DECIPHERING HLA-DQ ALLO-IMMUNOGENICITY IN TRANSPLANTATION BY SYSTEMATIC EMPIRICAL AND IN-SILICO ANALYSIS

Evangelyn Grace Matias[1], Desiree Elaine Austin[1], Karime França[1], Joey Scott Lockhart[1], Connor William Lantz[1], Chelsea Maguire[1], Anat R. Tambur[1]

Context

- HLA-DQ antibodies are strongly associated with the generation of de novo donor specific antibodies (dnDSA) and antibody mediated rejection (ABMR) leading to decreased allograft survival.[1]
- Immunologic risk of recipient/donor HLA-mismatch using computational approaches have limitations as they assign same weight to all mismatches.[2]
  - We performed Adsorption/Elution studies on sera from the time of first dnDSA detection to identify those mismatches that guide first humoral response, thus demonstrating higher immunogenic barrier.

Key Findings

- Not all amino acid mismatches contribute equally to the generation of HLA-DQ de novo DSA.
- In-silico antibody recognition patterns from the results of the single antigen bead assay, followed by adsorption studies, validate critical regions of interest that contribute to antibody specificity.
- Immunogenic risk is increased with HLA-DQα*05-heterodimer mismatches due to an increased number of potential immunogenic targets.

Study Design

Cohort selection

All SAB testing indicating presence of HLA-class II antibodies performed between 1/1/2012-12/31/2022
Number of samples = 3219

↓

First transplant, Kidney only, transplant recipients
N = 1463

Excluding:
- Recipients of transplant outside of NU
- Recipients of multi-organ transplantation
- Recipients of repeat transplant

↓

Presence of HLA typing with at least 2-field resolution for DRB1/DQA1/DQB1 (or DNA that was used for study-required typing for both recipient and donor
N = 343

Excluding:
- Recipients without relevant high-resolution typing, or absence of DNA to perform required typing for both patient and donor

↓

Absence of any preformed HLA class II antibodies pre-transplant (at least 2 samples)
N = 178

Excluding:
- Recipients with HLA-DQ matched donor
- Recipients with any pre-formed HLA class II antibodies prior to transplant

↓

Generation of HLA-DQ dnDSA with at least moderate level, persisting in more than one test
N = 130

Excluding:
- Recipients with potential memory responses (DSA detected < 12 weeks post-transplant)
- Recipients with dnDSA to loci other than HLA-DQ

↓

Sufficient serum volume
Enrolled in Study
Number of recipients = 97

Excluding:
- Recipients without sufficient left-over serum samples to perform adsorption studies

Final Cohort:

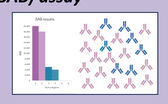
- 47.4 ± 17.8 years old
- 55.7% male
- 39% Caucasian
  28% Hispanic
  26% African American
  7% "Other"
- 51.5% deceased donor kidney
- 78.4% Induction: Alemtuzumab
- Maintenance:
  Calcineurin inhibitor,
  Mycophenolate mofetil,
  Prednisone

Study optimization

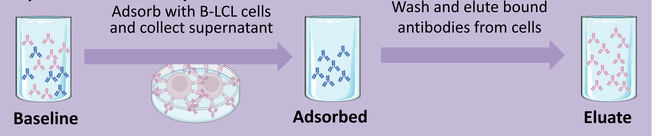
- Adsorption using homozygous B-lymphoblastoid (B-LCL) cells expressing HLA-DQ antigens, instead of single antigen beads coated with a single HLA-DQ antigen ("single single" beads)
- Dilution of the serum to determine the baseline antibody titer and optimize the adsorption of the target antibodies
- Testing different concentrations of cells used for adsorption
- Testing different incubation times on the amount of antibodies eluted
- Effect of the number of washes during elution
- Effect of an additional adsorption step (single vs double adsorption)

Methods

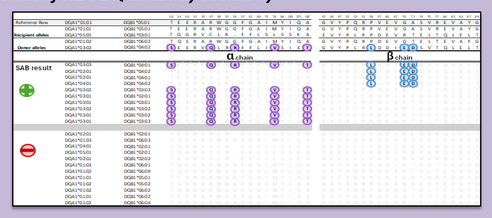
Baseline single antigen bead (SAB) assay



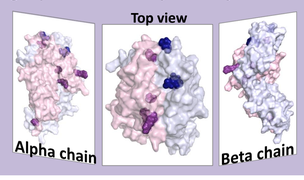
Adsorption and elution experiment



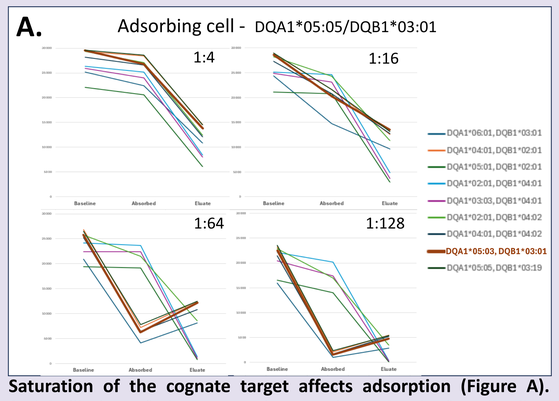
Contextualization of HLA-DQ antibody reactivity



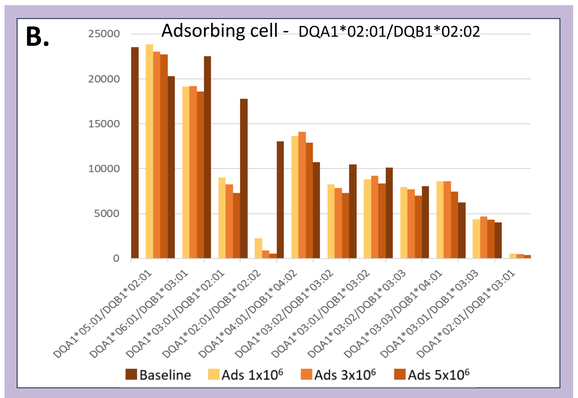
Visualization of ROIs using AlphaFold2 and PyMOL softwares



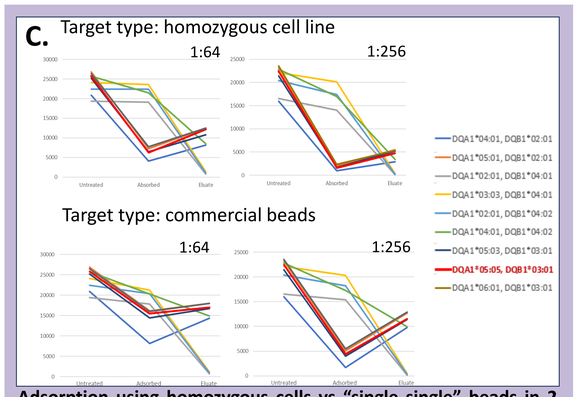
Results



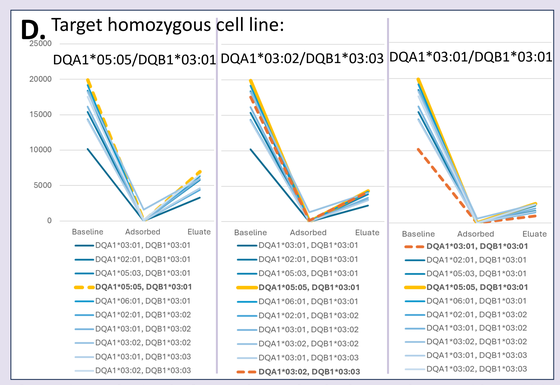
Saturation of the cognate target affects adsorption (Figure A). Serial dilution of the serum decreased the level of antibodies, overcoming the saturation of the cognate targets, thereby allowing full adsorption of antibodies with specificity to DQA1*05:05/DQB1*03:01 (maroon).



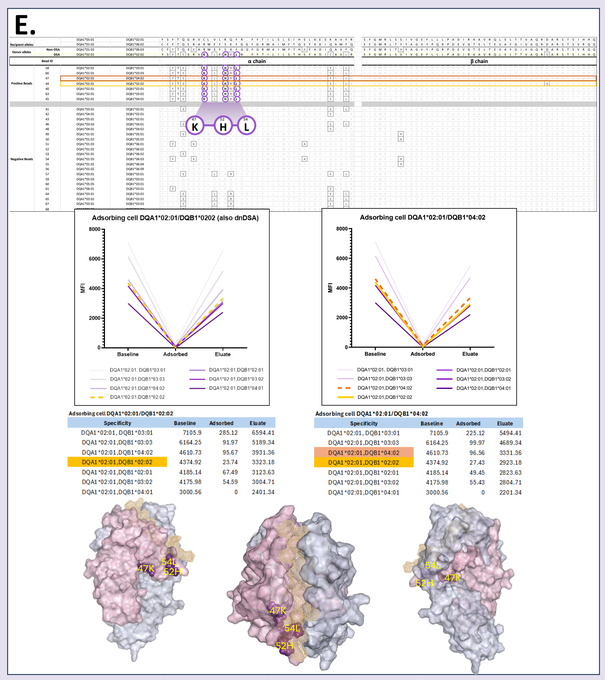
Adsorption of antibodies using different number of target cells (Figure B). Incubation of the serum with 3 different concentrations of the target homozygous cell lines showing minimal changes in MFI during adsorption.



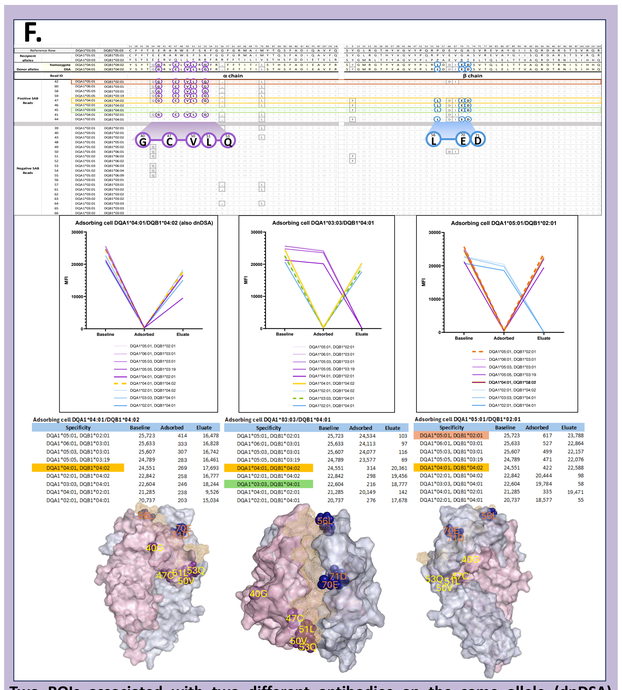
Adsorption using homozygous cells vs "single single" beads in 2 serum concentrations (Figure C). A more complete adsorption was observed using a homogyzous cell line (upper panel) potentially due to a more physiologic 3-dimensional expression of the HLA-DQ molecule.



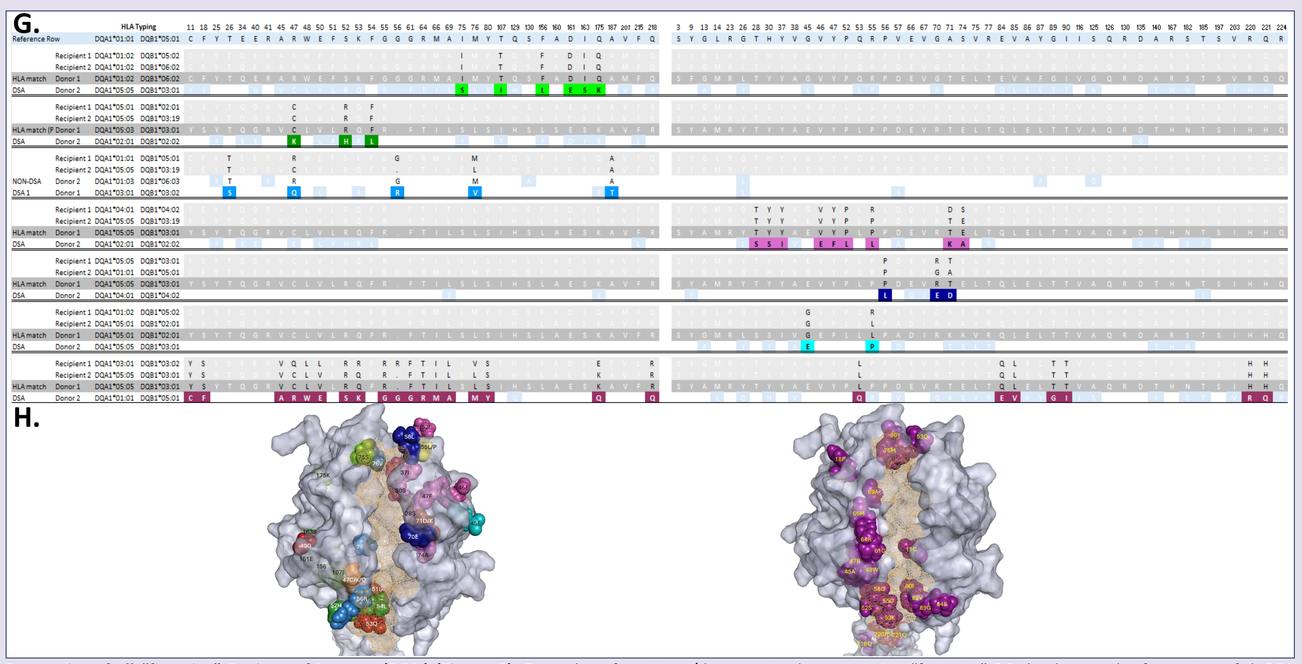
Information obtained from the Adsorbed sample provides critical information required to assess the level of antibody binding to the target (Figure D). While all antibodies were adsorbed by the 3 homozygous cells, not all antibodies are eluted, which may be interpreted as partial adsorption, while the Adsorbed sample demonstrates clear complete adsorption.



One ROI associated with a single dnDSA (Figure E). Antibody recognition pattern showing one ROI on the α chain. Adsorption using DQA1*02:01/DQB1*02:02 cells (dnDSA), and DQA1*02:01/ DQB1*04:02 cells, showed complete adsorption as both express the α-ROI. In silico modeling of the α-ROIs in relation with the peptide binding groove.



Two ROIs associated with two different antibodies on the same allele (dnDSA) (Figure F). Antibody recognition pattern showing two ROIs, one on the α-chain and one on the β-chain. Adsorption using DQA1*04:01/DQB1*04:02 showed complete adsorption, however, using cells that have only one of the ROIs demonstrated adsorption of only some of the antibodies (different patterns for the different cells). In silico analysis showing the location of the two α-ROIs on the surface of the HLA-DQ molecule.



Repertoire of all "first-tier" Regions of Interest (ROIs) (Figure G). Examples of recipient/donor pairs demonstrating "first-tier" ROI leading to the formation of dnDSA analyzed from the first serum sample documenting the humoral response. Since many amino acid sequences are inherited in linkage disequilibrium, it is not clear whether all amino acids demonstrated here are indeed critical to determine the specificity of the dnDSA, although all other amino acid mismatches highlighted in light blue are definitely not required. This demonstrates that some amino acid mismatches are more immunogenic than others. The last patient presented seem to have a very elaborate ROI, but this is likely due to the fact that all these amino acids travel in linkage and the more critical mismatches cannot be deciphered by adsorption/elution studies (likely requiring site-directed mutagenesis approach).
Visualization of ROIs on an HLA-DQ molecule (Figure H). Left depiction corresponds to the top 6 ROIs from the table; Right depiction corresponds to the bottom ROIs.

[1] Comprehensive Transplant Center, Department of Surgery, Northwestern University, Chicago, IL, USA

Funding: NIHR01AI170728-01A1 (Tambur)

References:
[1] Isaacson D, S.J., Gmeiner MW, Copley HC, Kosmoliaptsis V, Tambur AR, *HLA-DQ Mismatches Lead to More Unacceptable Antigens, Greater Sensitization, and Increased Disparities in Repeat Transplant Candidates.* Journal of the American Society of Nephrology, 2022. **33**: p. 2293-2305.
[2] Saleem N, D.R., Tambur AR, *Molecular Histocompatibility Beyond Tears: The Next Generation Version.* Human Immunology, 2022. **83**: p. 233-240.