Differences in expression of gut-homing receptors on CD4(+) T cells in black and white HIV-negative men who have sex with men

Colleen Kelley, Emory University
Lilin Lai, Emory University
Chris Ibegbu, Emory University
Eli Rosenberg, Emory University
Surinder Kaur, Emory University
Kalpana Patel, Emory University
Mark Mulligan, Emory University
Vincent Marconi, Emory University
Patrick Sullivan, Emory University
Rama Amara, Emory University

Journal Title: AIDS
Volume: Volume 30, Number 8
Publisher: Lippincott, Williams & Wilkins | 2016-05-15, Pages 1302-1308
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1097/QAD.0000000000001062
Permanent URL: https://pid.emory.edu/ark:/25593/s2rvn

Final published version: http://dx.doi.org/10.1097/QAD.0000000000001062

Copyright information:
© 2016 Wolters Kluwer Health, Inc. All rights reserved.

Accessed September 12, 2020 7:07 PM EDT
Differences in Expression of Gut-homing Receptors on CD4+T cells in Black and White HIV-negative Men Who Have Sex with Men

Colleen F. Kelley, MD, MPH1,2, Lilin Lai, MD1, Chris Ibegbu, PhD3, Eli S. Rosenberg, PhD2, Surinder Kaur3, Kalpana Patel3, Mark J. Mulligan, MD1, Vincent C. Marconi, MD1,4, Patrick S. Sullivan, DVM, PhD2, and Rama R. Amara, PhD3

1Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine
2Department of Epidemiology, Rollins School of Public Health, Emory University
3Yerkes National Primate Research Center and the Emory Vaccine Center, Emory University, Atlanta, GA, 30329
4Hubert Department of Global Health, Rollins School of Public Health, Emory University School of Medicine

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 α4β7, the gut-homing integrin, compared to WMSM. Higher expression of α4β7 on blood CD4+ cells correlated with higher percentage of proliferating CD4+α4β7+ cells in rectal tissue suggesting increased trafficking of potential HIV target cells to rectal mucosa could increase HIV susceptibility among BMSM.

Keywords

α4β7 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
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, 100μl 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), β7 (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 β⁷⁹⁴ and for co-expression of HLA-DR and CD38. Previous data showed that staining the β⁷⁹⁴ monomer identifies cells positive for the heterodimer α₄β₇.\[6\] 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+β⁷⁹⁴ 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+β⁷⁹⁴ cells (median 16.0 vs. 11.6%; Mann Whitney (MW) test, p<.0001) and density of the β7 integrin on CD4+ β⁷⁹⁴ 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 β7 expression on CD4+ cells.

In order to further examine the association between T cell memory subsets and co-expression of HIV co-receptors, 100μl 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), α₄β₇ (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 α₄β₇. In this subset of MSM, there were no significant differences in median percentage of total CD4+ α₄β₇+ cells or in median percentage of CD4+ cells that co-expressed CCR5 and α₄β₇. In both racial groups, T_CM and T_EM CD4+ cells showed significantly higher density of α₄β₇ 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 α₄β₇ 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=.01) CD4+ cells subsets (Figure 1e).

To determine whether blood α₄β₇ GMFI of CD4+ α₄β₇+ 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), α4β7 (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 α4β7 on memory CD4+α4β7+ cells in blood and rectum (Spearman r=0.6; p=0.006). In addition, the blood α4β7 GMFI on memory CD4+α4β7+ cells also positively correlated with the percentage of CD4+ memory rectal MMCs that co-expressed α4β7 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 α4β7 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, α4β7, Ki67, and CCR5 (Spearman r=0.32; p=0.09). Blood CD4+ memory α4β7 GMFI was not associated with overall percentage of CD4+ memory MMCs that expressed CCR5, α4β7, HLA-DR, or CD38.

Naïve and effector lymphocytes utilize the α4β7 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 α4β7.[10–12] In addition to its importance in lymphocyte trafficking, α4β7 may also be critical early in the transmission event because HIV target cells that express α4β7 may be preferentially infected[13–15] and depleted in acute HIV infection[16] and, most importantly, because blockade of α4β7 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 α4β7 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.

Acknowledgments

We would like to thank the CFAR Immunology and Pre-Clinical Virology Core Laboratories for assistance in conducting assays.

Funding: This work was supported by the National Institutes of Health: KL2TR000455 (to CFK), K23AI108335 (to CFK), R01MH085600 (to PSS), R37AI112787 (to RRA), the Georgia Research Alliance (MMJ), Action Cycling Atlanta (MMJ), The Atlanta Clinical and Translational Science Institute UL1TR000454, National Center for Research Resources P51RR169, the Office of Research Infrastructure Programs/OD P51OD11107, and the Emory Center for AIDS Research P30 AI050409.
References


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 \( \beta_7 \) on CD4+\( \beta_7 \) cells. B) Mann Whitney non-parametric test of medians for the percentage of CD4+\( \beta_7 \) cells and CD4+\( \beta_7 \) 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−, \( T_C \)= CD4+CCR7+, CD45RO+, \( T_E \)= CD4+CCR7−CD45RO+. GMFI=geometric mean fluorescence intensity of \( \alpha_4\beta_7 \) on CD4+ \( \alpha_4\beta_7 \) cells. D) Representative \( \alpha_4\beta_7 \) fluorescence intensity of CD4+ T cell memory subsets. E) Mann Whitney test of median \( \alpha_4\beta_7 \) GMFI on CD4+ \( \alpha_4\beta_7 \) 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 \( \alpha_4\beta_7 \) GMFI on CD4+ \( \alpha_4\beta_7 \) T cells and the percentage of rectal CD4+ T cells that co-express \( \alpha_4\beta_7 \) and Ki67.