Inhibition of MHC Class II CLIP Binding by HLA Gene Editing

Edward Prage[1], Vibha Jha[1], Saghar Kaabinejadian[2], Hooman Yari[2], William Hildebrand[2], Brian Freed[1], Christina Roark[1]
[1]ClinImmune The Center for Clinical Immunology, Department of Medicine, University of Colorado, Aurora, CO 80045 USA
[2]Dept of Microbiology and Immunology, College of Medicine, University of Oklahoma Health Campus, Oklahoma City, OK 73104 USA

Introduction

The DRB1 locus of the major histocompatibility complex (MHC) class II is associated with increased risk of autoimmune disease. Specifically, DRβ1*04:01 is associated with rheumatoid arthritis susceptibility, due to peptide presentation to CD4+ T cells.

Previously, we have shown that a single amino acid change in DRβ1*04:01 blocks collagen peptide binding and presentation, but increases binding of native vimentin. We therefore investigated other HLA gene edits in an attempt to decrease all high affinity peptide binding.

Here, we investigate the effect on class II-associated invariant chain peptide (CLIP) binding by introducing single amino acid changes at positions 71 (pocket 4), 86 (pocket 1), and 82 (pocket 2) of DRβ1*04:01.

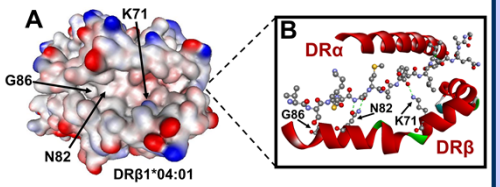


Figure 1. A) Structural model of DRβ1*04:01, highlighting the locations of mutated positions within the peptide binding groove. B) The interaction of K71, G86, and N82 with CLIP, shown as ball-and-stick, with the DRα and DRβ subunits represented by red ribbons. Hydrogen bonds are illustrated as green dashes.

Asparagine-82 Is Conserved

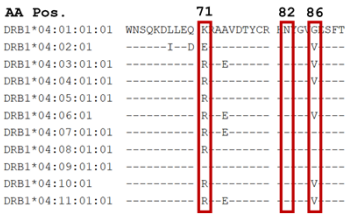


Figure 2. Amino acid sequence alignment of DR4 alleles. While there is variability within positions 71 and 86, position 82 is conserved.

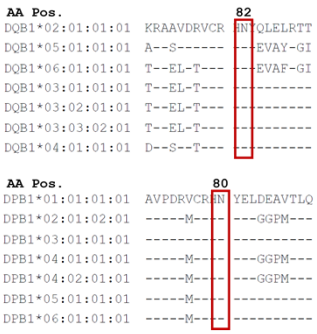


Figure 3. Amino acid sequence alignment of DQB1 and DPB1 alleles. Asparagine at position 82 (position 80 for DPB1) is conserved in HLA class II.

Methods

Peptide identification: T2 cell lines expressing DRβ1*04:01, DRβ1*04:01^{K71E}, DRβ1*04:01^{G86L}, or DRβ1*04:01^{N82L}, in the absence of other MHC class II molecules, were expanded in culture and snap frozen in LN2. Frozen cells were pulverized, resuspended in lysis buffer, and cleared by ultracentrifugation. DRβ1 molecules were then extracted and purified by affinity chromatography. The resulting mixture of peptides and DRβ1 molecules was separated by HPLC and applied to tandem mass spectrometry (MS/MS). Database searching was then performed to identify peptide sequences.

Peptide binding: Experiments were performed utilizing the same cell lines as above, harvested in log-phase growth. Cells were incubated overnight with biotinylated peptides, washed, and lightly fixed with formaldehyde to prevent loss of bound peptide. The resulting bound peptides were conjugated with PE-labeled streptavidin and analyzed by flow cytometry.

CLIP Peptide Discovery

Initial MS/MS data show overlapping, yet distinct, peptide repertoires for DRβ1*04:01, DRβ1*04:01^{K71E}, DRβ1*04:01^{G86L}, and DRβ1*04:01^{N82L}. Of particular interest, the N82L mutation appears to completely abrogate the binding of the immunodominant peptide of CLIP (residues 86-100, PVSKMRMATPLLMQA), while all other DRβ1*04:01 variants maintain binding.



Figure 4. CLIP peptides discovered by MS/MS. Partial sequence of CLIP, centering on residues 86-100, is shown in grey highlight, with peptides discovered by MS/MS represented by the blue bars.

DRβ1*04:01^{N82L} Does Not Bind CLIP

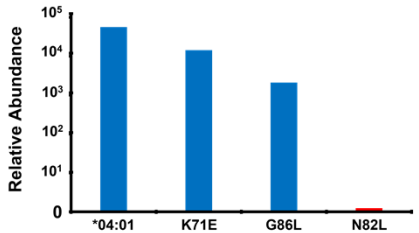


Figure 5. Relative abundance of CLIP peptides, as discovered by MS/MS. Bound CLIP peptides containing residues 86-100 were eluted from DRβ1*04:01, DRβ1*04:01^{K71E}, and DRβ1*04:01^{G86L}, while none were eluted from DRβ1*04:01^{N82L}.

N82L Blocks Peptide Binding

The MS/MS data are corroborated by flow cytometric peptide binding experiments, showing that DRβ1*04:01^{K71E} and DRβ1*04:01^{G86L} bind CLIP$^{86-100}$ similarly to DRβ1*04:01, while DRβ1*04:01^{N82L} does not bind. In addition, the N82L gene edit appears to prevent binding of other high affinity peptides of DRβ1*04:01 variants, including immunodominant peptides from influenza HA$^{306-381}$, collagen$^{258-272}$, and native vimentin$^{66-78}$.

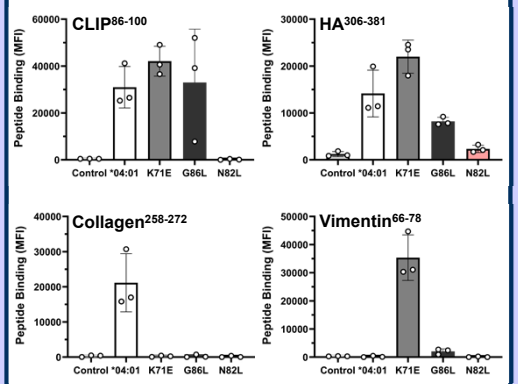


Figure 6. Binding of high affinity peptides. T2 cells that do not express MHC class II were used as a negative control. DRβ1*04:01^{N82L} does not bind CLIP$^{86-100}$ while wild-type DRβ1*04:01 and the K71E and G86L variants do. Binding of HA$^{306-381}$ is substantially diminished for the N82L mutant. While DRβ1*04:01 binds collagen$^{258-272}$, the other variants do not. DRβ1*04:01^{K71E} binds native vimentin$^{66-78}$ while the other variants display little-to-no binding.

Conclusion

This study highlights the influence of single amino acid residues, with respect to peptide binding by DRβ1*04:01. The DRβ1*04:01^{N82L} mutant displays unique binding affinity, as compared to DRβ1*04:01, DRβ1*04:01^{K71E}, and DRβ1*04:01^{G86L}. The inability of DRβ1*04:01^{N82L} to bind CLIP and other high affinity peptides suggests an important role that the conserved asparagine at position 82 plays in the composition of the peptide repertoire of MHC class II proteins. Future MS/MS and peptide-binding experiments will be aimed at elucidating the role of position 82 in the peptide binding domain of MHC class II.

Future Directions

Initial peptidomics trials by MS/MS have revealed a distinct, but partially overlapping, peptide repertoire of DRβ1*04:01^{N82L} as compared to DRβ1*04:01. Future experiments will use MS/MS to verify the identity of these unique and shared peptides. The flow cytometric peptide binding assay will then be used to investigate binding affinity to DRβ1*04:01, DRβ1*04:01^{N82L}, and other variants.

Worth noting, the T2 cell line used in the above described experiments do not express HLA-DM, a molecule that binds MHC class II, promotes release of bound CLIP, and facilitates the loading of antigen-derived peptides for presentation to CD4+ T cells. In order to further investigate the role HLA-DM plays in the binding and release of CLIP from DRβ1*04:01, we will design new experiments using cell lines that express HLA-DM along with variants of DRβ1*04:01.