

High Resolution Analysis of Renal Allograft rejection – HLA specific antibodies

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Aim of this study

To analyse the HLA mismatch and alloantibody specificity at the highest available resolution in 97 consecutive renal transplant recipients who lost their allograft to rejection over ten years at a single centre

The results for the HLA and eplet mismatch analysis and its relationship to the HLA specific antibody associated with the renal allograft loss is presented

Methods

We used nucleotide sequencing to define the HLA-A*, -B*, -C*, -DRB1*, -DRB3*, -DRB4*, -DRB5*, -DQB1* and -DPB1* alleles at the two field level. The HLA-DQA1* and -DPA1* specificities were similarly defined using high resolution PCR-SSP

The high resolution HLA mismatch between donor and recipient was analysed using the HLA Matchmaker programme. This defines eplets as spatially defined amino-acid motifs representing likely epitopes or partial epitopes to which alloantibody can bind

The antibodies associated with rejection of the renal allograft were analysed using single antigen beads and the specificity was correlated with the eplet mismatch

Results 1 – HR TYPING

The high resolution HLA typing revealed 51 “rare” or unexpected alleles at HLA-A, 56 at HLA-B and 21 at HLA-C

For HLA-DRB1* there were 85 “rare” alleles which was significantly higher than for the HLA-A locus (Chi^2 . 9.7 $p < 0.002$), the HLA-B locus (Chi^2 6.8 $p < 0.009$) and the HLA-C locus (Chi^2 43 $p < 0.00001$).

This resulted in 47% of the donor recipient pairs having unrecognised mismatches at the HLA-A*, -B* and -DRB1* loci alone

Results 2 – “Rare” allele eplet mismatches

	HLA-A*	HLA-B*	HLA-C*	HLA-DRB1*
Number of “rare” alleles	51 (13.1%)	56 (14.4%)	21 (5.4%)	85 (21.9%)
Eplet changes	40	13	0	119
Eplet changes per allele	78% (p<0.001)	23%	0	140%

Although the number of mismatched alleles was similar between HLA-A and B there were significantly more eplet mismatches at HLA-A ($p < 0.001$)

The higher number of “rare” alleles detected at the DRB1 locus resulted in significantly more potential eplet changes than the HLA-A locus ($\chi^2 4.7$ $p = 0.03$) with more eplet changes than allele changes

HLA Class I specific antibody response

Class I Ab. responders	N = 73			Antibody response		
HLA-A HR MM 86	HLA-B HR MM 94	HLA-C HR MM 90		Consistent HLA-A eplet	Consistent HLA-B eplet	Consistent HLA-C eplet
Eplet MM Total 622	Eplet MM Total 526	Eplet MM Total 426		Total 453	Total 291	Total 200
<u>Eplets per MM 7.3</u>	<u>Eplets per MM 6</u>	<u>Eplets per MM 5</u>		70% of possible eplets	56% of possible Eplets	44% of possible eplets
P<0.001				P<0.001	Chi sq. 19	

Story so far

>50% of the donor recipient pairs had mismatches not detected by standard HLA typing

There were significantly more eplet mismatches per allele at HLA-A than –B

This resulted in “more” antibody response to HLA-A as analysed by consistent eplet

HLA-A has more repetitive eplets than HLA-B (e.g. HLA-A1 & -A36)?

HLA-B polymorphism is focussed on the groove – recognised more effectively by T cells than antibody. HLA-B has had more selection pressure by pathogens (e.g. HIV controller study)?

What about HLA Class II?

Mismatch	N = 81					
<u>HLA MM</u>	DRB1	DRB3/4/5	DQA1	DQB1	DPA1	DPB1
Total	90	59	82	85	36	93
Mean	1.11	0.72	1	1.05	0.44	1.15
<u>Eplet MM</u>	DRB1	DRB3/4/5	DQA1	DQB1	DPA1	DPB1
Total	416	290	606	726	114	309
Mean	5.1	3.6	7.5	9	1.4	3.8

Class II mismatch - antibody responders 55 v. non-responders 26

Ab Resp.	DRB1	DRB3/4/5	DQA1	DQB1	DPA1	DPB1
HLA MM	66	43	61	63	25	67
Mean	1.2	0.78	1.1	1.14	0.45	1.2
Eplet MM	319	198	517	577	84	219
Mean	5.8	3.6	9.4	10.5	1.5	4
<u>Non-Resp.</u>	DRB1	DRB3/4/5	DQA1	DQB1	DPA1	DPB1
HLA MM	24	16	21	22	11	26
Mean	0.9	0.3	0.8	0.84	0.4	1
Eplet MM	97	92	89	149	30	90
Mean	3.7	3.5	3.4	5.7	1.1	3.5

Class II Ab. responders by consistent eplet

Eplet MM						
	DRB1	DRB3/4/5	DQA1	DQB1	DPA1	DPB1
Eplets	319	198	517	577	84	219
Mean	5.8	3.6	9.4	10.5	1.5	4
Early Ab. by eplet						
	DRB1	DRB3/4/5	DQA1	DQB1	DPA1	DPB1
Eplet	104	101	59	289	13	52
Mean	1.9	1.8	1	5.3	0.2	1

Class II discussion

HLA-DQ has significantly more eplets per MM than DR or DP

There is evidence that DQ eplet mismatch is associated with an antibody response to HLA Class II

When analysed by consistent eplets DQB1 dominates the Class II antibody response

Are we perhaps looking at separation of function of DR, DQ and DP in a similar way to Class I?

Consistent Class I eplets?

91 with good Class I data/results

2 with no Class I mismatch at HR (or Class II!)

14 had prior antibody, 6 showed no Class 1 antibody

41 had de novo Class 1 antibodies related to the mismatch (and the eplets...)

But 28 didn't – is this the third party antibody we've read about?

Let's analyse the antibody in another way - number of luminex SAB antigens positive, cRF and specificity...

HLA-A	HLA-B	HLA-C	cRF	Specificity
0	1	0	0	82
1	0	0	0	76
0	1	1	1	73, Cw17
0	2	2	1	73,76 Cw17,18
0	0	1	1	Cw17
0	1	0	4	13
0	0	1	7	Cw2
1	1	0	15	32,27
1	0	4	21	80, Cw6,15,17,18
0	3	0	27	8,45,75
0	2	1	29	49,62 Cw6
0	4	0	36	7,48,60,81
0	6	0	40	44,45,47,51,63,78
2	1	4	32	34,66,76 Cw1,6,12,15
4	0	0	33	3,32,36,74
0	6	3	60	8,44,45,73,76,82 Cw2,15,17
1	15	2	54	66,13,27,42,47,48,54,55,56,60,61,73,81,82 Cw17
2	3	3	66	24,30,8,37,Cw5,17,18
0	4	1	17	13,60,61,48,Cw17
1	2	0	43	11,37,44,74,76 wk2
3	2	0	24	24,25,26,37,47
0	5	0	10	45,46,57,58,76
1	0	4	51	wk1,Cw1,2,15,16
0	6	0	40	44,45,57,58,76,82
0	1	0	27	7,45,81
5	3	0	37	25,26,34,43,66,44,75,76
2	0	1	18	23,24,Cw7
5	0	0	8	1102,25,26,43,76

The 28 that produced some “HLA Class I specific” antibody that didn’t correlate with the mismatch or eplet analysis in detail

15 of these look like artefacts ? in blue with cRF 0-40

13 were more problematic to explain – run on the other beads

Specificity SAB kit 1	Specificity SAB kit 2
8,44,45, 73 ,76,82 Cw2,15, 17	29,32,33,36,6602,80 B13,1405/6,27,39,46,58,63,71, 73 ,75, Cw0202,0401,14,16,17,
66,13, 27,42 ,47,48,54, 55,56,60,61 ,73, 81,82 Cw17	7, 27,42 ,52, 55,56,60,61,81 ,82
24,30, 8,37 ,Cw5,17,18	8 ,18,2705,35, 37 ,41,42,4403,53,54,61,82
13,60,61,48,Cw17	B52
11,37,44,74,76 wk2	A2,1102,6802,37
24,25,26,37,47	Neg
45,46,57,58,76	Neg
wk1,Cw1,2,15,16	A0205
B44,45,57,58,76,82	B1512, 8202
B7,45,81	NT
A25,26,34,43,66,44,75,76	Neg
A23,24 ,Cw7	A23,24
1102,25,26,43 ,76	1102,25 wk,26,43,66,

Conclusion

Only 3 (of the 28 donor/recipient pairs that lost their allograft to rejection and had no eplets consistent with the HLA mismatch at HR) had a plausible HLA specific antibody

Each Luminex manufacturers kit throws up spurious nonsense which are probably artefacts of the method rather than alloantibody – third party antibody?

**What about the 3? Transfusion or prior sensitisation?
Investigation continues....**

(ask me about the sera with consistent Class 1 antibody)

Conclusions

Two field HLA typing reveals unexpected solid organ mismatches

The eplet mismatches generated by these unexpected mismatches may generate “unexpected” antibody specificities

Two field HLA typing may improve the ability for “virtual” crossmatching in sensitised patients by allowing more accurate assessment of donor epitope mismatches

Two field typing may have its uses in solid organ transplantation?

Speculation....

Do HLA-A, -B and -Cw antigens have specialised functions - like HLA-E and -G?

HLA-C is principle ligand for NK and NK receptor bearing cells (KIR,LILR etc) HLA-B is major focus for CD8 T cells via TCR-HLA-A ?? Rapid response to new infections?

Similarly for HLA Class II? Do DR, DQ and DP have the separate specialised function?

If there are many B cell clones as indicated by eplet analysis, how many CD4 T cell clones involved?

Is it justifiable to base patient antibody definition on just one manufacturers kit?

Acknowledgements



Paula Mobillo – the work

Olivia Shaw – Serology

Robert Collins – DNA typing