HIV

Integrin $\alpha_4\beta_7$ expression on peripheral blood CD4+ T cells predicts HIV acquisition and disease progression outcomes

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The gastrointestinal (GI) mucosa is central to HIV pathogenesis, and the integrin $\alpha_4\beta_7$ promotes the homing of immune cells to this site, including those that serve as viral targets. Data from simian immunodeficiency virus (SIV) animal models suggest that $\alpha_4\beta_7$ blockade provides prophylactic and therapeutic benefits. We show that pre-HIV infection frequencies of $\alpha_4\beta_7^+$ peripheral blood CD4+ T cells, independent of other T cell phenotypes and genital inflammation, were associated with increased rates of HIV acquisition in South African women. A similar acquisition effect was observed in a Kenyan cohort and in nonhuman primates (NHPs) after intravaginal SIV challenge. This association was stronger when infection was caused by HIV strains containing V2 envelope motifs with a preference for $\alpha_4\beta_7$ binding. In addition, pre-HIV $\alpha_4\beta_7^+$ CD4+ T cells predicted a higher set-point viral load and a greater than twofold increased rate of CD4+ T cell decline. These results were confirmed in SIV-infected NHPs. Increased frequencies of pre-HIV $\alpha_4\beta_7^+$ CD4+ T cells were also associated with higher postinfection expression of lipopolysaccharide binding protein, a microbial translocation marker, suggestive of more extensive gut damage. CD4+ T cells expressing $\alpha_4\beta_7$ were rapidly depleted very early in HIV infection, particularly from the GI mucosa, and were not restored by early antiretroviral therapy. This study provides a link between $\alpha_4\beta_7$ expression and HIV clinical outcomes in humans, in line with observations made in NHPs. Given the availability of a clinically approved anti-$\alpha_4\beta_7$ monoclonal antibody for treatment of inflammatory bowel disease, these data support further evaluation of targeting $\alpha_4\beta_7$ integrin as a clinical intervention during HIV infection.

INTRODUCTION

Several lines of evidence suggest that the gastrointestinal (GI) mucosa and the associated lymphoid tissue play a critical role in HIV pathogenesis. Infections by HIV and simian immunodeficiency virus (SIV) rapidly deplete CD4+ T cells from this site during the first weeks of infection (*1*–*3*), and the resulting extensive damage to the homeostasis of gut tissue persists into chronic HIV infection. One consequence of the extensive gut damage is the translocation of gut-resident bacterial products into the blood, which has been proposed to be a major source of chronic immune activation that drives HIV pathogenesis (*4*).

The homing of immune cells to the inductive and effector sites of the large and small intestine is facilitated by the expression of the integrin $\alpha_4\beta_7$ on these cells and its preferred ligand MAdCAM-1, which is constitutively expressed on the high endothelial venules of all GI tissues (*5*). Several studies suggest that $\alpha_4\beta_7$-expressing CD4+ T cells are important in HIV pathogenesis. These include direct binding of $\alpha_4\beta_7$ to some HIV strains (*6*–*9*), not as an HIV entry co-receptor per se, but rather as a molecule that may facilitate attachment of the virus to its optimal target cells (*10*–*12*). This role for $\alpha_4\beta_7$ in facilitating localization and attachment of virus to cells may be particularly important during HIV transmission, when availability of target cells is a rate-limiting step for the virus. Many laboratories have shown that CD4+ T cells expressing $\alpha_4\beta_7$ are preferentially infected both in vitro and ex vivo (*6*, *8*, *12*–*14*), including during acute SIV infection and in experiments using HIV clade C viruses (*15*), the predominant clade in South Africa. We have previously shown that $\alpha_4\beta_7$ expression on HIV target cells in the female reproductive tract (FRT) was associated with other markers of optimal HIV target cells, including CCR5 expression (*16*).

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The in vivo implications of modulating $\alpha_4\beta_7$ have been highlighted by studies that have used a primatized anti-$\alpha_4\beta_7$ monoclonal antibody (mAb), with heterodimer specificity in nonhuman primates (NHPs). When administered just before and during acute intravenous SIVmac239 infection, anti-$\alpha_4\beta_7$ mAb mediated moderate reductions in plasma viral load (VL) but substantial reductions in gut proviral DNA (*17*). More striking results were obtained when anti-$\alpha_4\beta_7$ mAb was administered before low-dose repeated vaginal challenge with SIVmac251; a significant delay in SIV acquisition was observed (*18*). Animals that eventually acquired SIV showed markedly reduced damage to gut-associated lymphoid tissue (GALT), in addition to other lymphoid and mucosal tissues. When given in combination with antiretroviral therapy (ART), anti-$\alpha_4\beta_7$ promoted potent therapeutic effects, leading to posttreatment virological control in eight of eight treated animals compared to zero of seven controls (*19*). These findings suggest that $\alpha_4\beta_7$-expressing cells play a central role in SIV transmission and pathogenesis.

One important gap in the literature is the role of $\alpha_4\beta_7$ at the time of HIV exposure in humans. Here, we report evidence that frequencies of $\alpha_4\beta_7$-expressing $CD4^+$ T cells predict both increased risk of HIV acquisition and more rapid disease progression in a cohort of high-risk women from KwaZulu-Natal, South Africa. Data from other cohorts and NHPs further support that $\alpha_4\beta_7$-expressing $CD4^+$ T cells are important HIV targets. With a number of anti-$\alpha_4\beta_7$ blockers in various stages of clinical development, these findings inform the potential translation of these drugs in the treatment of HIV disease.

RESULTS

Study participants

We compared $\alpha_4\beta_7$ integrin expression on $CD4^+$ T cells in blood samples from individuals who later acquired HIV to controls that remained HIV-uninfected for the duration of the CAPRISA 004 study. Cases were sampled at the last available pre-HIV infection visit, with a sampling median of 110 days [interquartile range (IQR), 65 to 182] before HIV infection. Controls (n = 106) were matched to cases (n = 59) at a 2:1 ratio based on study arm, age (5-year window), and month of enrollment. No major differences between cases and controls were observed for a number of demographic, clinical, and behavioral variables (Table 1). Additional analyses were conducted in study cohorts from Kenya, Uganda and the RV254/SEARCH 010 cohort in Thailand (tables S1 to S3). Further data from SIV-challenged NHPs were included to determine consistency between primate species.

Pre-HIV infection frequencies of β_7^{Hi} $CD4^+$ T cells and HIV acquisition

Previous studies have demonstrated that β_7^{Hi} cells in the blood are >99% $\alpha_4\beta_7^+$ (*6*, *20*); therefore, β_7^{Hi} $CD45RA^-$ gating was used to quantify $\alpha_4\beta_7$ expression on $CD4^+$ T cells. We identified three populations of $CD4^+$ T cells based on the relative density of the integrin β_7 and CD45RA expression that were consistently measured across the study population: β_7^{Hi} $CD45RA^-$, β_7^{Int} $CD45RA^+$, and β_7^{Neg} $CD45RA^-$ (Fig. 1A). Because it is difficult to sample cases immediately before HIV infection, we investigated the stability of β_7^{Hi} frequencies in blood samples over multiple HIV-uninfected visits in a subset of participants (range, two to five visits; Fig. 1B). The median coefficient of variation (CV) was 15% (IQR, 11 to 36), indicating relatively stable expression in most individuals before HIV infection. We then determined whether any association between HIV infection and higher frequencies of $\beta7^{Hi}$ $CD4^+$ T cells might be explained by sampling carried out closer to the estimated time of HIV infection (Fig. 1C). Our analysis indicates that this was not the case; the frequencies of β_7^{Hi} $CD4^+$ T cells were consistent among cases and controls regardless of sample timing, congruent with the stability data presented in Fig. 1B.

In our primary end point analysis, the frequency of β_7^{Hi} cells was higher in samples from cases (median, 9.7%; IQR, 8.1 to 12.3%) than controls (median, 8.7%; IQR, 6.5 to 10.7%). In conditional logistic regression analyses, each percent of pre-HIV infection β_7^{Hi} $CD4^+$ T cells correlated with 17% increased risk of HIV acquisition [odds ratio (OR), 1.17; 95% confidence interval (CI), 1.05 to 1.32; $P = 0.007$; Fig. 1D]. In contrast, no significant associations were observed for either β_7^{Int} or β_7^{Neg} populations and HIV acquisition (P = 0.242 and 0.882, respectively; fig. S1).

We next carried out multivariable modeling to adjust for variables that may confound the HIV acquisition analysis. Integrin β_7^{Hi} cell frequency remained associated with HIV acquisition with a similar effect estimate after adjusting for study site, herpes simplex virus type-2 (HSV-2) serostatus, abnormal vaginal discharge, number of sexual partners and sex acts per month, condom, and depomedroxyprogesterone acetate (DMPA) usage [adjusted OR (aOR), 1.16; 95% CI, 1.03 to 1.32; $P = 0.016$; Table 2]. We also adjusted the analysis for genital inflammation [defined as $\geq$5 of 9 proinflammatory cytokines in the upper quartile (*21*)] and found that frequencies of β_7^{Hi} cells remained a predictor of HIV outcome in a model that included all of the other covariates listed above (aOR, 1.15; 95% CI, 1.02 to 1.30; $P = 0.028$). In addition, frequencies of β_7^{Hi} cells remained a predictor of HIV outcome in a model that included both $CD4^+$ T cell activation (aOR, 1.18; 95% CI, 1.05 to 1.33; $P = 0.005$) and $CD8^+$ T cell activation (aOR, 1.18; 95% CI, 1.05 to 1.32; $P = 0.007$) defined by HLA-DR and CD38 coexpression. We carried out further phenotypic profiling to compare abundance of CCR5, Ki67, CD38, and HLA-DR expression between the three main β_7 subsets (fig. S2). β_7^{Hi} cells were comparable to at least one of the other β_7-associated subsets with respect to

Table 1. Characteristics of the CAPRISA 004 study population. IQR, interquartile range.

Variable	Cases (n = 59) Median (IQR)	Controls (n = 106) Median (IQR)	P value
Age*	23 (20–25)	22 (20–28)	0.864
Urban site	19/59 (32.2)	40/106 (37.7)	0.503
Tenofovir (TFV) arm*	23/59 (39.0)	43/106 (40.6)	0.87
DMPA use	50/59 (84.7)	85/106 (80.2)	0.532
Vaginal discharge	25/59 (42.4)	35/106 (33.0)	0.242
Ulcers	1/59 (1.7)	6/106 (5.7)	0.423
HSV-2 serostatus (at trial entry)	32/59 (54.2)	53/106 (50.0)	0.629
Sex acts, past 30 days	5 (2.5–6.6)	5 (3–8)	0.281
Parity	1 (1,2)	1 (1,1)	0.878

*Part of the matching criteria.

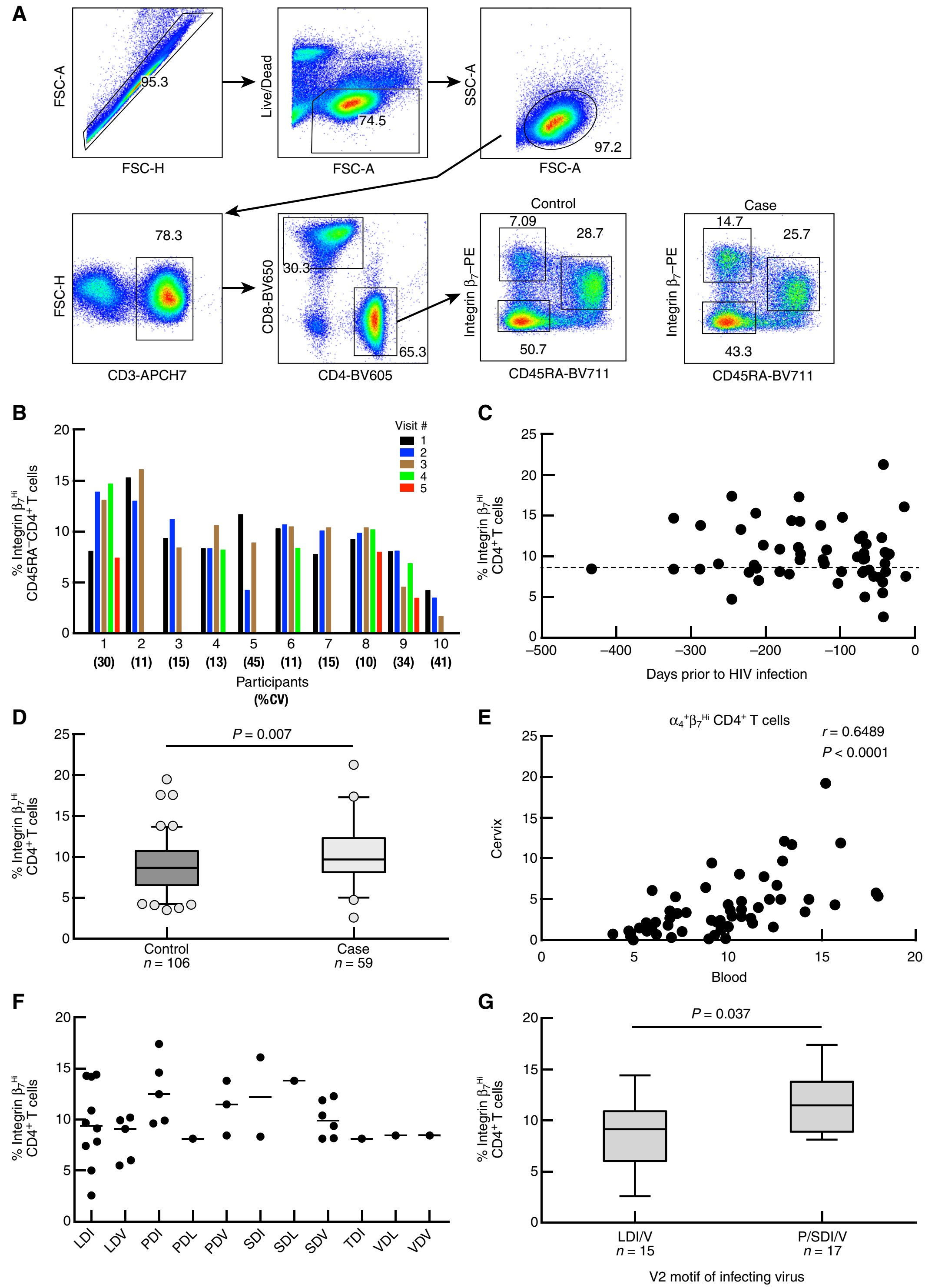

Fig. 1. Effect of preinfection β_7^{Hi} CD45RA$^-$CD4$^+$ T cell frequency on HIV acquisition risk in CAPRISA 004 study. (**A**) Parent gating strategy for the analysis of frozen PBMCs from the CAPRISA 004 study. The staining profile of PBMCs from a representative HIV-uninfected participant is shown. β_7 gating is shown for one representative control and one case sample obtained at an HIV-uninfected time point. (**B**) Samples from 10 patients (*x* axis) were assayed from three to five HIV-uninfected visits (colored bars) depending on sample availability. Median coefficient of variation (CV) between HIV-uninfected time points for every individual was calculated. Individual CVs are indicated in boldface and in parentheses in the graph. (**C**) Sampling time points for the pre-HIV flow cytometry measurements. The number of days before HIV infection that the sample was obtained (*x* axis) is plotted against β_7^{Hi} frequency (*y* axis). The dashed line represents the median integrin β_7^{Hi} expression by CD4$^+$ T cells. (**D**) Frequency of β_7^{Hi} CD45RA$^-$CD4$^+$ T cells in cases ($n = 59$) and controls ($n = 106$). Conditional logistic regression analysis was used to measure the effect of preinfection β_7^{Hi} frequency on HIV acquisition. (**E**) Spearman correlation between $\alpha_4\beta_7^{Hi}$ CD4$^+$ T cell frequency between the cervix and blood in the Nairobi/Uganda study ($n = 54$) (**F**) Infecting viral V2 motifs and pre-HIV frequencies of β_7^{Hi} CD4$^+$ T cells. (**G**) Pre-HIV frequencies of β_7^{Hi} CD4$^+$ T cells in cases infected by viruses with V2 loops containing the P/SDI/V and LDI/V motifs ($n = 32$). Differences between groups were analyzed using unpaired *t* test.

Table 2. Multivariable analysis of HIV acquisition and integrin β_7^{Hi} CD4$^+$ T cells using conditional logistic regression.

Variable		*P*	aOR	95% CI	
				Lower	Upper
Integrin β_7^{Hi} CD45RA$^-$CD4$^+$ T cells		0.016	1.163	1.029	1.316
Urban study site		0.091	0.349	0.103	1.181
HSV-2 seropositive at trial entry		0.223	1.606	0.750	3.438
Abnormal vaginal discharge		0.248	1.646	0.707	3.831
Number of new casual partners in the last 30 days		0.669	0.916	0.614	1.367
Median no. of sex acts per month		0.100	1.110	0.980	1.258
Condom use	Never	0.486	1*	—	—
	Always	0.134	0.399	0.120	1.327
	Occasionally	0.371	0.623	0.221	1.757
	Most times	0.740	0.824	0.262	2.587
DMPA use		0.294	1.880	0.578	6.120

*Reference category.

phenotypic markers that have been associated with HIV pathogenesis, further indicating that the observed effect is specific to β_7^{Hi} expression.

The HIV acquisition results were examined in an additional 41 participants (11 cases and 30 controls) from an independent cohort of female sex workers (FSW) from Nairobi (table S3), with a very similar OR for HIV acquisition risk observed as in CAPRISA 004 (OR, 1.19; 95% CI, 0.94 to 1.52; $P = 0.148$; table S4). Combining the data from the two cohorts ($n = 206$), we found that each percent increase in β_7^{Hi} expression correlated with an 18% increase in HIV risk (OR, 1.18; 95% CI, 1.06 to 1.31; $P = 0.002$). These data demonstrate that $\alpha_4\beta_7$ is a consistent predictor of HIV acquisition risk in two independent human cohorts.

We further analyzed preinfection $\alpha_4\beta_7$ expression on blood CD4$^+$ T cells as a predictor of SIV acquisition in NHPs exposed to weekly intravaginal challenges with SIVmac251. These animals were rhesus macaques (RMs) that were in the control arm [irrelevant immunoglobulin G (IgG)] of a published study (*18*). After adjusting for age, parity, and menses, RMs with higher $\alpha_4\beta_7$ frequency acquired SIV more rapidly than RMs with lower $\alpha_4\beta_7$ frequencies, although this did not reach statistical significance [adjusted HR (aHR), 1.20/% $\alpha_4\beta_7$; 95% CI, 0.99 to 1.44; $P = 0.057$; fig. S3]. These results are congruent with the previous NHP observations (*14*, *22*) and our human cohort data, suggesting that $\alpha_4\beta_7$ expression on CD4$^+$ T cells is associated with both HIV and SIV infection risk.

Expression of $\alpha_4\beta_7$ integrin by CD4$^+$ T cells in corresponding blood and cervical samples

We next explored whether the frequency of $\alpha_4\beta_7^+$ CD4$^+$ T cells in the blood reflected frequencies of these cells in the FRT, the main site of HIV exposure during heterosexual transmission. Although cervical specimens were not available in CAPRISA 004, we evaluated whether $\alpha_4^+\beta_7^{Hi}$ cells in the blood correlated with $\alpha_4^+\beta_7^{Hi}$ cells from endocervical cytobrushes in women from Uganda and Kenya (Fig. 1E). Positive correlations were observed consistently between cohorts (combined $r = 0.65$; $P < 0.0001$; $n = 57$). These data suggest that assessing systemic β_7^{Hi} cells is likely reflective of $\alpha_4^+\beta_7^{Hi}$ abundance in the FRT.

Pre-HIV infection frequencies of β_7^{Hi} CD4$^+$ T cells and early HIV env sequences

Several reports have suggested that $\alpha_4\beta_7$ can bind to the gp120 second variable loop (V2) of some strains of HIV envelope (Env) directly (*7*, *9*, *23*, *24*). Specifically, the P/SDI/V V2 motif has been associated with increased $\alpha_4\beta_7$-dependent in vitro replication and is overrepresented in the South African epidemic, particularly in KwaZulu-Natal (*7*, *15*). With these observations in mind, we hypothesized that the frequency of β_7^{Hi} cells before HIV infection would correlate with the V2 sequences of early-transmitting viruses encoding P/SDI/V motif. Sequences of acute/early HIV Envs were available for 32 CAPRISA 004 participants at a median of 5 weeks after HIV infection (IQR, 3 to 7). Participants infected by viruses with V2 loops containing the P/SDI/V motif had a higher frequency of pre-HIV β_7^{Hi} cells than those infected by viruses with LDI/V motifs (median, 11.5; IQR, 8.9 to 13.8; versus median, 9.1; IQR, 6.0 to 10.9; $P = 0.0366$; Fig. 1, F and G). These data suggest that the risk of HIV acquisition mediated by $\alpha_4\beta_7$ might be particularly pronounced when exposure involves viruses containing certain V2 motifs associated with enhanced $\alpha_4\beta_7$ binding.

Pre-HIV infection frequencies of β_7^{Hi} CD4$^+$ T cells and HIV disease progression

To determine whether pre-HIV infection frequencies of β_7^{Hi} CD4$^+$ T cells predicted the rate of HIV disease progression, we correlated the frequency of these cells with both peak and set-point VL, the rate of CD4$^+$ T cell decline before ART initiation, and the median CD4/CD8 ratio after HIV infection (Fig. 2). A correlation was observed between β_7^{Hi} cell frequency and set-point VL (average of >180 day measurements; $r = 0.345$; $P = 0.016$; Fig. 2A) and a trend with peak VL (<180 days after HIV; $r = 0.232$, $P = 0.112$; Fig. 2B). In contrast, no correlations were observed for β_7^{Int} or β_7^{Neg} cells (fig. S4, A and B).

The frequency of β_7^{Hi} cells was a strong predictor of CD4$^+$ T cell decline below 500 cells/μl; individuals with β_7^{Hi} cells above the median of β_7^{Hi} expression progressed to CD4$^+$ T cell count of <500 at more than twice the rate of those below the β_7^{Hi} median (HR, 2.38; 95% CI, 1.25 to 4.51; $P = 0.008$; Fig. 2C). At day 500, about 80% of

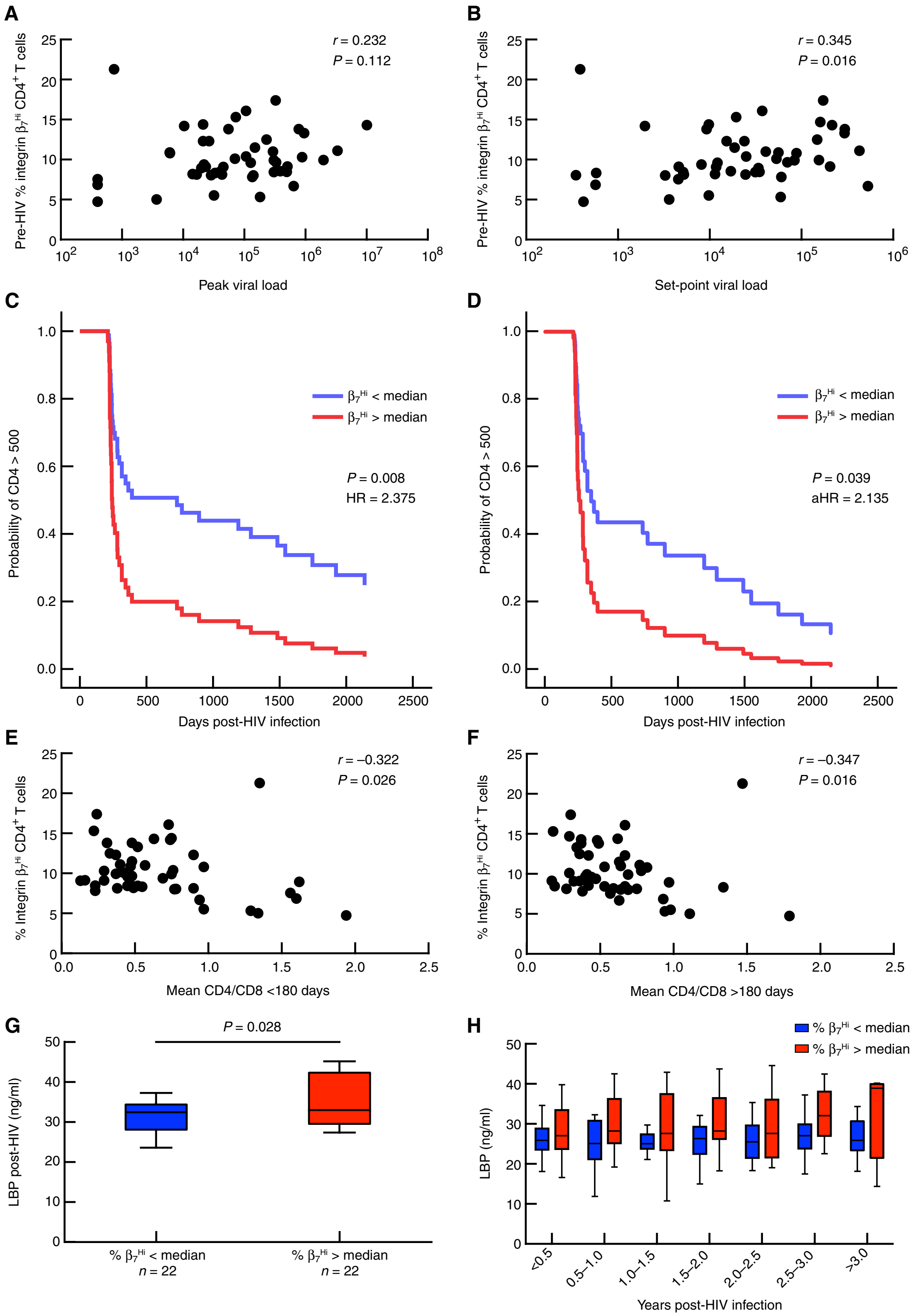

Fig. 2. Effect of preinfection β_7^{Hi} CD45RA$^-$CD4$^+$ T cell frequency on disease progression in patients that became infected in CAPRISA 004/002 study. (**A**) Correlation between preinfection β_7^{Hi} frequency and peak viral load (VL) (n = 49). (**B**) Correlation between preinfection β_7^{Hi} frequency and set-point VL (n = 49). (**C**) Frequency of preinfection β_7^{Hi} cells as a predictor of CD4$^+$ T cell decline of <500 cells/μl (n = 48), analyzed using Cox regression models. (**D**) Preinfection β_7^{Hi} cells as a predictor of CD4$^+$ T cell decline of <500 cells/μl in a multivariable Cox regression model correcting for age, study site, study arm, set-point VL, depomedroxyprogesterone acetate (DMPA) use, and HSV-2 status at baseline. (**E**) Correlation between preinfection β_7^{Hi} frequency and mean CD4/CD8 ratio <180 days after infection (n = 48). (**F**) Correlation between preinfection β_7^{Hi} frequency and mean CD4/CD8 ratio >180 days after infection (n = 48). (**G**) Median postinfection plasma expression of lipopolysaccharide binding protein (LBP) expression in cases with preinfection β_7^{Hi} CD4$^+$ T cell expression above (n = 22) and below (n = 22) the median. (**H**) Longitudinal plasma LBP expression at 6-month intervals in CAPRISA 002 cases. Linear mixed models were used to compare LBP expression over time. Spearman correlation was used to analyze associations between the two variables.

those above the β_7^{Hi} median had progressed, compared to about 50% of those below the β_7^{Hi} median. In multivariable Cox regression models (Table 3), inclusion of plasma VL as a covariate had an impact on the strength of association, suggesting that the β_7^{Hi}-associated rate of disease progression might be mediated, in part, by higher levels of HIV replication. However, in the full model, including VL and other important covariates (age, study site, study arm, DMPA use, and HSV-2 status at baseline), pre-HIV frequency of β_7^{Hi} cells remained a significant predictor of the rate of CD4$^+$ T cell decline (aHR, 2.14; 95% CI, 1.04 to 4.39; P = 0.039; Fig. 2D).

Table 3. Multivariable analysis of CD4$^+$ T cell decline and integrin β_7^{Hi} CD4$^+$ T cells using Cox regression.

Variable	*P*	aHR	95% CI	
			Lower	Lower
Integrin β_7^{Hi} CD45RA$^-$ CD4$^+$ T cells	0.039	2.135	1.039	4.389
Age	0.041	1.106	1.004	1.219
Urban study site	0.520	0.747	0.308	1.814
Study arm	0.159	0.598	0.293	1.223
Log_{10}pVL (set point)	<0.001	2.538	1.649	3.905
DMPA use	0.829	1.126	0.384	3.304
HSV-2 seropositive at baseline	0.952	0.975	0.429	2.217

Furthermore, we observed an inverse association between pre-HIV β_7^{Hi} cells and post-HIV CD4/CD8 ratio, another marker of disease progression ($r = -0.322$ and $P = 0.026$ for measurements <180 days after HIV; $r = -0.347$ and $P = 0.016$ for measurements >180 days after HIV; Fig. 2, E and F) (*25*). Again, neither β_7^{Int} nor β_7^{Neg} cell frequencies were associated with CD4$^+$ T cell decline below 500 cells/μl or CD4/CD8 ratio (fig. S4, C to E). We did not find any associations between activation markers expressed on bulk preinfection CD4$^+$ T cells and disease progression (fig. S5 and table S5).

To establish a link between human and NHP data, we characterized $\alpha_4\beta_7^+$ CD4$^+$ T cell frequencies in 14 NHPs before intravenous injection with SIVmac239. Nine animals had low frequency (<30%), whereas the remaining five animals had higher frequency (>30%) of $\alpha_4\beta_7^+$ on CD4$^+$ T cells; these patterns of expression were similar in both the blood and the gut tissue. RMs with higher $\alpha_4\beta_7$ expression experienced higher set-point VL than animals with lower $\alpha_4\beta_7$ expression (median from week 3 to 16, 331,680 versus 82,230 copies/ml), which was statistically significant in a linear mixed model analysis ($P = 0.033$; fig. S6A). As observed in humans, RMs with higher $\alpha_4\beta_7$ expression had a faster rate of CD4$^+$ T cell decline ($P < 0.001$; fig. S6B), demonstrating consistency between different primate species.

We hypothesized that the association between β_7^{Hi} cells and the rate of HIV disease progression might be mediated by more efficient transit of virus-infected cells into the GALT, leading to more extensive gut damage and its associated pathogenic effects. To test this hypothesis, we measured plasma expression of microbial translocation markers prospectively after acute HIV until ART initiation. We found that expression of lipopolysaccharide binding protein (LBP) was elevated in CAPRISA 004 participants with β_7^{Hi} cell frequencies above the median at all visits after HIV infection, whether compared as median values (Fig. 2G) or at 6-month intervals (β, 0.54; 95% CI, 0.06 to 1.03; $P = 0.028$; Fig. 2H). To determine whether this was simply a reflection of a more rapid progression, we used linear mixed models adjusting for VL and CD4/CD8 ratio, measured at the same time points as LBP. LBP remained associated with higher β_7^{Hi} frequency in the adjusted models (β, 0.59; 95% CI, 0.01 to 1.16; $P = 0.045$). In addition, we quantified plasma expression of another two commonly used markers, intestinal fatty acid–binding protein (I-FABP) and sCD14. We did not observe a statistically significant difference in I-FABP and sCD14 expression (table S6) in relation to β_7^{Hi} frequency.

Depletion of β_7^{Hi} CD4$^+$ T cells in the blood and GI mucosa during acute HIV infection

To determine the impact of acute HIV infection on the β_7^{Hi} CD4$^+$ T cell population early in HIV infection in both the blood and the GI tract, we compared frequencies of both CCR5$^+$ and β_7^{Hi} cells at Fiebig (F) stages I, II, and III in participants enrolled in the RV254 early infection cohort to chronic HIV infected and uninfected participants (Fig. 3) (*26*). In the blood, β_7^{Hi} cells increased transiently in FI, followed by a modest drop in those recruited from FII onward (Fig. 3A). Similar kinetics were observed for blood CCR5 depletion, where frequency of CCR5$^+$ cells remained comparable to HIV-uninfected expression levels in FI and decreased during FII and FIII (Fig. 3B). In contrast in the GI tract, β_7^{Hi} depletion was evident in all Fiebig stages, with major depletion occurring from FI (Fig. 3C). This occurred more rapidly than CCR5 depletion, where the major loss of CCR5$^+$ cells was only evident during the transition from FII to FIII (Fig. 3D). These data confirm that $\alpha_4\beta_7^+$ CD4$^+$ T cells are depleted very early in HIV infection, particularly in the gut, providing a potential explanation as to why these cells predicted higher rates of HIV acquisition and disease progression in CAPRISA 004 study.

Because ART was initiated upon diagnosis in RV254, we were not able to assess disease progression in this cohort. However, we determined the impact of ART on β_7^{Hi} and CCR5$^+$ CD4$^+$ T cell frequencies in both colon biopsies and blood over 2 years after HIV treatment, which was initiated at the time of HIV diagnosis in very early infection (FI or FIII). In participants who initiated ART in either FI or FIII, 2 years of ART failed to restore blood β_7^{Hi} CD4$^+$ T cells (Fig. 3E). The frequencies of β_7^{Hi} CD4$^+$ T cells in peripheral blood did not increase during treatment. Similarly, ART initiation failed to restore blood CCR5$^+$ CD4$^+$ T cells (Fig. 3F). However, the impact of ART on β_7^{Hi} and CCR5$^+$ CD4$^+$ T cells in the colon was strikingly different. The frequency of colonic β_7^{Hi} CD4$^+$ T cells in patients who initiated ART in either FI or FIII was already reduced at initial baseline measurement, and these expression levels showed no sign of recovery after 24 months of treatment (Fig. 3G). In contrast, in participants who initiated ART in FI, colonic CCR5$^+$ CD4$^+$ T cells were not depleted, and their frequency at 24 months of therapy was maintained at a level similar to healthy controls. Participants who initiated therapy in FIII showed an initial loss and a gradual, "near" recovery by month 24 (Fig. 3H). These data suggest that β_7^{Hi} CD4$^+$ T cell depletion occurs very early during acute HIV infection, especially in the GI tract, compared to blood. It is also clear that ART is unable to restore the frequency of those CD4$^+$ T cells in the GI tract, even when provided at the earliest time point, when gut damage is relatively minimal compared with chronic HIV.

DISCUSSION

The integrin $\alpha_4\beta_7$ plays an important role in promoting immune cell trafficking to the inductive and effector sites of the GI tract, both of which are irreversibly damaged during acute HIV infection. The main aim of the present study was to evaluate the role of $\alpha_4\beta_7$ integrin in humans at risk of HIV infection, an important step toward the translation of these promising preclinical studies. In line with our a priori hypotheses, expression of $\alpha_4\beta_7$ on blood memory CD4$^+$ T cells measured before HIV infection in South African women predicted both higher risk of HIV acquisition and a more rapid rate of HIV disease progression.

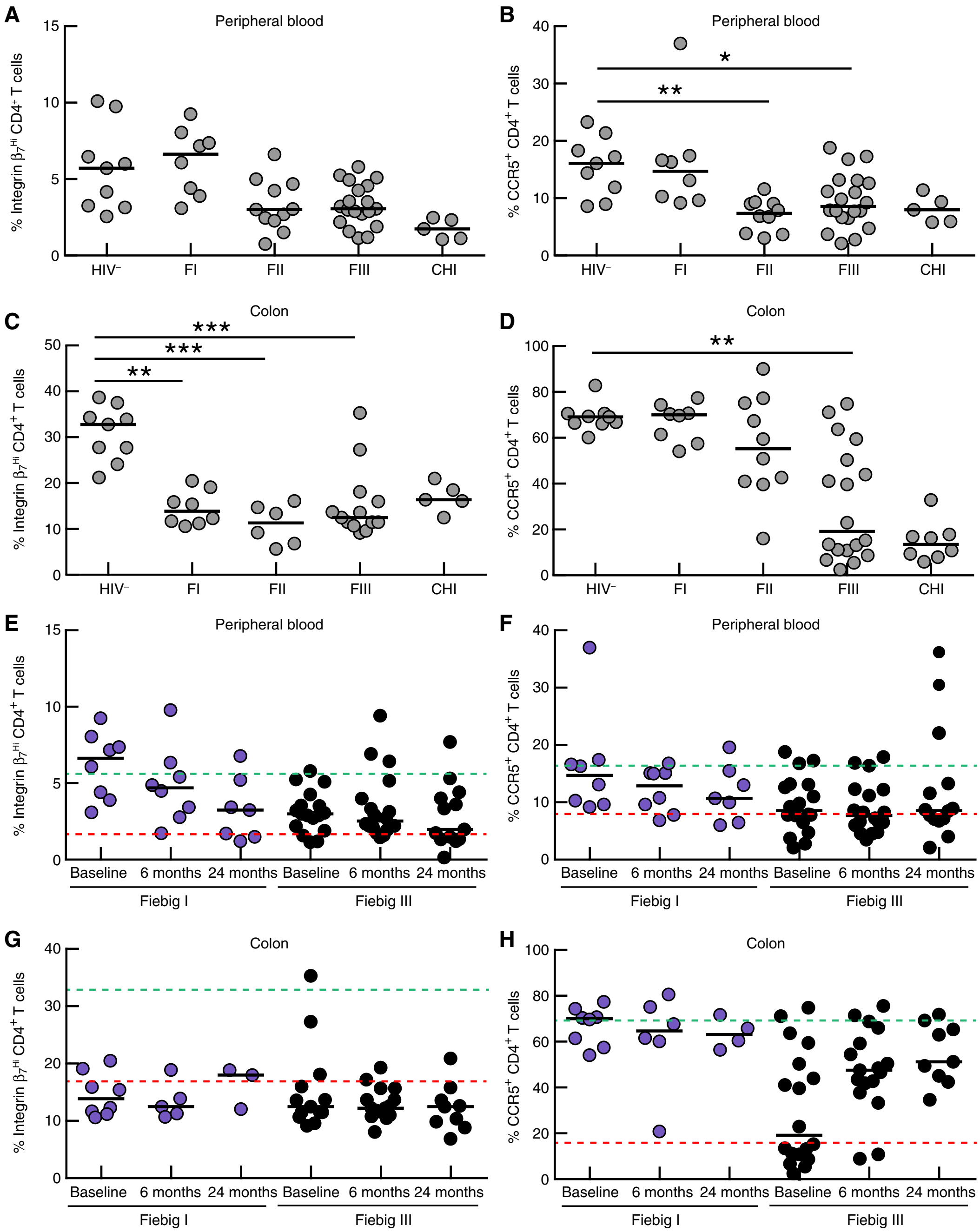

Fig. 3. Depletion and post-ART recovery of β_7^{Hi} and $CCR5^+$ $CD4^+$ T cells in peripheral blood and cells isolated from the sigmoid colon during acute infection in RV254. (**A** and **B**) Frequencies of β_7^{Hi} (A) and $CCR5^+$ (B) $CD4^+$ T cells in the peripheral blood at different Fiebig (F) stages [HIV-uninfected (HIV^-; $n = 9$), FI ($n = 8$), FII ($n = 11$), FIII ($n = 20$), and chronic HIV–infected (CHI; $n = 5$)]. (**C**) Frequencies of β_7^{Hi} $CD4^+$ T cells in the colon [HIV^- ($n = 9$), FI ($n = 8$), FII ($n = 6$), FIII ($n = 13$), and CHI ($n = 5$)]. (**D**) Frequencies of $CCR5^+$ $CD4^+$ T cells in the colon [HIV^- ($n = 9$), FI ($n = 8$), FII ($n = 10$), FIII ($n = 18$), and CHI ($n = 8$)]. (**E** and **F**) Frequencies of β_7^{Hi} (E) and $CCR5^+$ (F) $CD4^+$ T cells in the peripheral blood after antiretroviral therapy (ART) initiation in either FI [purple data points; at baseline ($n = 8$), 6 months ($n = 8$), and 24 months ($n = 7$)] or FIII [black data points; at baseline ($n = 18$), 6 months ($n = 18$), and 24 months ($n = 14$)]. (**G**) Frequencies of β_7^{Hi} $CD4^+$ T cells in the colon after ART initiation in either FI [purple data points; baseline ($n = 8$), 6 months ($n = 5$), and 24 months ($n = 3$)] or FIII [black data points; baseline ($n = 13$), 6 months ($n = 15$), and 24 months ($n = 9$)] (**H**) $CCR5^+$ $CD4^+$ T cells in the colon after ART initiation in either FI [purple data points; at baseline ($n = 8$), 6 months ($n = 6$), and 24 months ($n = 4$)] or FIII [black data points; at baseline ($n = 18$), 6 months ($n = 17$), and 24 months ($n = 9$)]. The dashed lines represent the median of cells in healthy control participants (green; $n = 9$) and in CHI patients (red; $n = 5$). For (A) to (D), Kruskal-Wallis test was used, followed by a Dunn's multiple comparisons test, to look for differences between HIV^- group and groups at different Fiebig stages. For (E) to (H), Friedman test was used to test for significant differences within each ART initiation group (FI and FIII). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

Although the association of $\alpha_4\beta_7$ expression and HIV acquisition was relatively modest, results were consistent in independent cohorts in two different countries and in NHPs. Higher preinfection $\alpha_4\beta_7$ frequency was previously associated with susceptibility to rectal SIV infection (*14*, *22*). In addition, in a low-dose vaginal challenge, SIV infection was significantly delayed by blocking $\alpha_4\beta_7$ using Act1 (*18*). Consistencies in ORs for HIV acquisition risk between CAPRISA 004 and the Kenyan FSW cohort demonstrate that $\alpha_4\beta_7$ associates with HIV acquisition risk in women from different geographic locations. Reported findings demonstrate consistency in humans and show that results can be translated between human and NHPs.

Interactions between $\alpha_4\beta_7$ and HIV Env may assist the virus in locating its ideal target cells (*27*). The findings herein show that higher frequency of $\alpha_4\beta_7$ $CD4^+$ T cells was associated with preferential infection by HIV-1 containing gp120 V2 motifs (P/SDV/I) that have been associated with higher $\alpha_4\beta_7$ binding and are overrepresented in clade C sequences from KwaZulu-Natal, South Africa, the region where the CAPRISA 004 study was conducted (*7*, *15*). The $\alpha_4\beta_7$-binding motif has been implicated as an important epitope for HIV antibody responses, including both broadly neutralizing antibodies (bNAbs) (*28*, *29*) and those that correlated with protection against HIV infection in the RV144 vaccine study (*30*). The role of preinfection $\alpha_4\beta_7$ expression on HIV pathogenesis likely depends on the nature of the transmitting virus.

The frequency of $\alpha_4\beta_7$ expression had a strong impact on the rate of HIV disease progression, in particular, as measured by the rate of $CD4^+$ T cell decline. As observed with HIV acquisition, the progression effect was highly similar between RMs and humans. In addition, the data from RV254 suggest that $\alpha_4\beta_7^+$ $CD4^+$ T cells are targeted very early in the blood and gut. In humans, a high proportion of initial HIV-target cells are likely $\alpha_4\beta_7^+$, and the rapid gut depletion of $CD4^+$ T cells may be driven by the preferential depletion of cells expressing $\alpha_4\beta_7$ (even earlier than CCR5). Therefore, in individuals with higher frequency of $\alpha_4\beta_7^+$ cells, one may expect increased viral replication and associated destruction of many CD4 compartments, including the GI mucosa, the latter of which has a major effect on disrupting immune homeostasis. Although we were not able to link rapid gut depletion with pre-HIV $\alpha_4\beta_7$ due to lack of pre-HIV samples in RV254 and lack of gut sampling in CAPRISA 004, our finding of raised LBP expression at all stages of untreated HIV infection in individuals with higher $\alpha_4\beta_7$ expression supports this concept, linking $\alpha_4\beta_7$ expression with subsequent microbial translocation and gut damage. Although we did not observe similar associations with I-FABP and sCD14, this could be due to recent reports that have suggested that these markers may not be specific to microbial translocation and are influenced by other causes of GI disease (*31*–*33*) and monocyte activation (*34*), respectively.

Initiation of ART, particularly at early stages of HIV infection (*35*), restores some immune cell populations but rarely to their preinfection frequency and/or function (*36*). Most of $CD4^+$ T cell depletion occurs in the GALT during primary infection (*3*, *37*), and ART administration at first detection of VL failed to prevent depletion or facilitate reconstitution of gut $\alpha_4\beta_7^+$ $CD4^+$ T cells in RV254. The fact that ART alone does not lead to immune restoration, but ART, in combination with anti-$\alpha_4\beta_7$, did so in NHPs (*19*), suggests that interventions in addition to ART may be needed to achieve functional $CD4^+$ T cell restoration.

One of the limitations of our study is the lack of paired cervical and blood sampling, which is not available in CAPRISA 004. Nevertheless, data from two independent East African cohorts demonstrate that the frequency of $\alpha_4\beta_7^+$ cells correlated strongly between blood and cervical $CD4^+$ T cells, suggesting that the correlation between increased frequencies of blood $\alpha_4\beta_7^+$ $CD4^+$ T cells and HIV acquisition may be explained, at least in part, by a higher concentration of target cells at the site of HIV exposure (*38*). Although the results presented here are specific to heterosexual transmission of HIV, previous NHP studies support a similar role for $\alpha_4\beta_7$ during other modes of exposure.

This study defines the importance of $\alpha_4\beta_7$ in HIV acquisition and disease progression in a prospective natural history study of high-risk women. One model to explain these data is that preferential infection of $\alpha_4\beta_7^+$ $CD4^+$ T cells at the time of HIV exposure leads to more pronounced local infection of these cells, which then migrate rapidly to the gut mucosal and lymphoid tissue, where rapid viral replication contributes to establishment of the latent HIV reservoir. Recent work by our group has suggested that the administration of a primatized analog of the anti-$\alpha_4\beta_7$ mAb in SIV-infected macaques receiving early ART leads to sustained spontaneous control of SIV and repopulation of GI tract with $CD4^+$ T cells in the absence of further treatment (*19*). Combined with the findings of the current study, these data and those from NHPs suggest that $\alpha_4\beta_7$ integrin might be a useful target for HIV prevention and/or treatment in humans. Further evaluation of this concept is clinically feasible, given that a humanized version of the Act1 mAb clone (called vedolizumab) has been proven safe and effective and is U.S. Food and Drug Administration–approved for the treatment of adults with moderate to severe ulcerative colitis and Crohn's disease (*39*, *40*).

MATERIALS AND METHODS

Study design

We carried out a nested retrospective case-control analysis to correlate the expression of integrin β_7^{Hi} on $CD4^+$ T cells to rates of HIV acquisition. Cases then followed prospectively to compare disease progression outcomes stratified by β_7^{Hi} $CD4^+$ T cells at pre-HIV infection time points. The main outcomes for disease progression were set point, and peak HIV VLs measured after infection before the initiation of ART. Survival analyses were used to compare the time to $CD4^+$ T cell decline below 500 cells/μl. In the RV254 study, different acute HIV stages were compared cross-sectionally at diagnosis and followed longitudinally after early ART initiation. All experiments were performed in a blinded fashion. Analogous studies were conducted in NHPs, as described further in Supplementary Materials and Methods. The detailed study cohorts, sample collection, and processing information are provided in Supplementary Materials and Methods. The sample size for each experiment is included in the figures and/or figure legends. Primary data are located in table S7.

Flow cytometry analysis

Peripheral blood mononuclear cells (PBMCs) were thawed, washed to remove the cryopreserving fluid, then rested for 3 hours (RPMI 1640 supplemented with 10% fetal bovine serum) at 37°C and 5% CO_2, and stained with a panel of antibodies designed to profile β_7 expression on different $CD4^+$ T cell subsets in terms of their memory, activation, and target cell properties. Detailed methods can be found in Supplementary Materials and Methods.

Soluble biomarker analysis

Plasma LBP expression was measured using Human LBP DuoSet ELISA DY870-05 (R&D Systems Inc.). All assays were performed following the manufacturer's instructions. Samples with values below the lower detection limit were assigned the value half the lower limit of quantification (LLOQ/2).

Viral sequencing

The sequence of transmitted/founder *Envs* were inferred as the consensus of acute/early sequences obtained from a median of 5 weeks after HIV infection (IQR, 3 to 7.25; range, 2 to 13 weeks). Viral *Env* sequences were generated by Sanger sequencing of amplicons generated by single genome amplication of viral RNA, performed as previously described (*41*, *42*). Additional methods can be found in Supplementary Materials and Methods.

Statistical analysis

To compare HIV acquisition risk, we carried out conditional logistic regression with strata defined by matching criteria that was used to select cases and controls. The main explanatory variables in all models included three integrin β_7–defined subsets, each modeled separately in bivariate and multivariable models adjusting for a number of potential confounding variables. Disease progression rates were evaluated using several readouts, including correlation analyses (Spearman rank correlation) with HIV VL and CD4/CD8 ratio, measured both as peak (highest value in the first 180 days of infection) and set point (average value in measurements made after 180 days of infection until ART initiation). Rates of $CD4^+$ T cell decline were compared in bivariate and multivariable Cox regression models, with the end point defined as any two $CD4^+$ T cell counts below 500/μl before ART initiation. Survival analyses excluded $CD4^+$ T cell counts measured during the first 180 days of follow-up; these were censored to exclude transient CD4 drops during acute HIV infection, as previously described (*43*). D'Agostino and Pearson omnibus normality test was used for Gaussian distribution of the data. For data that did not follow normal distribution, nonparametric tests, including Kruskal-Wallis test and Spearman correlation, were performed. All statistics are two-tailed. Linear mixed models were used to compare LBP expression prospectively, with β_7^{Hi} above and below the median as the primary predictor, and adjustments were made for progression variables, including VL and CD4/CD8 ratio.

SUPPLEMENTARY MATERIALS

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Materials and Methods
Fig. S1. Effect of preinfection β_7^{int} $CD45RA^+CD4^+$ and β_7^{Neg} $CD45RA^-CD4^+$ T cell frequency on HIV acquisition.
Fig. S2. Phenotype of β_7^{Hi} $CD45RA^-CD4^+$, $\beta 7^{int}$ $CD45RA^+CD4^+$, and $\beta 7^{Neg}$ $CD45RA^-CD4^+$ T cells.
Fig. S3. Effect of preinfection $\alpha_4\beta_7^+$ $CD4^+$ T cell frequency in the blood on SIV acquisition in NHPs exposed to weekly intravaginal challenges with SIVmac251.
Fig. S4. Effect of preinfection β_7^{int} $CD45RA^+CD4^+$ and β_7^{Neg} $CD45RA^-CD4^+$ T cell frequency on disease progression in patients that became infected in CAPRISA 004/002 study.
Fig. S5. Gating strategy used for the analysis of frozen PBMCs from the CAPRISA 004 study.
Fig. S6. Effect of preinfection $\alpha_4\beta_7^+$ $CD4^+$ T cell frequency in the gut and the blood on disease progression in RMs after intravenous injection with SIVmac239.
Table S1. Characteristics of the Ugandan and Kenyan study populations.
Table S2. Cohort characteristics (RV254 study).
Table S3. Cohort characteristics (FSW cohort; Nairobi, Kenya).
Table S4. Analysis of HIV acquisition and integrin β_7^{Hi} $CD4^+$ T cells using conditional logistic regression in CAPRISA 004 (n = 165), in FSW cohort from Nairobi (n = 41), and in the combined cohort analysis (n = 206).
Table S5. Correlation of other immunological markers on bulk $CD4^+$ T cells and $CD4^+$ T cell decline of <500 cells/μl in the CAPRISA 004 study.
Table S6. Effect of preinfection integrin β_7^{Hi} $CD4^+$ T cell frequency on postinfection plasma I-FABP and sCD14 expression using linear mixed model analysis adjusting for VL and CD4/CD8 ratio.
Table S7. Primary data (provided as an Excel file).
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Integrin $\alpha_4\beta_7$ expression on peripheral blood $CD4^+$ T cells predicts HIV acquisition and disease progression outcomes

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Indicative integrins in HIV

The gut is thought to be a major viral reservoir in HIV infection, and studies in nonhuman primates suggest that targeting the $\alpha_4\beta_7$ integrin on T cells may be a viable therapy. Sivro *et al*. now extend these findings to humans by examining HIV acquisition in multiple African cohorts. Higher frequencies of $\alpha_4\beta_7^+$ circulating $CD4^+$ T cells before infection were associated with increased HIV acquisition, viral load at set point, and more rapid $CD4^+$ T cell decline. These exciting data confirm that integrin targeting could help reduce the spread of HIV.