*Identification of the First HIV-1 p24 Peptide for the Amerindian HLA-B*39:02 Allele*

0293

Maribel Soto-Nava[1], Lucero Félix-Soto[1], Dafne Díaz-Rivera[1], Humberto Valenzuela-Ponce[1], Aldo Alejandro Arvizu Flores[2],Mariela Cervantes-Valenzuela[1], Cora Sosa-Flores[1], Xavier Flores-Andrade[1], Vanessa Dávila-Conn[1] Santiago Avila-Rios[1]

[1]Centro en Investigación en Enfermedades Infecciosas, (CIENI), Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, INER, Mexico City, Mexico [2] Departamento de Ciencias Químico Biológicas, Universidad de Sonora, Mexico.

BACKGROUND

HIV evolution is driven by immunogenetic factors, particularly HLA alleles. Our group previously demonstrated that *HLA-B*39:02 relatively common in Mexico*, has been associated with better HIV outcomes in Mexico and selected polymorphisms in p24-Gag; however, the HIV-specific responses restricted by this allele remain undefined. Here, we characterized an *HLA-B*39:02*–restricted p24 epitope that induces strong immune responses. Furthermore, competitive peptide assays revealed that polymorphisms selected by *HLA-B*39:02* alter peptide HLA binding affinity.

METHODS

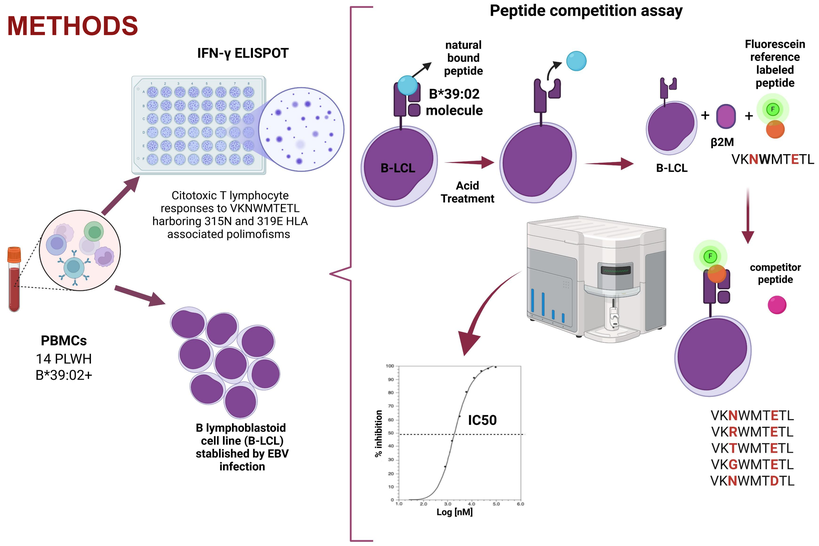


Figure 1. Competition-Based Cellular Peptide Binding Assay workflow. We enrolled 14 men living with HIV who expressed *HLA-B*39:02* and had received <60 days of antiretroviral therapy (ART). CD8 T-cell responses were measured by IFN-γ ELISpot using overlapping 9-mer p24 peptides spanning previously identified *HLA-B*39:02*-associated sites (positions 315 and 319). *HLA-B*39:02* binding affinity was measured using a competition-based peptide-binding assay with variant peptides carrying substitutions at 315 (R, T, G) and 319 (D). Positive ELISpot responses and IC50 values were compared using the Mann–Whitney test.

CONCLUSIONS

*HLA-B*39:02*, common in Mexico but rare globally, is associated with improved HIV outcomes and drives HIV adaptation in p24 Gag. We identified the first confirmed *HLA-B*39:02*-restricted p24 epitope, VKNWMTETL, which elicited strong CD8 T-cell responses. Naturally occurring variants within this epitope reduced *HLA-B*39:02* binding affinity, consistent with immune escape. These results nominate VKNWMTETL as a relevant target for vaccine and immunotherapy design in populations where *HLA-B*39:02* is prevalent.

1. Avila-Rios, S. (2009). Unique features of HLA-mediated HIV evolution in a Mexican cohort: A comparative study. Retrovirology, 6. https://doi.org/10.1186/1742-4690-6-72 **2.** Carlson, J. M. (2015). HIV-1 adaptation to HLA: A window into virus-host immune interactions. Trends in Microbiology, 23(4), 212–224. https://doi.org/10.1016/j.tim.2014.12.008 **3.** Goulder, P. J. R., & Watkins, D. I. (2008). Impact of MHC class I diversity on immune control of immunodeficiency virus replication. Nature Reviews Immunology, 8(8), 619–630. https://doi.org/10.1038/nri2357. **4.** Kessler, B.(2003). Development, validation, and application of competition-based peptide binding assays for 13 prevalent human leukocyte antigen (HLA) class I alleles. *Human Immunology*, 64(3), 245-255. doi:10.1016/S0198-8859(03)00005-5. **5**. Soto-Nava, M., (2018). Weaker HLA Footprints on HIV in the Unique and Highly Genetically Admixed Host Population of Mexico. Journal of Virology, 92(2). https://doi.org/10.1128/jvi.01128-17 **6.** Valenzuela-Ponce H (2018). Novel HLA class I associations with HIV-1 control in a unique genetically admixed population. Sci Rep. 2018. Apr 17;8(1):6111. doi: 10.1038/s41598-018-23849-7.PMID: 29666450.

RESULTS

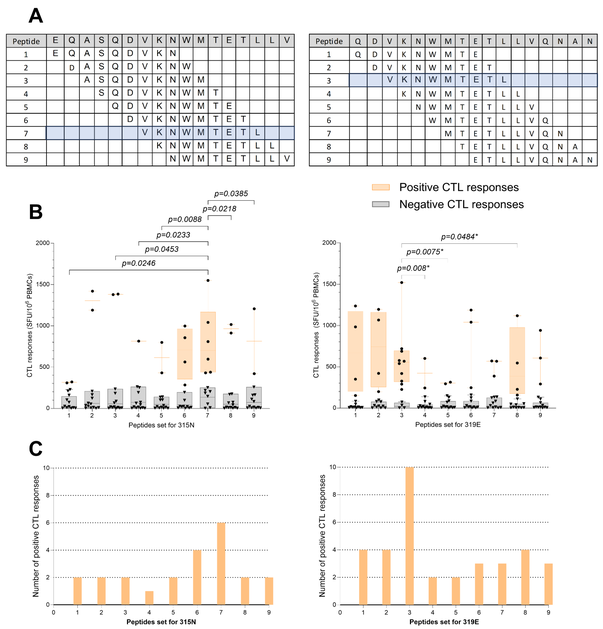


Figure 2. CTL responses to peptide sets spanning positions 315 and 319 measured by IFN-γ ELISpot. A. Overlapping 9-mer peptide sets spanning site 315 (left) and site 319 (right). **B.** Responses shown as spot-forming units (SFU) per 10^6 PBMCs. Positive responses (DFR method) are shown in orange; negative responses are shown in gray. Each point is the mean of duplicates. **C.** Number of positive responses for each peptide set.

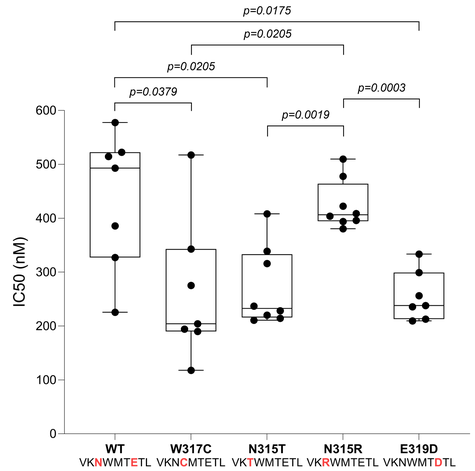


Figure 3. IC50 comparison for peptides with substitutions at positions 315 and 319. Box plots show IC50 (nM) for each competitor peptide

VKNWMTETL is a small piece of the HIV that is shown to the immune system by a genetic variant called *HLA-B*39:02*. This variant is common in Mexico and Central America and may help design better vaccines or immune therapies.

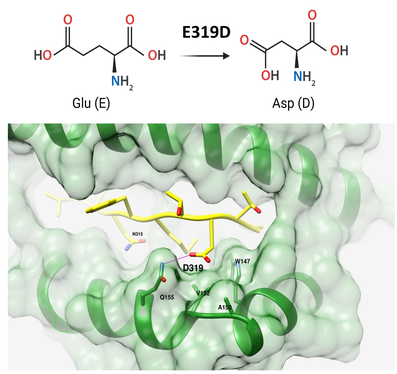


Figure 4. Effect of the 319 substitution on VKNWMTETL binding to HLA-B*39:02. Glu (E) →Asp (D) substitution at 319 position has a R group smaller and less flexible. The R group allows the orientation of Asp319 to α2-helix Gln (Q)155 which is more hydrophobic.

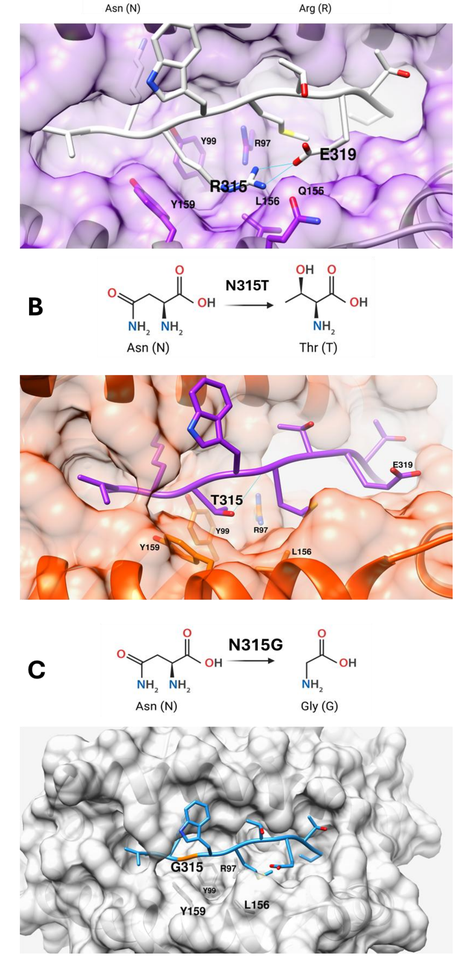


Figure 5. Predicted structural effects of position-315 variants on peptide binding to *HLA-B*39:02*. A. Asn (N) → Arg (R) substitution at position 315 alters side-chain orientation, disrupting the hydrogen bond with R97 and forming a new interaction with E319.

B. Asn (N) →Thr (T) substitution at position 315 disrupts the hydrogen bond with R97 but preserves interaction with Y99 and forms a new backbone hydrogen bond.

C. Asn (N) →Gly (G) substitution at position 315 abolishes the hydrogen bond with R97 and reduces peptide affinity for HLA-B39:02; water-mediated interactions would be thermodynamically unfavorable.All models were generated using AlphaFold3.

Contact: maribel.soto.nava@gmail.com