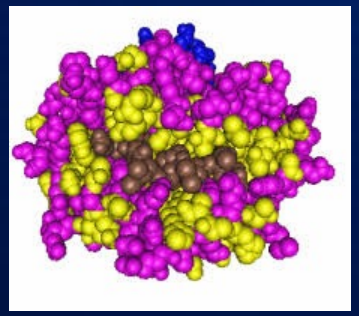
Epitope Matching - The MUST Addition in Donor Selection Algorithm



The 30th International Congress of The Transplantation Society (TTS 2024)

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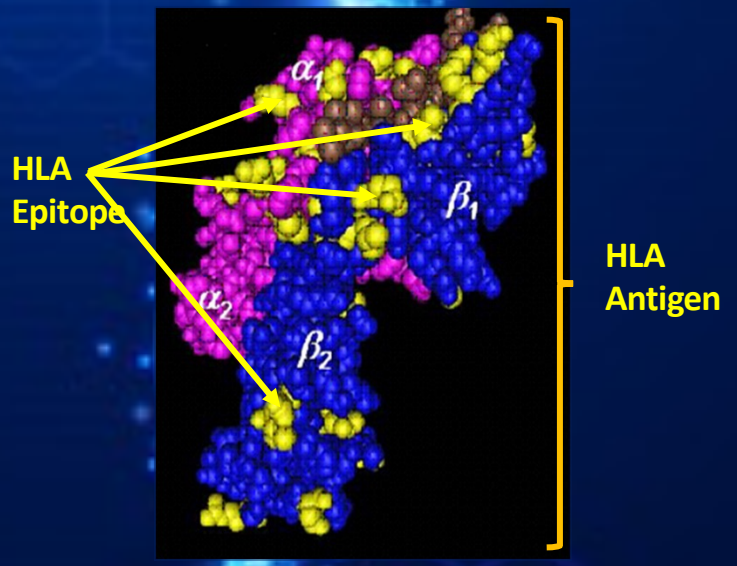
Introduction

- Concerns
  - Chronic rejection accounts for 63% of graft failure after the first year post-transplant
- Limitations with Current donor selection algorithm
  - Fails in predicting *de novo* DSA formation - catalyst for chronic rejection
  - Antibodies are not specific for an HLA antigen but for an epitope present on the HLA molecule
  - Epitopes - exposed amino acid(s) at the surface of the HLA molecule, accessible for antibody binding

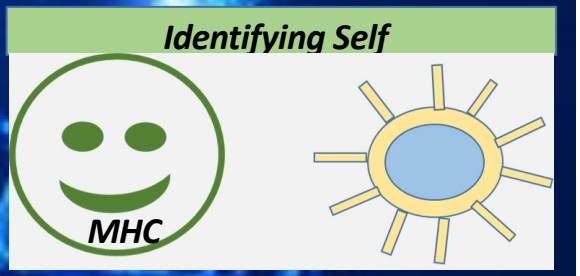


HLA chain (pink) with β2-microglobulin (blue)showing epitope or eplts in yellow color

Epitopes can be private (present on a single HLA) or public (shared by multiple HLA antigens)

Materials, Methods and Ethics Approval

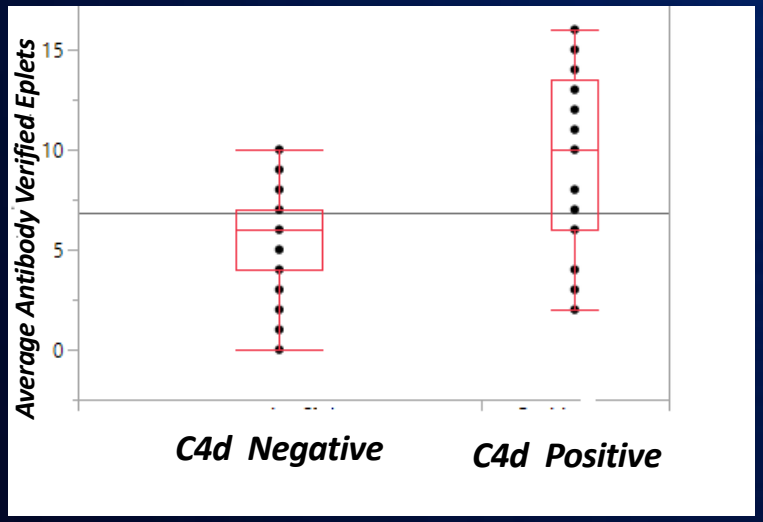
- **HLA Typing**
  - HLA typing using low resolution and high resolution PCR
- **HLAMatchmaker**
  - To determine non-self eplet by imputing 04 digit typing to the software
- **Epitope registry**
  - Non-self eplets Database of theoretical and confirmed HLA eplets recognizable by B-Cell receptors. Epitopes that are verified experimentally with specific antibodies
- **Genepop & Arlequin**
  - To calculate molecular diversity indices, including allele and haplotype frequencies
- **Ethics Approval**
  - Approved by Deakin University Human Research Ethics Committee Ref No: 2018-330



*Algorithm for assessing donor: recipient HLA compatibility at the level of structurally defined HLA targets called '**eplets**'.*
Antibody-verified eplets are eplets that have been verified by human monoclonal HLA antibodies

Results

Study Of Eplet Mismatched Loads Against Biopsy Findings



In C4d negative (n=62) patients there were an *average* 5.58 eplets, while in C4d positive (n=29) patients there is an *average* of 9.55 eplets. p <0.0001

Study Of HLA Haplotype Frequency In Indian Population

Results are only shown for polymorphic loci

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het
1	31670	22	0.86836	0.87554
2	31844	38	0.89813	0.91797
3	31844	18	0.83934	0.86215
Mean	31786.000	26.000	0.86861	0.88522
s.d.	100.459	10.583	0.02939	0.02914

Molecular diversity for HLA-A,B,DRB1 alleles (n=16900) for 13 states of India using Arlequin shows standard deviation of 0.020 indicating high polymorphism

Number of epiopte mis-matches between donor and recipient 'the predictor of transplant outcomes'

Practical Application: Case Study

R/D	HLA A	HLA A	HLAB	HLAB	HLA DRB1	HLA DRB1	HLA DQB1	HLA DQB1
Recipient Daughter	02:01	30:01	38:01	51:01	03:01	15:01	02:01	30:01
Donor Mother	01:01 (MFI 584)	30:01	51:01	57:01 (MFI 245)	03:01	10:01 (MFI 764)	01:01 (MFI 584)	30:01

SAB profile Class I	MFI
B*15:02	19203
B*15:12	18978.5
B*50:01	18921
B*27:08	18647
B*15:01	18577
B*07:02	18224

SAB profile Class II	MFI
DRB1*16:02	6618
DRB1*01:02	2330
DRB1*13:01	2119
DRB1*01:01	1252
DRB1*16:02	6618
DRB1*01:02	2330

No Donor Specific antibodies

↓

Transplant Done

MFI for Single bead antigen assay is exhibited against corresponding donor antigen in light green color and shows negative expression for donor specific antigens

Practical Application: Case Study

Haplotmatched & No donor specific antibodies

Rejection

Why?

Epitope sharing between donor specific antigens and antigens against which recipient pre-formed antibodies

- B*15:02 shares epitope (44RMA) with donor specific B*57:01 antigen showing MFI 19203
- B*07:02 shares epitope (69AA) with donor specific B*57:01 antigen showing MFI 18224
- DRB1*01:02, shares epitope (13FE) with donor specific DRB1*10:01 antigen showing MFI 2330

HLA antibodies are primary cause of transplant rejection; they recognize epitopes that can be structurally defined

Understating of Structural characteristics of epitope is the new beginning.....

Conclusion

- Number of antibody verified eplets correlated with biopsy findings forC4D analysis
- HLA is highly polymorphic in India
- Epitope matching can identify 'unacceptable mismatches' which may lead to rejection and de novo donor specific development.
- Therefore , epitope matching may be an effective tool for reducing chronic antibody mediated rejection and improving long term graft outcomes.

'Epitope matching' the MUST addition in donor selection algorithms

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