Using HDX-MS to Map the Epitope of Anti-HLA-B*08:01 Antibodies

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Abstract

The HLA Epitope Registry[1] provides a database for defined HLA epitopes, including a proposed shared 66IF+163TEW epitope for HLA-B*08:01 and HLA-B*59:01. However, the molecular basis of this proposed epitope remains incompletely defined. To investigate differences in antibody recognition of these alleles, we characterized two monoclonal antibodies with overlapping reactivity: mAb-1 bound mainly to HLA-B*08:01, whereas mAb-2 bound to both HLA-B*08:01 and HLA-B*59:01. Binding was evaluated by single-antigen bead assay and flow cytometry, and antibody-HLA interaction footprints were mapped by hydrogen-deuterium exchange mass spectrometry (HDX-MS), with pull-down assays used to assess antigen recovery. HDX-MS revealed distinct protection patterns: mAb-1 primarily shielded residues 36–67, whereas mAb-2 showed a more discrete footprint involving residues 36–38, 62–67, and 161–167, with different time-dependent exchange behavior. Only one antibody showed relatively strong recovery of HLA-B*08:01 in pull-down experiments. These findings demonstrate that antibodies with overlapping serologic reactivity can engage HLA-B*08:01 through distinct molecular interactions and support HDX-MS as a useful approach for resolving antibody-HLA binding mechanisms.

Materials and methods

Antibody characterization

Antibody binding was evaluated using the LABScreen™ single antigen bead (SAB) assay and confirmed by flow cytometry with recombinant cells expressing HLA-B*08:01 or HLA-B*59:01.

HDX-MS

For HDX-MS, 3.6 µg of antigen and 26 antibody µg of mAb-1 or 13 µg of mAb-2 were mixed, exposed to D_2O at multiple time points, and then quenched under denaturing conditions. Regions involved in binding show reduced deuterium uptake, indicating that they are more shielded by the antibody. Samples were separated on an UltiMate™ 3000 HPLC system and analyzed on an Orbitrap™ Elite mass spectrometer. Peptide mapping data was processed and were analyzed using HDExaminer™ v2.5.1 software.

Pull-down assay

To assess binding differences, both antibodies were tested using *in vitro* pull-down experiments with donor-derived HLA class I antigen preparations using protein A/G magnetic beads.

Results

mAb-1 and mAb-2 showed overlapping but distinct HDX-MS interaction footprints on HLA-B*08:01. Both antibodies showed protection within the **36-67** region, with mAb-1 showing broader protection and mAb-2 showing a more discrete protection pattern. mAb-2 showed additional protection at residues **161-167**, along with different time-dependent deuterium-exchange behavior compared with mAb-1. Another difference between the antibodies was observed at residues **181-201**, where mAb-2 showed higher deuterium exchange than mAb-1, suggesting altered local dynamics consistent with a binding-induced conformational change in HLA-B*08:01. Consistent with these distinct interaction profiles, mAb-2 showed stronger HLA-B*08:01 recovery than mAb-1 in the pull-down assay.

Figure 1. Single-antigen bead assay results for (A) mAb-1 and (B) mAb-2. Both antibodies bound to HLA-B*08:01, whereas mAb-2 also showed strong binding to HLA-B*59:01.

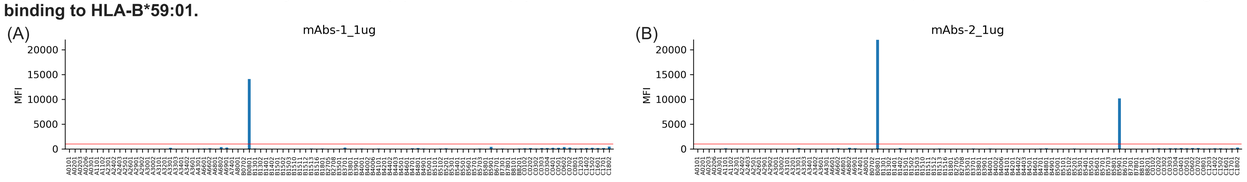


Figure 2. HDX-MS maps of HLA-B*08:01 bound by mAb-1 or mAb-2 after labeling in D_2O for 30, 100, 1,000, and 10,000 s. Colors indicate the extent of deuterium uptake: blue represents lower uptake (greater shielding), and red represents higher uptake (less shielding). Regions with lower uptake are interpreted as sites involved in antibody binding or regions affected by binding. Residue numbering shown in the figure uses full-length precursor numbering, including the 24-amino acid signal peptide.

(A) mAb-1/HLA-B*08:01, showing reduced exchange mainly in residues **36-67** (**60-91** in the figure where numbering includes the 24-amino acid signal peptide).

(B) mAb-2/HLA-B*08:01, showing reduced exchange in residues **36-38**, **62-67**, and **161-167** (**60–62**, **86–91**, and **185–191** in the figure). mAb-2/HLA-B*08:01 shows higher exchange in residues **181-201** (**205-225** in the figure) compared to mAb-1/HLA-B*08:01.

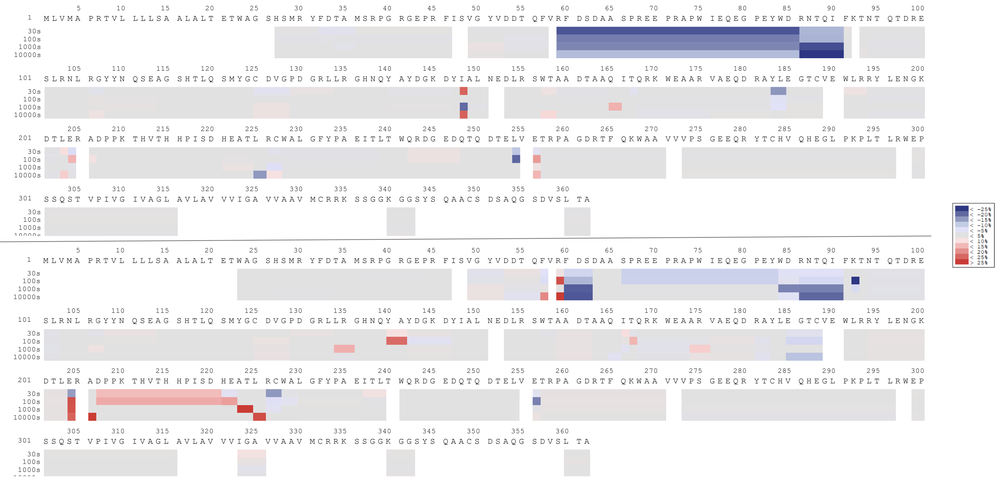


Figure 3. Structural visualization of the HLA-B*08:01 residues identified by HDX-MS as part of the antibody interaction footprint along with their sequence alignment.

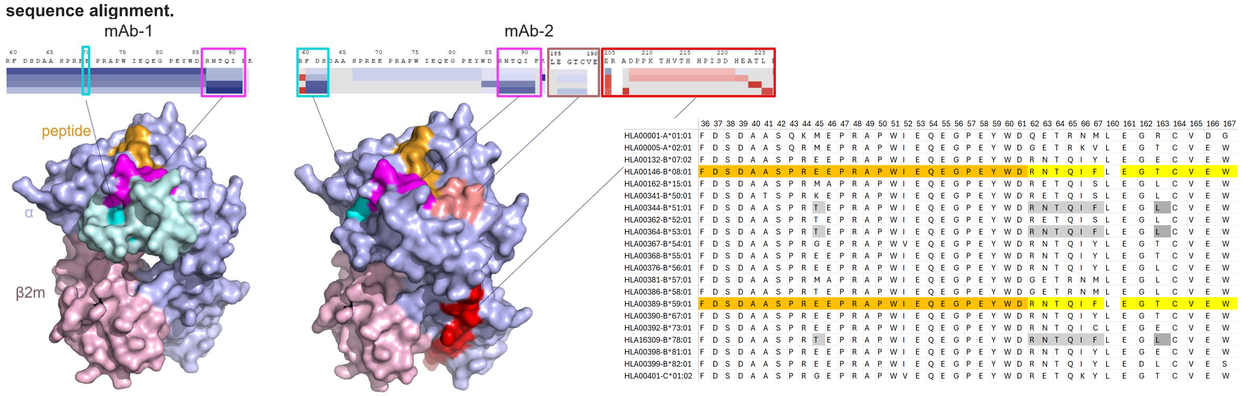
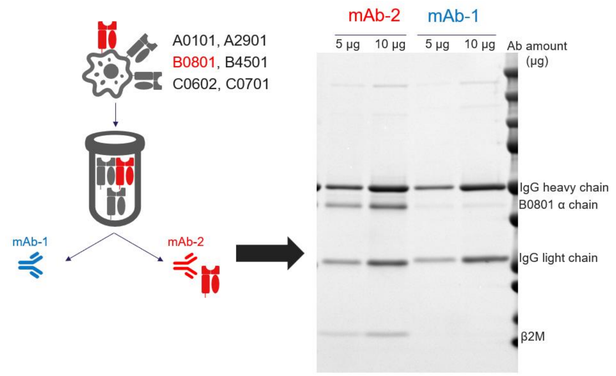


Figure 4. Pull-down assay of HLA-B*08:01 from donor-derived HLA class I antigen preparations. Donor HLA typing was A*01:01, A*29:01, B*08:01, B*45:01, C*06:02, and C*07:01. Either 5 or 10 µg of mAb-1 or mAb-2 was incubated with HLA class I antigens, and antibody-antigen complexes were isolated using Protein A/G magnetic beads. Pulldown antigens were analyzed using SDS-PAGE.



Conclusions

Although both mAb-1 and mAb-2 antibodies bound to HLA-B*08:01, HDX-MS identified distinct molecular interaction profiles. These findings highlight the complexity of antibody-HLA interactions and support the utility of HDX-MS in distinguishing antibodies with similar reactivity patterns but different binding mechanisms.

References

1. Duquesnoy, R. J., M. Marrari, L. C. D. da M Sousa, et al. “16th IHIW: A Website for Antibody-Defined HLA Epitope Registry.” International Journal of Immunogenetics 40, no. 1 (2013): 54–59. https://doi.org/10.1111/iji.12017.

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