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Differences in Expression of Gut-homing Receptors on CD4+T cells in Black and White HIV-negative Men Who Have Sex with Men

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Summary

HIV incidence rates are higher among black MSM (BMSM) as compared to MSM of other race/ethnicities in the US. We found that blood memory CD4+ cells from BMSM express higher levels of $\alpha 4\beta 7$, the gut-homing integrin, compared to WMSM. Higher expression of $\alpha 4\beta 7$ on blood CD4+ cells correlated with higher percentage of proliferating CD4+ $\alpha 4\beta 7$ + cells in rectal tissue suggesting increased trafficking of potential HIV target cells to rectal mucosa could increase HIV susceptibility among BMSM.

Keywords

$\alpha 4\beta 7$ integrin; men who have sex with men; HIV susceptibility; HIV disparities

RESEARCH LETTER

Reasons for higher HIV incidence rates among BMSM compared to MSM of other race/ethnicities in the US are multifactorial.[1, 2] Some have suggested that differences in immunologic susceptibility could contribute to racial disparities in HIV acquisition seen

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The data were presented in part at the 19th Conference on Retroviruses and Opportunistic Infections; Seattle, Washington; March 2012.

Contributions: CFK conceived the study, recruited and enrolled human subjects, collected the specimens, analyzed the data, and wrote the manuscript. LL, CI, SK, KP performed assays and analyzed the data. MJM collaborated on the human subjects protocol and critically edited the research letter. VCM and PSS provided guidance throughout the study and critically edited the research letter. RRA oversaw the conduct of laboratory assays, provided guidance throughout the study, wrote and critically edited the research letter.

globally.[3] Therefore, we hypothesized that BMSM may have higher levels of T cell activation and HIV co-receptor expression than WMSM leading to increased HIV susceptibility by increasing target cell availability[4, 5] and undertook this pilot study to compare systemic cellular profiles between BMSM and WMSM. We recruited sequentially enrolling HIV-negative MSM from the InvolveMENT study, a longitudinal cohort of BMSM and WMSM aged 18–39 years in Atlanta, Georgia, into this substudy.[1, 2]

From the first 22 BMSM and 39 WMSM enrolled, 100 μ l of whole blood was stained with fluorescently labeled antibodies to cell surface markers CD3 (Immunotech), CD4 (BD Biosciences), CD8 (BD Biosciences), HLA-DR (Immunotech), CD38 (Immunotech), β 7 (BD Biosciences), and CCR5 (BD Biosciences) and analyzed by 4-color flow cytometry with FACSCalibur platform. CD3+CD4+ cells were analyzed for expression of CCR5 or β 7^{high} and for co-expression of HLA-DR and CD38. Previous data showed that staining the β 7^{high} monomer identifies cells positive for the heterodimer α 4 β 7.[6] CD3+CD8+ cells were analyzed for HLA-DR/CD38 co-expression. The geometric mean fluorescence intensity (GMFI), a measure of receptor density/cell, was determined for CD4+CCR5+ and CD4+ β 7^{high} cells. There were no significant differences in percentage or GMFI of CD4+CCR5+ cells or percentage of CD4+ or CD8+ cells that co-expressed HLA-DR and CD38 (Figure 1A, 1B). Interestingly, we found the median percentage of CD4+ β 7^{high} cells (median 16.0 vs. 11.6%; Mann Whitney (MW) test, $p < .0001$) and density of the β 7 integrin on CD4+ β 7^{high} cells as measured by GMFI (median GMFI 318 vs. 280; MW test; $p = .01$) was significantly higher in BMSM. There were no significant correlations between demographic (age), behavioral (number of anal intercourse partners, unprotected anal intercourse partners, condom use), or clinical factors (circumcision status, HSV-2, CMV seropositivity, presence of sexually transmitted infection) and β 7 expression on CD4+ cells.

In order to further examine the association between T cell memory subsets and co-expression of HIV co-receptors, 100 μ l of whole blood from an additional 36 BMSM and 20 WMSM was examined (Figure 1 C–E) using multi-color flow cytometry on LSRII platform with antibodies to cell surface markers CD4 (BD Biosciences), CD45RO (Biolegend), CCR7 (R&D Systems), α 4 β 7 (Act 1, NIH/NHP Reagent Resource), and CCR5 (BD Biosciences). Naïve (CCR7+CD45RO–), central memory (T_{CM} ; CCR7+, CD45RO+), and effector memory (T_{EM} ; CCR7–CD45RO+) CD4+ cells were analyzed for expression of CCR5 and/or α 4 β 7. In this subset of MSM, there were no significant differences in median percentage of total CD4+ α 4 β 7+ cells or in median percentage of CD4+ cells that co-expressed CCR5 and α 4 β 7. In both racial groups, T_{CM} and T_{EM} CD4+ cells showed significantly higher density of α 4 β 7 than naïve CD4+ cells (median GMFI $T_N=2546$, $T_{CM}=6138$, $T_{EM}=6283$; MW test, $p < .0001$) (Figure 1D). In addition, the density of α 4 β 7 remained significantly higher in BMSM for T_{CM} (median GMFI 6478 vs. 4909; MW test, $p = 0.0007$), and T_{EM} (median GMFI 6586 vs. 5243; MW test, $p = 0.01$) CD4+ cells subsets (Figure 1e).

To determine whether blood α 4 β 7 GMFI of CD4+ α 4 β 7+ cells was associated with cellular phenotypes in the rectal mucosa, the site of HIV exposure for most MSM[7], we utilized data from a separate study protocol with paired blood and rectal biopsy specimens available. This protocol enrolled 29 healthy, HIV-negative MSM and men who have sex with women

aged 18–45 to undergo peripheral blood and rectal biopsy sampling via office-based rigid sigmoidoscopy. This separate study protocol was not designed to examine racial disparities; therefore, we were unable to stratify this analysis by race. Blood was processed by Ficoll density gradient to separate PBMCs, and 5 pinch biopsies from the rectal mucosa were processed with collagenase digestion to separate mucosal mononuclear cells (MMCs).[8] PBMCs and MMCs were analyzed by flow cytometry on LSRII platform after staining with the following antibodies: CCR7 (R&D systems), $\alpha 4\beta 7$ (Act 1, NIH/NHP Reagent Resource), HLA-DR (BD Biosciences), CD45RO (Tonbo Biosciences), CCR5 (BD Biosciences), CD38 (Caltag Laboratories), CD45 (Tonbo Biosciences), CD8 (BD Biosciences), CD4 (Biolegend), Ki-67 (BD Biosciences) (Figure 1F). Live cells were identified by staining with Live/Dead Near-IR Dead Cell stain (Life Technologies). Memory CD4⁺ cells were defined by excluding CCR7⁺CD45RO⁻ cells, which constituted the majority of MMCs. We observed a significant correlation between the GMFI of $\alpha 4\beta 7$ on memory CD4⁺ $\alpha 4\beta 7$ ⁺ cells in blood and rectum (Spearman $r=0.6$; $p=0.006$). In addition, the blood $\alpha 4\beta 7$ GMFI on memory CD4⁺ $\alpha 4\beta 7$ ⁺ cells also positively correlated with the percentage of CD4⁺ memory rectal MMCs that co-expressed $\alpha 4\beta 7$ and the proliferation marker, Ki67 (Figure 1G; Spearman $r=0.41$; $p=0.03$). Also, there was a non-significant positive association between blood CD4⁺ memory $\alpha 4\beta 7$ GMFI and the percentage of CD4⁺ memory MMCs that expressed Ki67 alone (Spearman $r=0.35$; $p=0.06$) and co-expressed the three markers, $\alpha 4\beta 7$, Ki67, and CCR5 (Spearman $r=0.32$; $p=0.09$). Blood CD4⁺ memory $\alpha 4\beta 7$ GMFI was not associated with overall percentage of CD4⁺ memory MMCs that expressed CCR5, $\alpha 4\beta 7$, HLA-DR, or CD38.

Naïve and effector lymphocytes utilize the $\alpha 4\beta 7$ heterodimer to extravasate from blood into gut lymphatic tissues through interaction with MAdCAM-1.[9] Thus, the migration potential of CD4⁺ cells to colorectal mucosa can be predicted by cell surface expression of $\alpha 4\beta 7$. [10–12] In addition to its importance in lymphocyte trafficking, $\alpha 4\beta 7$ may also be critical early in the transmission event because HIV target cells that express $\alpha 4\beta 7$ may be preferentially infected [13–15] and depleted in acute HIV infection [16] and, most importantly, because blockade of $\alpha 4\beta 7$ with monoclonal antibody prior to SIV challenge resulted in a 50% reduction in SIV acquisition in rhesus macaques.[17] Taken together, our data suggest that BMSM express higher levels of $\alpha 4\beta 7$ on CD4⁺ cells, including memory CD4⁺ cells that are a primary target for HIV infection,[18] and that these differences may be associated with susceptibility to HIV infection in the rectal mucosa where nearly 70% of HIV transmissions are estimated to occur among MSM.[7] Larger, appropriately powered studies will be needed to examine this preliminary finding further and elucidate potential mechanisms.

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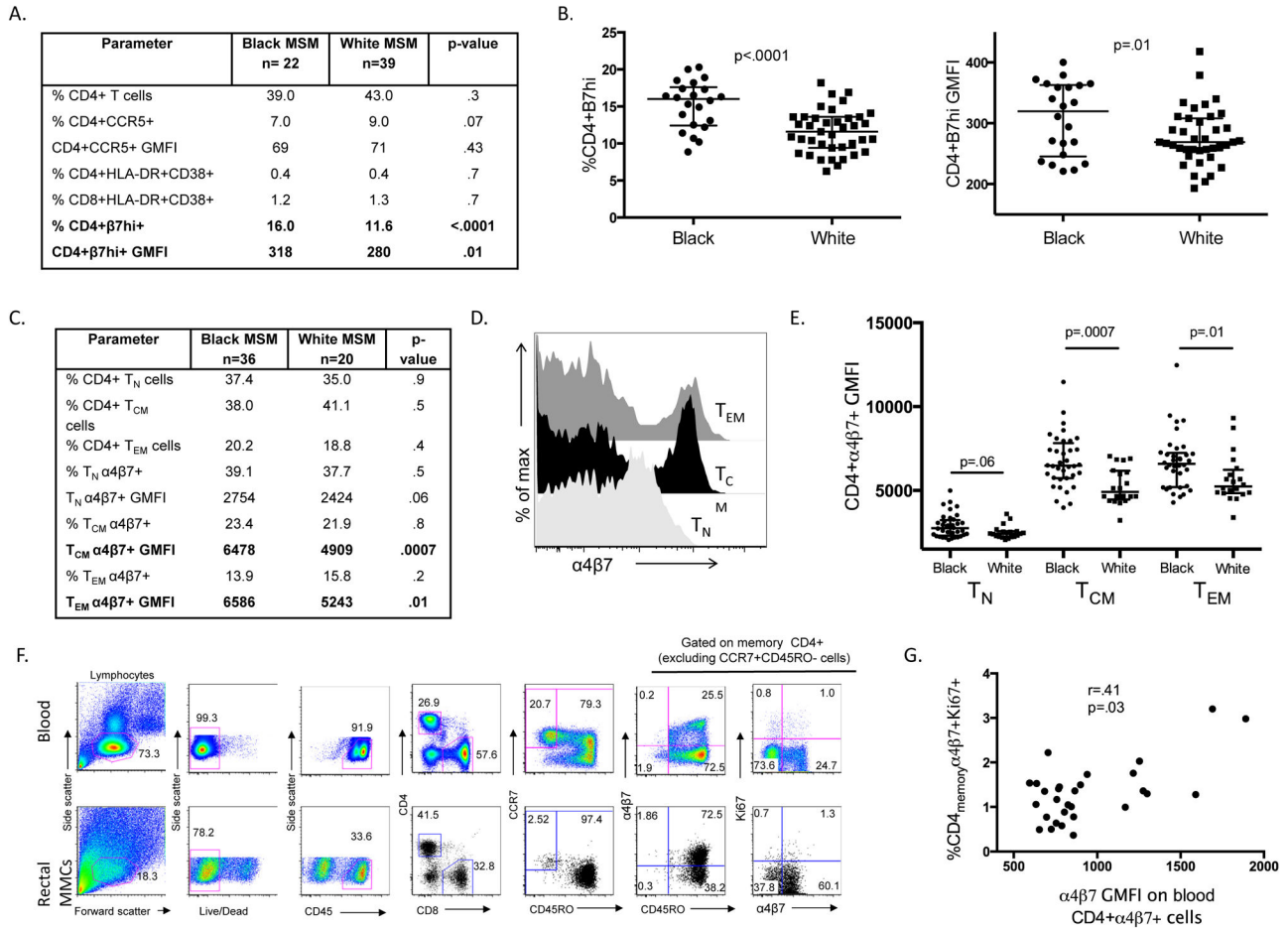


Figure.

A) Blood samples from black (n=22; circles) and white (n=39; squares) MSM were analyzed by flow cytometry. Values represent the median percentage of total T, CD4+, or CD8+ T cells expressing a given marker/s. GMFI=geometric mean fluorescence intensity of CCR5 on CD4+CCR5+ cells or β 7 on CD4+ β 7^{high} cells. B) Mann Whitney non-parametric test of medians for the percentage of CD4+ β 7^{high} cells and CD4+ β 7^{high} GMFI between black and white MSM. C) Blood samples from an additional black (n=36; circles) and white (n=20; squares) MSM were analyzed by flow cytometry. Values represent the median percentage of CD4+ T cells expressing a given marker. T_N= CD4+CCR7+CD45RO⁻, T_{CM}=CD4+CCR7+, CD45RO⁺, T_{EM}= CD4+CCR7-CD45RO⁺. GMFI=geometric mean fluorescence intensity of α 4 β 7 on CD4+ α 4 β 7+ cells. D) Representative α 4 β 7 fluorescence intensity of CD4+ T cell memory subsets. E) Mann Whitney test of median α 4 β 7 GMFI on CD4+ α 4 β 7+ T cells for black and white MSM in CD4+ T cells memory subsets. F) Representative gating strategy for blood and rectal mucosal mononuclear cells from one donor. G) Spearman correlation analysis between blood α 4 β 7 GMFI on CD4+ α 4 β 7+ T cells and the percentage of rectal CD4+ T cells that co-express α 4 β 7 and Ki67.