risk levels for HVTN 503 as we did in PP/COS [36]. Exposure to HIV may be relatively low among some serodiscordant couples due to low viremia in the HIV-positive partner [37–39] and the risk levels tend to be highest at the early stages of the relationship, waning as the relationship progresses [40]. While matching on baseline risk score in PP/COS ensured that HIV exposure level is comparable between cases and controls, this may have dampened associations in PP/COS compared to HVTN 503. We were unable to examine the effect of our immune phenotypes on CD4/CD8 ratio in PP/COS due to lack of data on the absolute $CD8^+$ T-cell count in this cohort. However, we were able to test HIV progression associations within this cohort by using CD4 and VL as HIV progression parameters. We acknowledge that our sample size limitations may have constrained our ability to detect significant effects in our HIV acquisition analyses. Additionally, due to our limited sample size, we were unable to conduct subgroup analyses in our progression analyses as we did in the acquisition analyses.

In conclusion, our study suggests that associations between pre-HIV $\alpha4\beta7^{hi}$ expression on $CD4^+$ T cells and HIV outcomes may vary between different populations. Our data suggest that $\alpha4\beta7^{hi}$ may more consistently predict disease progression compared to acquisition, where the interplay of exposure, susceptibility, and various cofactors makes prediction of acquisition more complex. While $\alpha4\beta7^{hi}$ cells are reliable targets for HIV, their abundance alone may not consistently predict HIV acquisition, which in turn depends on external factors and the specific population being studied. Our research emphasizes the need to recognize the population-specific nature of α4β7 associations with HIV.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. The authors thank the HVTN 503, Partners PrEP, and COS study participants who donated their specimens for this research.

Author contributions. Study design and funding acquisition were developed by A. Si., G. G., J. R. L., and L. R. M. Data acquisition and flow cytometry analysis were performed by T. E. O., H. M. N., A. Ma., and A. Ss. Data analysis and interpretation were the responsibility of T. E. O., M. M. O., A. Si., K. K. T., and L. R. M. Study logistics and provision of reagents were coordinated by N. J. and G. S. The HVTN cohort was managed by J. K., L. C., and G. G. The PP/COS cohorts were managed by K. K. T., C. C., N. M., A. Mu., and J. R. L. Writing of the original manuscript draft was done by T. E. O. and L. R. M. All authors have reviewed, edited, and approved the manuscript.

Data availability. Data will be made available upon reasonable request to the corresponding author.

Disclaimer. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Downloaded from https://academic.oup.com/jid/article/231/4/e770/7932397 by UNIV OF WITWATERSRAND user on 01 August 2025

Financial support. This work was supported by the Canadian Institutes of Health Research (grant number MSH-147782).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Guzzo C, Ichikawa D, Park C, et al. Virion incorporation of integrin α4β7 facilitates HIV-1 infection and intestinal homing. Sci Immunol **2017**; 2:1–30.
2. Liu Q, Lusso P. Integrin α4β7 in HIV-1 infection: a critical review. J Leukoc Biol **2020**; 108:627–32.
3. Cicala C, Martinelli E, Mcnally JP, et al. The integrin α4β7 forms a complex with cell-surface CD4 and defines a T-cell subset that is highly susceptible to infection by HIV-1. PNAS **2009**; 106:20877–82.
4. Kader M, Wang X, Piatak M, et al. $\alpha4^{+}\beta7^{hi}CD4^{+}$ memory T cells harbor most Th-17 cells and are preferentially infected during acute SIV infection. Mucosal Immunol **2009**; 2:439–49.
5. Wang Y, Lu X, Wu H, Li W. Gut-homing α4β7 $CD4^{+}$ T cells: potential key players in both acute HIV infection and HIV-associated cancers. Cell Mol Immunol **2018**; 15: 190–2.
6. Arthos J, Cicala C, Martinelli E, et al. HIV-1 envelope protein binds to and signals through integrin α4β7, the gut mucosal homing receptor for peripheral T cells. Nat Immunol **2008**; 9:301–9.
7. Richardson SI, Gray ES, Mkhize NN, et al. South African HIV-1 subtype C transmitted variants with a specific V2 motif show higher dependence on α4β7 for replication. Retrovirology **2015**; 12:54.
8. Byrareddy SN, Arthos J, Cicala C, et al. Sustained virologic control in SIV^{+} macaques after antiretroviral and α4β7 antibody therapy. Science **2016**; 354:197–202.
9. Iwamoto N, Mason RD, Song K, et al. Blocking a4b7 integrin binding to SIV does not improve virologic control. Science **2019**; 365:1033–6.
10. Di Mascio M, Lifson JD, Srinivasula S, et al. Evaluation of an antibody to α4β7 in the control of SIVmac239-nef-stop infection. Science **2019**; 365:1025–9.
11. Sivro A, Schuetz A, Sheward D, et al. Integrin α4β7 expression on peripheral blood $CD4^{+}$ T cells predicts HIV acquisition and disease progression outcomes. Sci Transl Med **2018**; 10:eaam6354.
12. Martin AR, Patel EU, Kirby C, et al. The association of α4β7 expression with HIV acquisition and disease progression in people who inject drugs and men who have sex with men: case control studies. EBioMedicine **2020**; 62:103102.
13. Gray GE, Moodie Z, Metch B, et al. Recombinant adenovirus type 5 HIV gag/pol/nef vaccine in South Africa: unblinded, long-term follow-up of the phase 2b HVTN 503/Phambili study. Lancet Infect Dis **2014**; 14:388–96.
14. Mujugira A, Baeten JM, Donnell D, et al. Characteristics of HIV-1 serodiscordant couples enrolled in a clinical trial of antiretroviral pre-exposure prophylaxis for HIV-1 prevention. PLoS One **2011**; 6:e25828.
15. Baeten JM, Donnell D, Ndase P, et al. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. N Engl J Med **2012**; 367:399–410.
16. Lingappa JR, Petrovski S, Kahle E, et al. Genomewide association study for determinants of HIV-1 acquisition and viral set point in HIV-1 serodiscordant couples with quantified virus exposure. PLoS One **2011**; 6:e28632.
17. Heffron R, Stalter R, Pyra M, et al. HIV risk associated with serum medroxyprogesterone acetate levels among women in east and southern Africa. AIDS **2019**; 33:735–44.
18. Mijiti Z, Song JW, Jiao YM, et al. $\alpha4\beta7^{high}$ $CD4^{+}$ T cells are prone to be infected by HIV-1 and associated with HIV-1 disease progression. HIV Med **2022**; 23:106–14.
19. Yi TJ, Shannon B, Prodger J, McKinnon L, Kaul R. Genital immunology and HIV susceptibility in young women. Am J Reprod Immunol **2013**; 69:74–9.
20. Baeten JM, Kahle E, Lingappa JR, et al. Genital HIV-1 RNA predicts risk of heterosexual HIV-1 transmission. Sci Transl Med **2011**; 3:77ra29.
21. Hemelaar J, Gouws E, Ghys PD, Osmanov S. Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004. AIDS **2006**; 20:W13–23.
22. Hemelaar J. The origin and diversity of the HIV-1 pandemic. Trends Mol Med **2012**; 18:182–92.
23. Bbosa N, Kaleebu P, Ssemwanga D. HIV subtype diversity worldwide. Curr Opin HIV AIDS **2019**; 14:153–60.
24. Nawaz F, Cicala C, van Ryk D, et al. The genotype of early-transmitting HIV gp120s promotes α4β7-reactivity, revealing $\alpha4\beta7^{+}/CD4^{+}$ T cells as key targets in mucosal transmission. PLoS Pathog **2011**; 7:e1001301.
25. McKinnon LR, Nagelkerke NJ, Kaul R, et al. HIV-1 clade D is associated with increased rates of CD4 decline in a Kenyan cohort. PLoS One **2012**; 7:e49797.
26. Gray GE, Allen M, Moodie Z, et al. Safety and efficacy of the HVTN 503/Phambili study of a clade-B–based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. Lancet Infect Dis **2011**; 11:507–15.
27. Hopkins KL, Laher F, Otwombe K, et al. Predictors of HVTN 503 MRK-AD5 HIV-1 gag/pol/nef vaccine induced immune responses. PLoS One **2014**; 9:e103446.

Downloaded from https://academic.oup.com/jid/article/231/4/e770/7932397 by UNIV OF WITWATERSRAND user on 01 August 2025

28. Benlahrech A, Harris J, Meiser A, et al. Adenovirus vector vaccination induces expansion of memory CD4 T cells with a mucosal homing phenotype that are readily susceptible to HIV-1. PNAS **2009**; 106:19940–5.
29. Bukh I, Calcedo R, Roy S, et al. Increased mucosal $CD4^+$ T cell activation in rhesus macaques following vaccination with an adenoviral vector. J Virol **2014**; 88:8468–78.
30. Richert-Spuhler LE, Pattacini L, Plews M, et al. Pre-exposure prophylaxis differentially alters circulating and mucosal immune cell activation in herpes simplex virus type 2 seropositive women. AIDS **2019**; 33: 2125–36.
31. Pattacini L, Murnane PM, Baeten JM, et al. Antiretroviral pre-exposure prophylaxis does not enhance immune responses to HIV in exposed but uninfected persons. J Infect Dis **2015**; 211:1943–52.
32. Taiwo B, Hunt PW, Gandhi RT, et al. $CD8^+$ T-cell activation in HIV-1-infected patients experiencing transient low-level viremia during antiretroviral therapy. J Acquir Immune Defic Syndr **2013**; 63:101–4.
33. Okoye AA, Picker LJ. $CD4^+$ T-cell depletion in HIV infection: mechanisms of immunological failure. Immunol Rev **2013**; 254:54–64.
34. Rodriguez-Garcia M, Barr FD, Crist SG, Fahey JV, Wira CR. Phenotype and susceptibility to HIV infection of $CD4^+$ Th17 cells in the human female reproductive tract. Mucosal Immunol **2014**; 7:1375–85.
35. Perciani CT, Jaoko W, Farah B, Ostrowski MA, Anzala O, MacDonald KS. αeβ7, α4β7 and α4β1 integrin contributions to T cell distribution in blood, cervix and rectal tissues: potential implications for HIV transmission. PLoS One **2018**; 13:e019248.
36. Kahle EM, Hughes JP, Lingappa JR, et al. An empiric risk scoring tool for identifying high-risk heterosexual HIV-1-serodiscordant couples for targeted HIV-1 prevention. J Acquir Immune Defic Syndr **2013**; 62:339–47.
37. Cohen MS, Chen YQ, McCauley M, et al. Antiretroviral therapy for the prevention of HIV-1 transmission. N Engl J Med **2016**; 375:830–9.
38. Rodger AJ, Cambiano V, Bruun T, et al. Sexual activity without condoms and risk of HIV transmission in serodifferent couples when the HIV-positive partner is using suppressive antiretroviral therapy. JAMA **2016**; 316:171–81.
39. Attia S, Egger M, Müller M, Zwahlen M, Low N. Sexual transmission of HIV according to viral load and antiretroviral therapy: systematic review and meta-analysis. AIDS **2009**; 23:1397–404.
40. Lasry A, Sansom SL, Wolitski RJ, et al. HIV sexual transmission risk among serodiscordant couples: assessing the effects of combining prevention strategies. AIDS **2014**; 28:1521–9.

Downloaded from https://academic.oup.com/jid/article/231/4/e770/7932397 by UNIV OF WITWATERSRAND user on 01 August 2025