

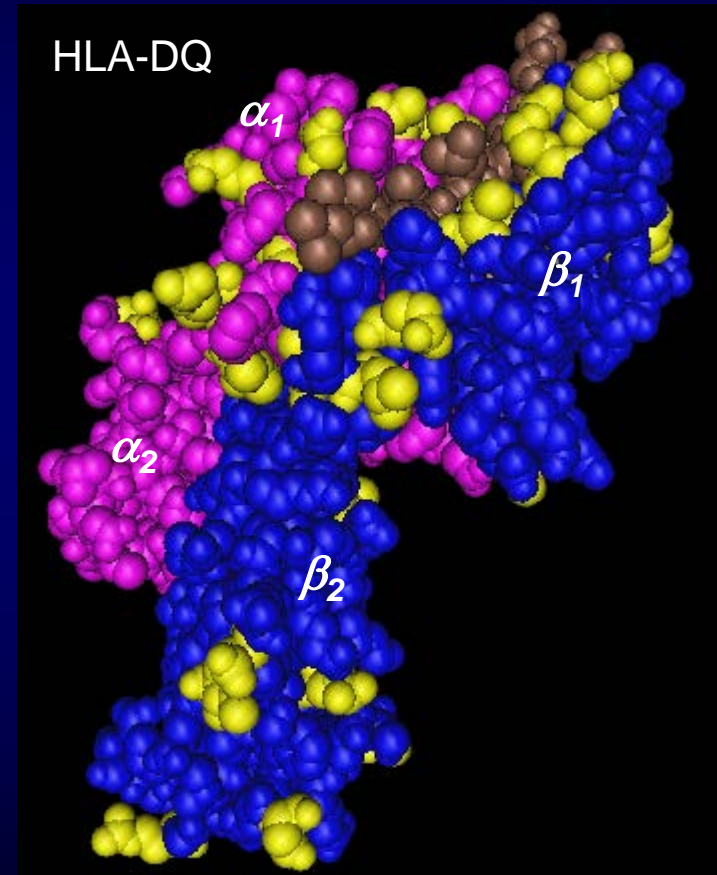
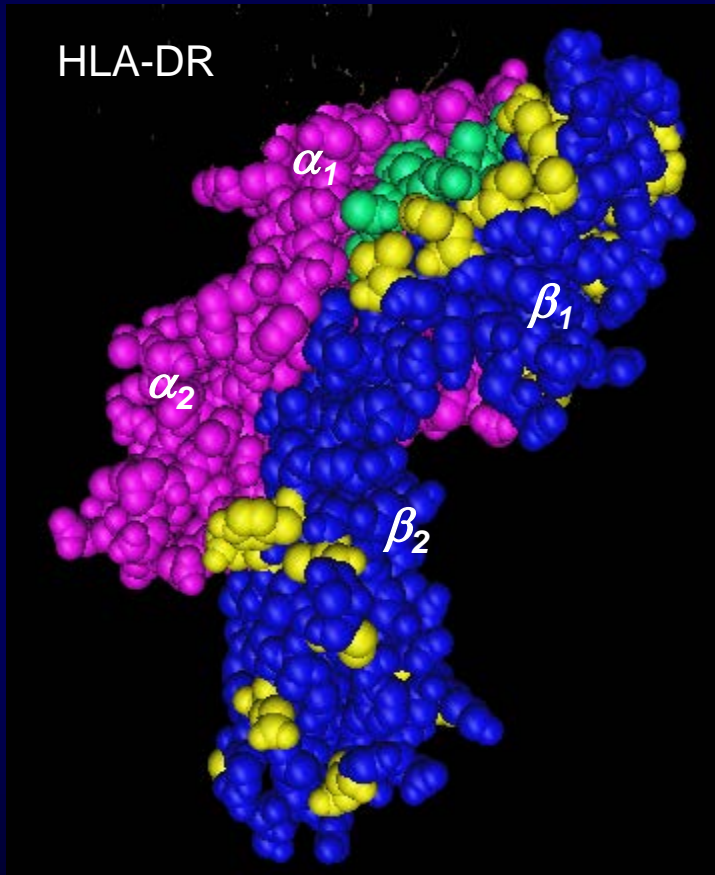
# Lecture 3

Antigenicity of HLA class II  
epitopes and MICA epitopes

# Class II HLA Antibodies Decrease Transplant Success

- DRB1 is “standard” for donor-recipient matching
- DRB3/4/5
  - Antibodies to DR51, DR52 and DR53
- DQB1 and DQA1
  - Relevance of DQB matching in transplantation
  - Patients make antibodies to DQB and DQA epitopes
- DPA1 and DPB1
  - Relevance of DP matching
  - Anti-DP antibodies in transplantation

## Topography of Polymorphic Residues on HLA-DR and HLA-DQ Molecules



# Class II Eplet Version of HLAMatchmaker

Duquesnoy, RJ and Askar, M: HLAMatchmaker: A Molecularly Based Algorithm for Histocompatibility Determination. V. Eplet Matching for HLA-DR, HLA-DQ and HLA-DP, *Human Immunology*, 68: 12-25, 2007

# 3.0 Angstrom Patches on DRB1,3,4,5

Polymorphic  
positions are  
underlined

Sequence Position	Molecular Location	Surface Exposure	3.0 Angstrom Patches						
<b>4</b>	Side	++	3	<u>4</u>	5				
<b>6</b>	Side	+	5	<u>6</u>	7	A15	A17		
<b>12</b>	Underside	±	<u>11</u>	<u>12</u>	<u>13</u>	29			
<b>14</b>	Underside	+	<u>13</u>	<u>14</u>	15	<u>16</u>	27	29	
<b>16</b>	Underside	+	15	<u>16</u>	17	<u>18</u>	<u>25</u>		
<b>18</b>	Side	+	17	<u>18</u>	19	23			
<b>25</b>	Side	++	<u>16</u>	24	<u>25</u>	<u>26</u>	43		
<b>26</b>	Side	±	<u>25</u>	<u>26</u>	27	42			
<b>31</b>	Underside	+	<u>10</u>	29	<u>30</u>	<u>31</u>	<u>32</u>		
<b>32</b>	Underside	+	<u>31</u>	<u>32</u>	<u>33</u>	35			
<b>33</b>	Underside	+	8	<u>32</u>	<u>33</u>	<u>34</u>			
<b>34</b>	Side	++	<u>33</u>	<u>34</u>	35	A83			
<b>40</b>	Side	+	<u>28</u>	39	<u>40</u>	<u>41</u>			
<b>41</b>	Side	+	<u>40</u>	<u>41</u>	42	43	<u>44</u>	45	
<b>44</b>	Side	+	<u>41</u>	43	<u>44</u>	45			
<b>47</b>	Side	+	<u>28</u>	46	<u>47</u>	<u>48</u>	62		
<b>48</b>	Side	++	<u>47</u>	<u>48</u>	49				
<b>51</b>	Side	+	37	50	<u>51</u>	52			
<b>57</b>	Top	+	56	<u>57</u>	<u>58</u>	61	A76	P13	
<b>58</b>	Top	+	54	<u>57</u>	<u>58</u>	<u>59</u>	62		
<b>59</b>	Top	++	<u>58</u>	<u>59</u>	<u>60</u>				
<b>60</b>	Top	++	<u>59</u>	<u>60</u>	61	63			
<b>67</b>	Top	+	66	<u>67</u>	68	<u>71</u>			
<b>70</b>	Top	+	<u>67</u>	69	<u>70</u>	<u>71</u>	<u>73</u>	P11	

DRB1,3,4,5

Contd

Sequence Position	Molecular Location	Surface Exposure	3.0 Angstrom Patches									
<b>71</b>	Top	$\pm$	<b><u>70</u></b>	<b><u>71</u></b>	72	<b><u>73</u></b>						
<b>73</b>	Top	+	69	72	<b><u>73</u></b>	<b><u>74</u></b>	<b><u>76</u></b>	<b><u>77</u></b>				
<b>74</b>	Top	+	<b><u>70</u></b>	<b><u>71</u></b>	72	<b><u>73</u></b>	<b><u>74</u></b>	79	P8	P9		
<b>76</b>	Top	++	<b><u>73</u></b>	75	<b><u>76</u></b>	<b><u>77</u></b>						
<b>77</b>	Top	++	<b><u>73</u></b>	<b><u>76</u></b>	<b><u>77</u></b>	78	P6					
<b>81</b>	Top	+	80	<b><u>81</u></b>	82	<b><u>85</u></b>	P4					
<b>85</b>	Top	+	84	<b><u>85</u></b>	<b><u>86</u></b>							
<b>86</b>	Side	$\pm$	82	<b><u>85</u></b>	<b><u>86</u></b>	87	90					
<b>96</b>	Side	++	95	<b><u>96</u></b>	97	<b><u>180</u></b>						
<b>98</b>	Side	++	97	<b><u>98</u></b>	99	<b><u>120</u></b>						
<b>104</b>	Side	+	103	<b><u>104</u></b>	<b><u>105</u></b>	107	114					
<b>105</b>	Side	++	<b><u>104</u></b>	<b><u>105</u></b>	106	107						
<b>108</b>	Side	++	107	<b><u>108</u></b>	109							
<b>112</b>	Bottom	++	108	111	<b><u>112</u></b>	113						
<b>120</b>	Side	+	98	119	<b><u>120</u></b>	121						
<b>133</b>	Bottom	++	132	<b><u>133</u></b>	134							
<b>135</b>	Bottom	++	134	<b><u>135</u></b>	136							
<b>140</b>	Side	++	139	<b><u>140</u></b>	141							
<b>142</b>	Side	++	138	141	<b><u>142</u></b>	143						
<b>149</b>	Side	++	148	<b><u>149</u></b>	150							
<b>180</b>	Side	+	96	177	179	<b><u>180</u></b>	<b><u>181</u></b>					
<b>181</b>	Side	++	<b><u>180</u></b>	<b><u>181</u></b>	182							
<b>183</b>	Side	+	182	<b><u>183</u></b>	184							
<b>187</b>	Side	++	186	<b><u>187</u></b>	188							
<b>189</b>	Bottom	++	188	<b><u>189</u></b>	190							

## Polymorphic Residues in 3 Angstrom DRB Patches

4	67	70	71	73
6	70	71	73	
11 12 13	73	74	76	77
13 14 16	70	71	73	74
16 18 25	73	76	77	
18	81	85		
16 25 26	85	86		
25 26	96	180		
10 30 31 32	98	120		
31 32 33	104	105		
8 32 33 34	108			
33 34	112			
28 40 41	120			
40 41 44	133			
41 44	135			
28 47 48	140			
47 48	142			
51	149			
57 58	180	181		
57 58 59	183			
58 59 60	187			
59 60	189			
67 71				

## DQA

2
18
21
25 26
40 41
44 45
34 47 48
48 51
50 51
48 50 51 52 53
51 52 53
51 52 53 54
51 53 54 55
54 55 56
53 55 56
59 61
64
66
69
75 76 79
75 76
75 79 80
107
129 130
138 139
153
156
160 161
163
175

## DQB

3
13 14
23
26
30 37 38
45 46
45 46 47
46 47
49
52 53
52 55 56
55 56 57
56 57
66 67
66 67 71
70 71
67 70 71
74 75
76 77
84 85
84 85 86 89
86 87
85 86 89 90
86 89 90
116
125 126
130
135
140
167 168
182

## DPA

18
28
50 51
72 73
83
111
127
160

## DPB

8
11
28
33
35 36
43 70
56 57
64 65
64 65 69
65 69 70
43 69 70
76
84 85
84 85 86
85 86 87
86 87
91
96
171
173

Polymorphic Residues in 3 Angstrom  
DQ and DP Patches



# Determination of the Polymorphic Residue Composition of Each Patch

- HLA Patch Generator: a Microsoft Excel Macro developed by **Grzegorz Dudek** (now at Czestchowa University of Technology, Poland)

# Eplet Numbers on HLA Class II Molecules

Locus	# Eplets
DRB	149
DQB	74
DQA	58
DPB	45
DPA	19

Duquesnoy and Askar, Human Immunology, 68: 12-25, 2007

# Class II Epitope Studies by Terasaki's group

- Deng CT, Cai J, Tarsitani C, El-Awar N, Lachmann N, Ozawa M. HLA Class II DQ Epitopes. *Clinical Transplants 2006*. 2006:115-22.
- Deng CT, El-Awar N, Ozawa M, et al. Human leukocyte antigen class II DQ alpha and beta epitopes identified from sera of kidney allograft recipients. *Transplantation*. 2008;**86**:452-9.
- El-Awar N, Terasaki PI, Cai J, et al. Epitopes of the HLA-A, B, C, DR, DQ and MICA antigens. *Clinical Transplants*. 2007:175-94.
- Cai J, Terasaki PI, Mao Q, et al. Development of nondonor-specific HLA-DR antibodies in allograft recipients is associated with shared epitopes with mismatched donor DR antigens. *American Journal of Transplantation*. 2006;**6**:2947-54.
- Cai J, Kohanof S, Terasaki P. HLA-DR Antibody Epitopes. In: Checka MaT, PI, ed. *Clinical Transplants 2006*. Los Angeles, CA: Terasaki Foundation Laboratory, 2006:103-14.

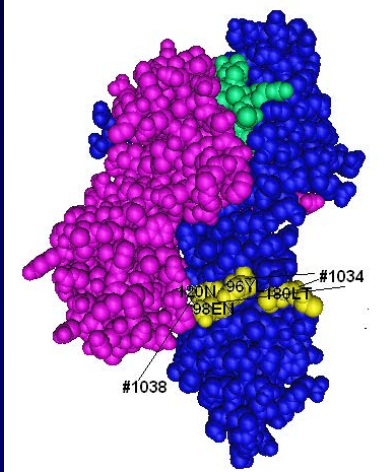
# Comparison between Terasaki's Class II epitopes HLA Matchmaker-defined eplets

Locus	TerEps	Eplets	Eplet pairs	Eplets with substitutions	No eplets
DR	60	45	3	12	2
DQ	18	13	1	3	1

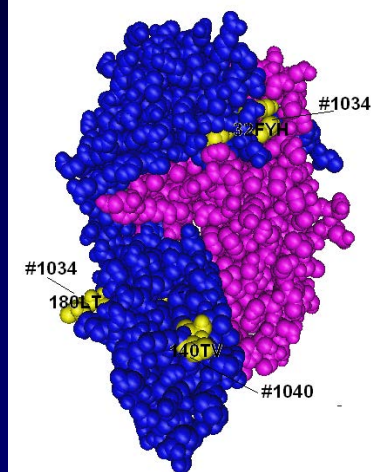
\* Marrari M and Duquesnoy RJ, *Tissue Antigens*, 74:134-146, 2009.

# Molecular locations of eplet equivalents of Terasaki's DRB Epitopes

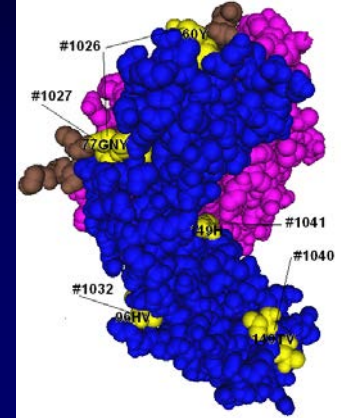
(a) DRB1\*0401 (view 1)



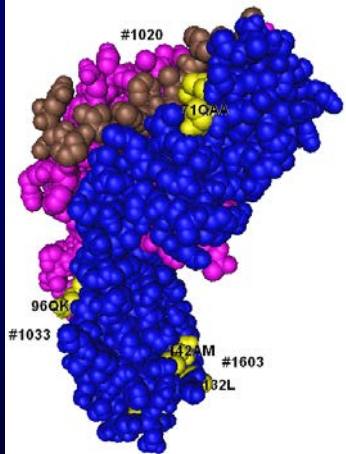
(b) DRB\*0401 (view 2)



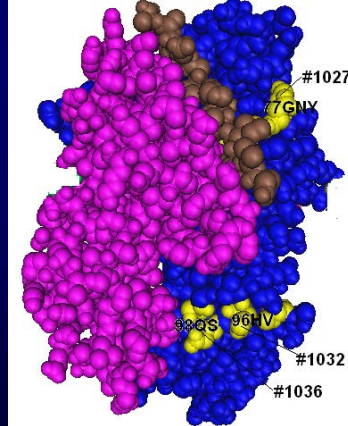
(c) DRB1\*0301



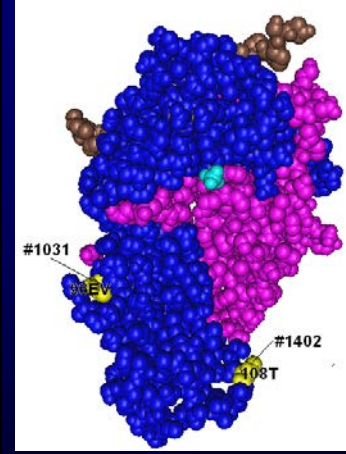
(d) DRB1\*1501



(e) DRB3\*010

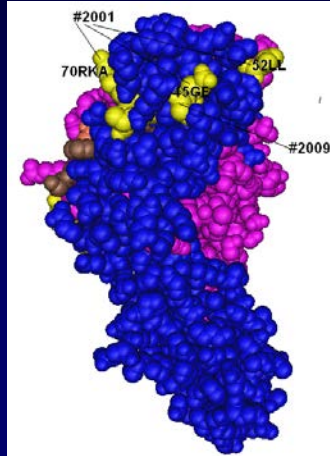


(f) DRB5\*0101

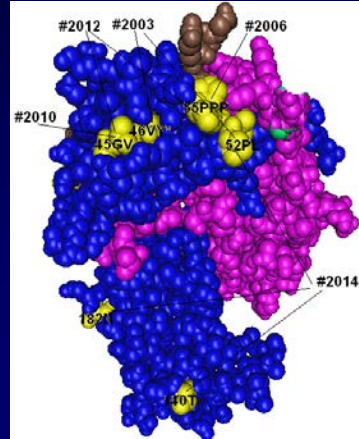


# Molecular locations of eplet equivalents of Terasaki's DQA and DQB Epitopes

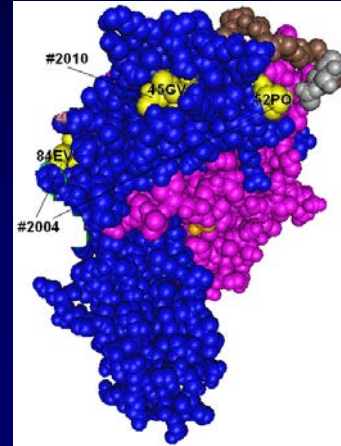
(a) DQA1\*0501-DQB1\*0201



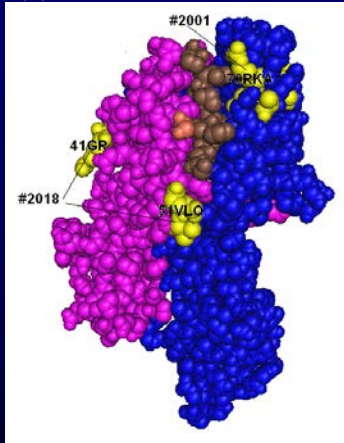
(c) DQA1\*0301-DQB1\*0302



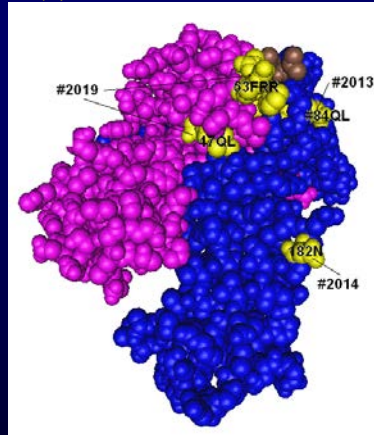
(e) DQA1\*0102-DQB1\*0602



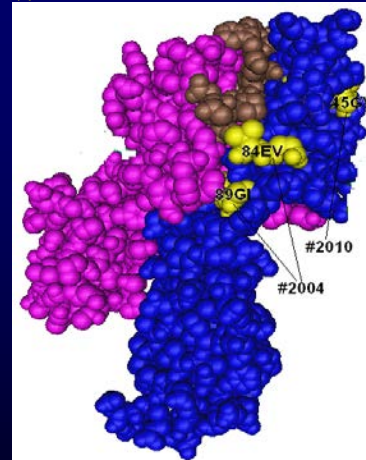
(b) DQA1\*0501-DQB1\*0201



(d) DQA1\*0301-DQB1\*0302



(f) DQA1\*0102-DQB1\*0602



# Class II Eplet Examples In HLA Epitope Registry

Locus	Eplet	Description	Eplet-Carrying Alleles in Luminex Panel
DRB	12TSE	12T,13S,14E	DRB1*01:01/02/03,09:01/02,10:01
DRB	57V	57V,58A,60S	DRB1*07:01,09:01,12:01;B3*01:01,03:01

Locus	Eplet	Description	Eplet-Carrying Alleles in Luminex Panel
DQB	56PA	56P,57A	DQB1*02:01, 02:02, 03:02
DQA	41RA <sub>2</sub>	40E,41R,45A / 129Q,130S	DQA1*01:01, 01:02, 01:04

Locus	Eplet	Description	Eplet-Carrying Alleles in Luminex Panel
DPB	65LK	65L,69K	DPB1*03,*14,*28
DPB DPB	56EE 56EE <sup>DR11</sup>	55D,56E,57E 55D,56E,57E* *DR11 has 57D,58E,59E	DPB1*02:01,*04:02,*08,*10,*18,*28 DPB1*02:01,*04:02,*08,*10,*18,*28 and DR11
DPA	50RA	50R,51A	DPA1*02:01,02:02,*04:01



# Antibody Verification of HLA Epitopes

- Duquesnoy RJ, Marrari M, Mulder A, da M. Sousa LCD, Da Silva AS, do Monte SJH. 'First Report on the Antibody Verification of HLA-ABC Epitopes Recorded in the HLA Epitope Registry'. *Tissue Antigens* 83:391-400, 2014
  - 97 HLA-ABC antibody-verified epitopes have been recorded, 62 correspond to eplets and 35 are defined by eplets paired with other residue configurations
- Duquesnoy RJ, Marrari M, Tambur A, da Mata Sousa LCD, Da Silva AS, do Monte SJH. 'First Report on the Antibody Verification of HLA-DR, HLA-DQ and HLA-DP Epitopes Recorded in the HLA Epitope Registry' *Human Immunology* 75:1097-1103, 2014
  - 24 HLA-DRB1/3/4/5, 15 DQB, 3 DQA and 8 DPB antibody-verified epitopes
- Duquesnoy RJ, Marrari M, Mosteck J, Da Silva AS, da Mata Sousa LCD, do Monte SJH. 'First Report on the Antibody Verification of MICA Epitopes Recorded in the HLA Epitope Registry' *Int J Immunogenetics* 41: 370-377, 2014
  - 21 MICA epitopes verified with antibodies which have primarily been tested in Luminex assays with single alleles



# Two examples of antibody-verified DRB epitopes

Patient:		DRB1*07:01,-; DRB4*01:01,-		DQB1*03:03,-; DQA1*02:01,-	
Allele	Epitope	MFI	Allele	Epitope	MFI
neg cont		41	DRB1*10:01	25R	7148
pos cont		7694	DRB1*11:01	25R	7518
DRB1*07:01	self	117	DRB1*11:01	25R	7518
DRB4*01:01	self	218	DRB1*12:01	25R	3538
DRB4*01:03		90	DRB1*13:01	25R	5303
DRB1*01:01	25R	9403	DRB1*13:03	25R	6910
DRB1*01:02	25R	10847	DRB1*14:01	25R	4359
DRB1*01:03	25R	7351	DRB1*15:01	25R	7293
DRB1*03:01	25R	6897	DRB1*15:02	25R	5544
DRB1*03:02	25R	2946	DRB1*16:01	25R	7347
DRB1*04:01	25R	7624	DRB3*01:01*	25R	414
DRB1*04:04	25R	7098	DRB3*02:02	25R	3967
DRB1*04:05	25R	4850	DRB5*01:01	25R	6833
DRB1*08:01	25R	6385	DRB5*02:02	25R	1848
DRB1*09:01	25R	3120	18 DQ heterodimers		184±118
* has 11R ; others have 11D/L/S/V					

High frequency  
epitope

	PANEL	OL MFI
	Positive control	10172
	Negative control	3
Immunizing allele:	96HK+ DRB1*03:01	20647
	96HK+ DRB1*03:02	17386
	96HK+ DRB1*08:01	16093
	96HK+ DRB1*11:01	17314
	96HK+ DRB1*11:04	15912
	96HK+ DRB1*12:01	13915
	96HK+ DRB1*12:02	15767
	96HK+ DRB1*13:01	19756
	96HK+ DRB1*13:03	22404
	96HK+ DRB1*14:01	19810
	Self Alleles	17 ± 3
	Other 96HK-negative Alleles	36 ± 51

# Serum antibodies against antibody-verified epitopes on DQB and DQA

Patient HLA	DRB1*04:01 DQB1*03:01	DRB1*15:01 DQB1*06:02	DRB4*01:01 DQA1*03:03	DRB5*01:01 DQA1*01:02	
Donor HLA	DRB1*07:01	DRB4*01:01			
One haplo mm	DQB1*02:01	DQA1*02:01	Enter		
Kit	Luminex Panel		Rx	DQB Eplet	DQA Eplet
OL	Negative Control		863		
OL	Positive Control		5161		
TEP	Negative Control		274		
TEP	Positive Control		8290		
OL	DQB1*02:02	DQA1*02:01	10277	45GE <sub>5</sub>	47KHL
OL	DQB1*02:01	DQA1*02:01	6673	45GE <sub>5</sub>	47KHL
OL	DQB1*02:01	DQA1*03:01	1936	45GE <sub>5</sub>	
OL	DQB1*02:01	DQA1*05:01	3985	45GE <sub>5</sub>	
TEP	DQB1*02:02	DQA1*02:01	13914	45GE <sub>5</sub>	47KHL
TEP	DQB1*02:02	DQA1*03:02	2956	45GE <sub>5</sub>	
TEP	DQB1*02:02	DQA1*05:01	2581	45GE <sub>5</sub>	
OL	DQB1*03:03	DQA1*02:01	11037		47KHL
OL	DQB1*04:01	DQA1*02:01	5958		47KHL
OL	DQB1*04:02	DQA1*02:01	9487		47KHL
TEP	DQB1*03:02	DQA1*02:01	13740		47KHL
TEP	DQB1*05:03	DQA1*02:01	9212		47KHL
OL (N=11)	DQB1*02/DQA1*02 neg		591±173		
TEP (N=12)	DQB1*02/DQA1*02 neg		743±429		
Note: less reactivity with 45GE <sub>5</sub> than with 47KHL					

# DRB Epitope Clinical Study

Why can Sensitization by a HLA-DR2 Mismatch lead to Antibodies that react also with HLA-DR1?

Marrari and Duquesnoy Human Immunology, 70:403-9, 2009

# 15th International Histocompatibility Workshop

## Project on HLA Epitope Immunogenicity

- A multilaboratory collaborative project to characterize these epitopes and also how often they induce specific antibodies in patients with rejected kidney transplants
- Serum analyses on more than 150 allograft nephrectomy cases contributed so far by more than 25 laboratories worldwide have yielded interesting information that has led to a better understanding of antibody recognition of HLA epitopes
- This report address the question how a single DR2 mismatch can lead to antibodies that also react with DR1
- Although DR1 and DR2 might share an epitope recognized by these antibodies, this interpretation is incorrect
- An HLAMatchmaker analysis offers a clearly different explanation

# 19 Allograft Nephrectomy Cases with DR2 or DR1 Mismatches in 15<sup>th</sup> International Workshop Project on Epitope Immunogenicity

Case	Contributor	Location	Recipient DR Antigens	Donor DR Mismatch
ADEL-1	James McCluskey	Adelaide, Australia	DR8, DR13	DR15
ADEL-2	James McCluskey	Adelaide, Australia	DR4, -	DR15
AV	Mary Younie	Bristol, UK	DR8, DR9	DR15
EM	Adriana Zeevi	Pittsburgh, USA	DR8, DR13	DR15
HC	Amy Hahn	Albany, USA	DR4, DR17	DR15
ND11	Constanze Schönemann	Berlin, Germany	DR4, DR7	DR15
RM 70532482	Andrew Lobashevsky	Indianapolis, USA	DR8, DR17	DR15
SA	Silvia Chrenova	Bratislava, Slovakia	DR4, DR13	DR15
ZS	Sandra Nehlsen-Cannarella	Detroit, USA	DR11, DR17	DR15
2910	Agathi Varnavidou	Nicosia, Cyprus	DR4, DR11	DR16
POL-8790	Marilyn Pollack	San Antonio, USA	DR8, DR17	DR16
4765	Maria Gerbase-DeLima	Sao Paulo, Brazil	DR4, DR7	DR1
290544	Tsuyoshi Sato	Sapporo, Japan	DR4, DR8	DR1
695723	Tsuyoshi Sato	Sapporo, Japan	DR12, DR14	DR1
ECU-1	Lorita Rebellato	Greenville, USA	DR11, DR13	DR1
MC 05048330	Andrew Lobashevsky	Indianapolis, USA	DR8, DR17	DR1
ND4	Constanze Schönemann	Berlin, Germany	DR4, DR13	DR1
SM	Amy Hahn	Albany, USA	DR4, DR14	DR1
VS	Silvia Chrenova	Bratislava, Slovakia	DR7, DR14	DR1

# Example of an antibody response to a DR15 mismatch

Patient type: DRB1\*0301, DRB1\*0801; DRB3\*0101

Immunizing donor: DRB1\*1501, DRB5\*0101

Allele		MFI	Allele		MFI	Mismatched Eplets
DRB1*0301	Self	120	DRB1*0101		3252	12LKF2,14FEH,25HRL,96EV
DRB1*0302		206	DRB1*0102		2683	12LKF2,14FEH,25HRL,96EV
DRB1*0401		317	DRB1*0103		2460	12LKF2,14FEH,25HRL,96EV
DRB1*0402		199	DRB1*0901		5057	14FEH,26KYH,31QGIY,40HFD
DRB1*0403		225	DRB1*0902		1754	14FEH,26KYH,31QGIY,40HFD
DRB1*0404		401	DRB1*1001		2474	12VKF3,14FEH,25HRL,96QV
DRB1*0405		181	DRB1*1501	IM	3695	142M3,71QAA,96QV
DRB1*0701		175	DRB1*1502		2281	142M3,71QAA,96QV
DRB1*0801	Self	173	DRB1*1601		2511	142M3,96QV
DRB1*1101		125	DRB1*1602		2239	142M3,70LDRA,96QV
DRB1*1201		214	DRB5*0101	IM	6099	31QDIY,40HFD,96EV,108T3
DRB1*1202		150	DRB5*0202		8384	6C,31QGIY,40HFD,71QAA,96EV,108T3
DRB1*1301		165				
DRB1*1303		90				
DRB1*1401		109				
DRB3*0101	Self	157				
DRB3*0202		241				
DRB4*0101		550				
DRB4*0103		710				

**The immunizing DR15 shares no eplets with DR1**

# Example of an antibody response to a DR15 mismatch

Patient type: DRB1\*0301, DRB1\*0801; DRB3\*0101

Immunizing donor: DRB1\*1501, DRB5\*0101

Allele		MFI	Allele		MFI	Mismatched Eplets
DRB1*0301	Self	120	DRB1*0101		3252	12LKF2,14FEH,25HRL,96EV
DRB1*0302		206	DRB1*0102		2683	12LKF2,14FEH,25HRL,96EV
DRB1*0401		317	DRB1*0103		2460	12LKF2,14FEH,25HRL,96EV
DRB1*0402		199	DRB1*0901		5057	14FEH,26KYH,31QGIY,40HFD
DRB1*0403		225	DRB1*0902		1754	14FEH,26KYH,31QGIY,40HFD
DRB1*0404		401	DRB1*1001		2474	12VKF3,14FEH,25HRL,96QV
DRB1*0405		181	DRB1*1501	IM	3695	142M3,71QAA,96QV
DRB1*0701		175	DRB1*1502		2281	142M3,71QAA,96QV
DRB1*0801	Self	173	DRB1*1601		2511	142M3,96QV
DRB1*1101		125	DRB1*1602		2239	142M3,70LDRA,96QV
DRB1*1201		214	DRB5*0101	IM	6099	31QDIY,40HFD,96EV,108T3
DRB1*1202		150	DRB5*0202		8384	6C,31QGIY,40HFD,71QAA,96EV,108T3
DRB1*1301		165				
DRB1*1303		90				
DRB1*1401		109				
DRB3*0101	Self	157				
DRB3*0202		241				
DRB4*0101		550				
DRB4*0103		710				

**The immunizing DR15 shares no eplets with DR1**

**DR1 shares 96EV with the immunizing DR51**



# Example of an antibody response to a DR15 mismatch

Patient type: DRB1\*0301, DRB1\*0801; DRB3\*0101

Immunizing donor: DRB1\*1501, DRB5\*0101

Allele		MFI	Allele		MFI	Mismatched Eplets
DRB1*0301	Self	120	DRB1*0101		3252	12LKF2,14FEH,25HRL,96EV
DRB1*0302		206	DRB1*0102		2683	12LKF2,14FEH,25HRL,96EV
DRB1*0401		317	DRB1*0103		2460	12LKF2,14FEH,25HRL,96EV
DRB1*0402		199	DRB1*0901		5057	14FEH,26KYH,31QGIY,40HFD
DRB1*0403		225	DRB1*0902		1754	14FEH,26KYH,31QGIY,40HFD
DRB1*0404		401	DRB1*1001		2474	12VKF3,14FEH,25HRL,96QV
DRB1*0405		181	DRB1*1501	IM	3695	142M3,71QAA,96QV
DRB1*0701		175	DRB1*1502		2281	142M3,71QAA,96QV
DRB1*0801	Self	173	DRB1*1601		2511	142M3,96QV
DRB1*1101		125	DRB1*1602		2239	142M3,70LDRA,96QV
DRB1*1201		214	DRB5*0101	IM	6099	31QDIY,40HFD,96EV,108T3
DRB1*1202		150	DRB5*0202		8384	6C,31QGIY,40HFD,71QAA,96EV,108T3
DRB1*1301		165				
DRB1*1303		90				
DRB1*1401		109				
DRB3*0101	Self	157				
DRB3*0202		241				
DRB4*0101		550				
DRB4*0103		710				

**The immunizing DR15 shares no eplets with DR1**

**DR1 shares 96EV with the immunizing DR51**

**Why do antibodies react with DR9, DR10 and DR16?**



# Example of an antibody response to a DR15 mismatch

Patient type: DRB1\*0301, DRB1\*0801; DRB3\*0101

Immunizing donor: DRB1\*1501, DRB5\*0101

Allele		MFI	Allele		MFI	Mismatched Eplets
DRB1*0301	Self	120	DRB1*0101		3252	12LKF2,14FEH,25HRL,96EV
DRB1*0302		206	DRB1*0102		2683	12LKF2,14FEH,25HRL,96EV
DRB1*0401		317	DRB1*0103		2460	12LKF2,14FEH,25HRL,96EV
DRB1*0402		199	DRB1*0901		5057	14FEH,26KYH,31QGIY,40HFD
DRB1*0403		225	DRB1*0902		1754	14FEH,26KYH,31QGIY,40HFD
DRB1*0404		401	DRB1*1001		2474	12VKF3,14FEH,25HRL,96QV
DRB1*0405		181	DRB1*1501	IM	3695	142M3,71QAA,96QV
DRB1*0701		175	DRB1*1502		2281	142M3,71QAA,96QV
DRB1*0801	Self	173	DRB1*1601		2511	142M3,96QV
DRB1*1101		125	DRB1*1602		2239	142M3,70LDRA,96QV
DRB1*1201		214	DRB5*0101	IM	6099	31QDIY,40HFD,96EV,108T3
DRB1*1202		150	DRB5*0202		8384	6C,31QGIY,40HFD,71QAA,96EV,108T3
DRB1*1301		165				
DRB1*1303		90				
DRB1*1401		109				
DRB3*0101	Self	157				
DRB3*0202		241				
DRB4*0101		550				
DRB4*0103		710				

DR10 and DR16 share 96QV with the immunizing DR15

# Example of an antibody response to a DR1 mismatch

Patient type: DRB1\*0701, DRB1\*1401; DRB3\*0202, DRB4\*0101

Immunizer: DRB1\*0101

Allele		MFI	Allele		MFI	Mismatched Eplets on Reactive Alleles
DRB1*0401		1494	DRB1*0101	IM	11942	12LKF2,14FEH,25HRL,71QRA,74QRAA,96EV
DRB1*0402		872	DRB1*0102		11167	12LKF2,14FEH,25HRL,71QRA,74QRAA,96EV
DRB1*0701	Self	2090	DRB1*0103		8023	12LKF2,14FEH,25HRL,96EV
DRB1*0801		818	DRB1*0405		3341	71QRA,74QRAA
DRB1*1101		826	DRB1*0901		3807	14FEH,26KYH,31QGIY,40HFD
DRB1*1201		928	DRB1*1001		4032	12VKF3,14FEH,25HRL
DRB1*1301		924	DRB5*0101		6584	31QDIY,40HFD,96EV,108T3
DRB1*1303		879	DRB5*0202		6995	6C,31QGIY,40HFD,96EV,108T3
DRB1*1401	Self	806	<p>DR2 gives negative reactions</p> <p>The reactive DR51 shares 96EV with the immunizing DR1</p> <p>The reactive DR9 and DR10 share 14FEH with the immunizing DR1</p> <p>The reactive DRB1*0405 shares 71QRA,74QRAA with the immunizing DR1</p>			
DRB1*1501		1656				
DRB1*1502		1852				
DRB1*1601		1140				
DRB1*0301		841				
DRB1*0303		953				
DRB3*0101		989				
DRB3*0202	Self	840				
DRB3*0301		765				
DRB4*0101	Self	1111				

# 96EV is an immunogenic eplet shared by DR1 and DR51

Sensitization induced by	DR51	Antibodies to 96EV
DR15	DRB5*0101	7/9 cases
DR16	DRB5*0202	2/2 cases
DR1	None	8/8 cases
Total		17/19 cases 90%

# Further support of the 96EV epitope

- Monoclonal antibody 137BL7
  - Loh MT, Chan SH and Ren EC: A monoclonal antibody with specificity to the HLA-DR1 and -DR51 antigens. *Tissue Antigens*. 42:100-4, 1993
- Monoclonal antibody against epitope #1055 shared between DR1 and DR51
  - Cai J, Kohanof S and Terasaki P: HLA-DR Antibody Epitopes. *Clinical Transplants* 2006, p 103.

# Conclusions

- These findings demonstrate the importance of DR51 in determining mismatch acceptability of DR1 and DR2
- Exposure to DR2-DR51 may lead to anti-96EV antibodies that render DR1 as an unacceptable mismatch
- Exposure to the DR1 may also lead to anti-96EV antibodies that render DR51 as an unacceptable mismatch. Consequently, all DR2-DR51 haplotypes should be considered as unacceptable mismatches
- This study provides further support of the usefulness of HLAMatchmaker in determining mismatch acceptability

# Antibody-verified DQ epitopes in the HLA Epitope Registry

<b>DQB1Epitope *provisional</b>	<b>Polymorphic Residue Descriptions</b>	<b>Epitope-carrying antigens and/or alleles in Luminex kits</b>	<b>Verified with**</b>	<b>References</b>
45EV	45E46V	DQ7	A, M, E	(16, 51-56)
45GE <sub>3</sub>	46E47F 52L55L 74A	DQ2	A, M, H, S, E	(16, 18, 19, 55-59)
45GV	45G46V47Y	DQ4,DQ5,DQ6,DQ8,DQ9	A, M, S, E	(51, 52, 54, 56, 60-62)
46VY <sub>2</sub>	46V47Y 52P	DQ3,DQ4,DQ5,DQ6 (nonDQ2)	A, M, E	(3, 55, 56, 63, 64)
52PL <sub>3</sub>	52P53L 140T 182N	DQ3,DQ4	A, M, E	(16, 56, 63-65)
52PQ <sub>2</sub>	52P53Q 84E85V	DQ5,DQ6	A, M, H, E	(3, 16, 18, 19, 55, 56, 66, 67)
52PR	52P55R	DQ4,DQ5,DQ6	A, M, H, E	(3, 16, 18, 19, 55, 56, 68)
55PP	52P53L55P56P	DQ3	A, M, H, E	(3, 16, 18, 19, 55, 56, 69)
74SR <sub>3</sub>	71A74S77R 116I 125S	DQ5	A, M, E	(3, 55, 56)
74SV	74S75V26G	DQ4,DQ5	A, H	(65, 70)
77R*	77R	DQ2, DQ5	A, E	(56)
77T*	77T	DQ3, DQ4, DQ6	A, E	(56)
84QL <sub>3</sub>	84Q85L86E89T 53L 125A	DQ2,DQ3,DQ4	A, E, M	(3, 56, 63, 71)
125SQ*	125S126Q	DQB1*05:01/03	A, E	(56)
140A*	140A	DQ2,DQ5,DQ6	A, E	(56)
<b>DQA1 Epitope</b>				
40G <sub>3</sub>	40G 47C 50V51L53Q	DQA4,DQA5,DQA6	A, E	(56, 63, 65, 72)
47KHL	47K52H54L	DQA2	A, E	(56, 63, 65)
75S <sub>3</sub>	75S 161E 175K	DQA5	A, E, H	(68, 72)

\*\* Antibody sources and methods are: A, alloserum; E, alloserum eluate; H, human mAb; M, mouse mAb; and S, site mutagenized alleles with residue substitutions.

# Antibody Reactivity with Epitopes on HLA-DQ Heterodimers

Anat R Tambur, Northwestern University

Sample size: Sera from 104 patients awaiting renal TX, with known history of antibodies to DQ antigens

76(73%) have anti-DQ antibodies

How frequent are antibodies against “self” DQ component?

37 (36%): Own *DQB1* with non-self *DQA1*

60 (58%): Own *DQA1* with non-self *DQB1*

26 (25%): Antibodies to both groups of molecules

DQ epitopes can comprise pairs of *DQA* + *DQB* eplets

Antibody reactivity should be assessed with DQ heterodimers rather than individual *DQA* and *DQB* chains

# Antibody-verified DPB epitopes in the HLA Epitope Registry

DPB1 Epitope *provisional	Polymorphic Residue Descriptions	Epitope-carrying alleles in Luminex kits	Defined by	References
35FV	35F36V	DP2,DP3,DPB1*04:02,DP5,DP6,DP8,DP9, DP10,DP14,DP17,DP18,DP19,DP23	A, M	(76-79)
56A*	56A57E	DP1,DPB1*04:01,DP5,DP11,DP13,DP19, DP23	A	(80)
56E	55D56E	DP1,DPB1*02:01,DP3,DPB1*04:02,DP6,DP9 DP10,DP14,DP16,DP17,DP18,DP28	A, H, M	(23, 77, 79, 81, 82)
56E <sup>DR11</sup>	55D56E	DP1,DPB1*02:01,DP3,DPB1*04:02,DP6,DP9 DP10,DP14,DP16,DP17,DP18,DP28 + DR11	A, H, M, S	(22, 30-34, 77, 80, 83)
57D	55D56E57D	DP3,DP6,DP9,DP14,DP17	A, M	(77, 79, 84, 85)
56E <sup>DR11</sup>	55D56E57E	DPB1*02:01,DPB1*04:02,DP8,DP10,DP18,D P28 and DR11	A, M	(22, 77, 84, 85)
84DEAV	84D85E86A87V	DP1,DP3,DP5,DP6,DP9,DP10,DP11, DP13,DP14,DP16,DP17,DP19	A, H, M	(23, 65, 77-80, 84-86)
85GPM	85G86P87M	DP2,DP4,DP15,DP18,DP23,DP28	A, M	(22, 77-80, 84, 87)

\*\* Antibody sources and methods are: A, alloserum; E, alloserum eluate; H, human mAb; M, mouse mAb; and S, site mutagenized alleles with residue substitutions.



# Cross-reactivity between DR11 and certain DPB alleles

Allele	57	58	59
DRB*11:01	D	E	E
	55	56	57
DPB1*02/04:02/08/10/18/28	D	E	E
DPB1*03/06/09/14/17	D	E	D

# Cross-reactivity between DR11 and certain DPB alleles

Allele	57	58	59
DRB*11:01	D	E	E
	55	56	57
DPB1*02/04:02/08/10/18/28	D	E	E
DPB1*03/06/09/14/17	D	E	D

Induced by	DR11	DR11	DP	DP	DR11	DR11	DP	DP	DP
Eplet	57DE	57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>	58EE	58EE <sup>DP</sup>	56EE	56EE <sup>DR11</sup>	57D
Description	57D58E	57D58E	55D56E	55D56E	57D58E59E	57D58E59E	55D56E57E	55D56E57E	55D56E57D
DRB1*11:01	57DE	57DE <sup>DP</sup>		56E <sup>DR11</sup>	58EE	58EE <sup>DP</sup>		56EE <sup>DR11</sup>	
DPB1*03:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>					57D
DPB1*06:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>					57D
DPB1*09:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>					57D
DPB1*14:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>					57D
DPB1*17:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>				56EE <sup>DR11</sup>	57D
DPB1*02:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>		58EE <sup>DP</sup>	56EE	56EE <sup>DR11</sup>	
DPB1*04:02		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>		58EE <sup>DP</sup>	56EE	56EE <sup>DR11</sup>	
DPB1*08:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>		58EE <sup>DP</sup>	56EE	56EE <sup>DR11</sup>	
DPB1*10:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>		58EE <sup>DP</sup>	56EE	56EE <sup>DR11</sup>	
DPB1*18:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>		58EE <sup>DP</sup>	56EE	56EE <sup>DR11</sup>	
DPB1*28:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>		58EE <sup>DP</sup>	56EE		
Antibody verified?	YES	YES	YES	YES	(YES)	NO	NO	YES	YES

# Cross-reactivity between DR11 and certain DPB alleles

Allele	57	58	59
DRB*11:01	D	E	E
	55	56	57
DPB1*02/04:02/08/10/18/28	D	E	E
DPB1*03/06/09/14/17	D	E	D

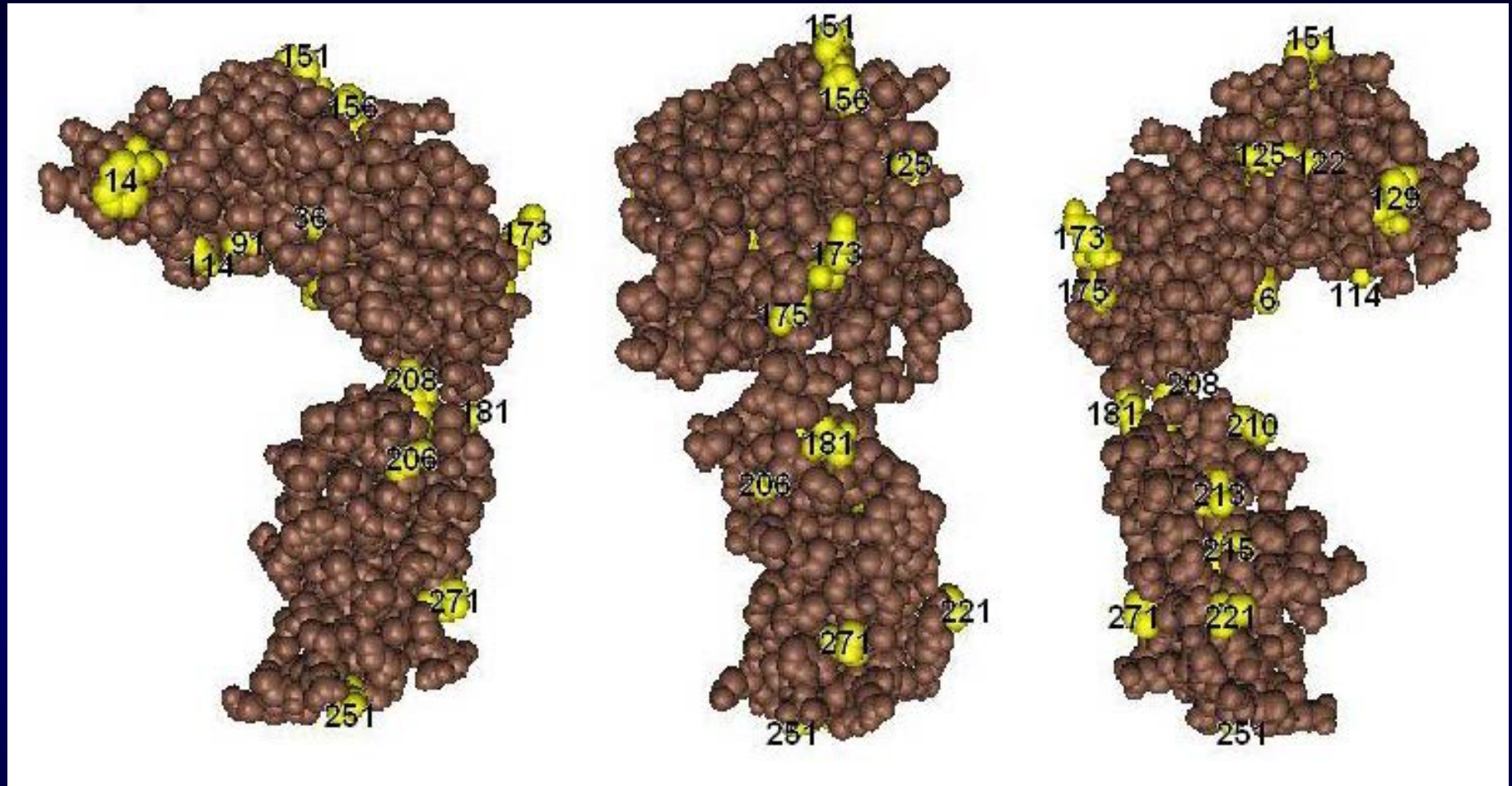
Induced by	DR11	DR11	DP	DP	DR11	DR11	DP	DP	DP
Eplet	57DE	57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>	58EE	58EE <sup>DP</sup>	56EE	56EE <sup>DR11</sup>	57D
Description	57D58E	57D58E	55D56E	55D56E	57D58E59E	57D58E59E	55D56E57E	55D56E57E	55D56E57D
DRB1*11:01	57DE	57DE <sup>DP</sup>		56E <sup>DR11</sup>	58EE	58EE <sup>DP</sup>		56EE <sup>DR11</sup>	
DPB1*03:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>					57D
DPB1*06:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>					57D
DPB1*09:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>					57D
DPB1*14:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>					57D
DPB1*17:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>				56EE <sup>DR11</sup>	57D
DPB1*02:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>		58EE <sup>DP</sup>	56EE	56EE <sup>DR11</sup>	
DPB1*04:02		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>		58EE <sup>DP</sup>	56EE	56EE <sup>DR11</sup>	
DPB1*08:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>		58EE <sup>DP</sup>	56EE	56EE <sup>DR11</sup>	
DPB1*10:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>		58EE <sup>DP</sup>	56EE	56EE <sup>DR11</sup>	
DPB1*18:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>		58EE <sup>DP</sup>	56EE	56EE <sup>DR11</sup>	
DPB1*28:01		57DE <sup>DP</sup>	56E	56E <sup>DR11</sup>		58EE <sup>DP</sup>	56EE		
Antibody verified?	YES	YES	YES	YES	(YES)	NO	NO	YES	YES
57DE <sup>DP</sup> = 56E <sup>DR11</sup>					57DE <sup>DP</sup> = 56E <sup>DR11</sup>				

# The Major Histocompatibility Complex class I-related chain A (MICA) antigens

- Highly polymorphic, stress-inducible cell-surface proteins encoded by genes on chromosome 6 mapping close to HLA-B and HLA-C
- Similar to class I HLA but no  $\beta$ 2-microglobulin and no peptide binding
- Ligand for NKG2D also expressed on CD8 $\alpha\beta$  T-cells and  $\gamma\delta$  T-cells
- Increased MICA expression on endothelial cells and following transplantation
- MICA mismatches can trigger alloantibody responses that are associated graft rejection and transplant failure
- Pre-transplant MICA antibodies represent significant risk factors for allograft rejection and lower graft survival

References: Bahram et al. Res. Immunol. 147:328, 1996. Steinle et al. Immunogenetics 53:279, 2001. Zwirner et al. Hum Immunol. 61:917, 2000. Zou et al. NEJM 357:1293, 2007. Mizutani et al. Amer. J Transplantation 5:2265, 2005

# Topography of Polymorphic Residues on MICA



Duquesnoy, Mosteck, Hariharan and Balazs: Structurally based epitope analysis of MICA antibody specificity patterns, *Human Immunology* 69: 826-832, 2008

# Eplet Positions on MICA

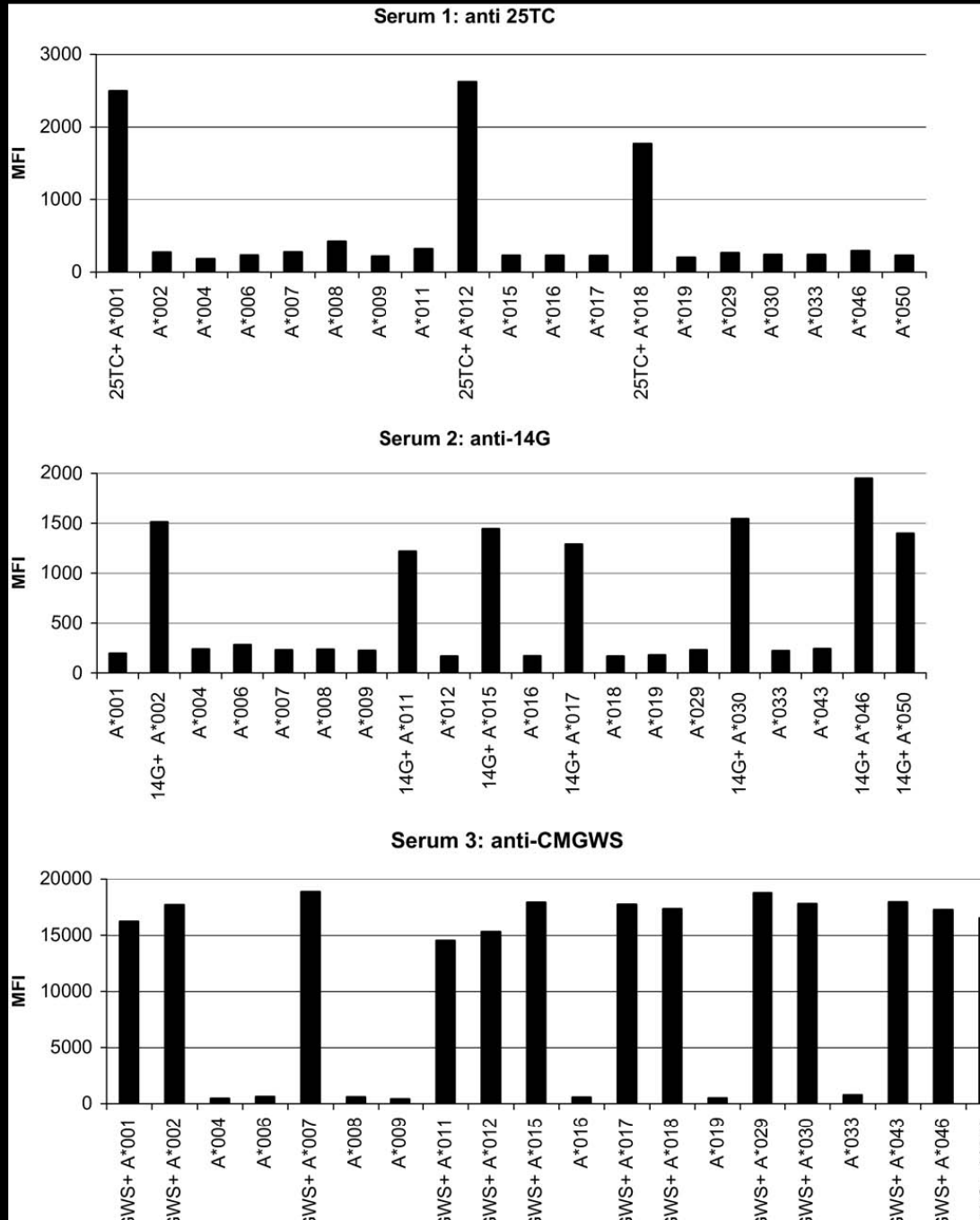
Position	Location	Surface Exposure	Eplet Positions		
6	underside	+	6		
14	side	++	14		
24	side	<u>+</u>	24	36	
24	side	<u>+</u>	24	26	36
26	side	+	26		
36	side	<u>+</u>	36		
91	underside	+	90	91	
105	side	++	105		
114	under	++	114		
122	side	+	122	91	
122	side	+	122	124	125
124	side	++	124		
125	side	++	125		
129	side	++	129		
142	top	<u>+</u>	142		
151	top	++	151		
156	top	++	156		
173	side	++	173	125	
173	side	++	173	125	175
175	side	+	175	125	
181	side	+	181		
206	side	+	206		
208	side	+	206	208	210
210	side	++	210		
213	side	++	213	215	
215	side	+	215		
221	side	++	221		
251	bottom	++	251		
256	bottom	++	256		
268	side	+	268		
271	side	++	271		



# Common Eplet Repertoire of MICA

EPLET	MICA Alleles
6P	*010*025
6R	non*010*025
14G	*002*011*013*014*015*017*020*022*023*030*034*035*036*041*044*046*047*050*052*053
14W	*001*004*005*006*007*008*009*010*012*016*018*019*021*024*025*026*027*028*029*031*032*033*037*038*039*040*042*043*045*048*049*051
24AC	*002*007*011*013*014*015*017*020*022*023*026*029*030*034*035*036*037*038*039*040*041*043*045*046*047*050*052*053
24AY	*004*005*006*008*009*010*016*019*024*025*027*028*031*032*033*042*044*048*049*051
24TC	*001*012*018*021
25AVC	*002*007*011*013*014*015*017*020*022*023*026*029*030*034*035*036*037*038*039*040*043*045*046*050*052*053
36C	*001*002*007*011*012*013*014*015*017*018*020*021*022*023*026*029*030*034*035*036*037*038*039*040*041*043*045*046*047*050*052*053
114G	non*014*015
114R	*014*015
122LQ	non*004*006*009*017*044*049
122LR	*017
122VQ	*004*006*009*044*049
123LTE	non*004*006*009*044*049
125E	non*001*031*040
125K2	*001*031*040
129M	*001*002*007*011*012*014*015*017*018*020*021*023*025*026*029*030*031*032*034*035*036*037*038*039*040*041*042*043*045*046*047*050*051*052
129V	*004*005*006*008*009*010*013*016*019*022*024*027*028*033*044*048*049*053
151M	non*011*034
151V	*011*034
156H	non*012*021*032*043
156L	*012*021*032
173EE	*004*006*008*009*010*013*014*016*019*022*024*027*028*033*036*044*048*049*053
173KE	*002*005*007*011*012*015*017*018*020*021*023*025*026*029*030*032*034*035*037*038*039*041*042*043*045*046*047*050*051*052
174EEG	*008*013*014*024*027*028*048*053
174EES	*004*006*009*010*016*019*022*033*036*044*049
174KEG	*002*005*007*011*012*015*017*018*020*021*023*025*026*029*030*034*035*037*038*039*041*042*043*045*046*047*050*051*052
174KKS	*001
175GE	*002*005*007*008*011*012*013*014*015*017*018*020*021*023*024*025*026*027*028*029*030*034*035*037*038*039*041*042*043*045*046*047*048*050*051*052*053
175SE	*004*006*009*010*016*019*022*032*033*036*044*049
181R	*004*014*032*044
181T	non*004*014*032*044
206G2	*001*002*007*011*012*013*014*015*017*018*020*021*023*025*026*028*029*030*031*032*036*040*041*043*045*046*047*050*051*052
206S2	*004*005*006*008*009*010*016*019*022*024*027*033*034*035*037*038*039*042*044*048*049*053
208GYW	*001*002*007*011*012*013*014*015*017*018*020*021*023*025*026*028*029*030*031*032*036*040*041*043*045*047*050*051*052
213I	*008*010*016*019*022*027*033*035*037*039*042*048*053
213T	*001*002*004*005*006*007*009*011*012*013*014*015*017*018*020*021*023*024*025*026*028*029*030*031*032*034*036*038*040*041*043*044*045*046*047*049*050*051*052
214TT	*004*005*006*009*024*034*038*044*049
221L	*016*039
221V	non*016*039
251Q	*001*002*004*006*007*009*011*012*014*015*017*018*020*021*023*024*025*026*028*029*030*031*032*034*036*038*040*041*043*044*046*047*049*050*051*052
251R	*005*008*010*013*016*019*022*027*033*035*037*039*042*048*053
271A	*011*030*047
271P	non*011*030*047

# Three patients with antibodies against MICA epitopes



Duquesnoy, Mostecky,  
 Hariharan and Balazs  
 Human Immunology 69:826-  
 832, 2008



# Antibody Verification of HLA Epitopes

- Duquesnoy RJ, Marrari M, Mulder A, da M. Sousa LCD, Da Silva AS, do Monte SJH. 'First Report on the Antibody Verification of HLA-ABC Epitopes Recorded in the HLA Epitope Registry'. *Tissue Antigens* 83:391-400, 2014
  - 97 HLA-ABC antibody-verified epitopes have been recorded, 62 correspond to eplets and 35 are defined by eplets paired with other residue configurations
- Duquesnoy RJ, Marrari M, Tambur A, da Mata Sousa LCD, Da Silva AS, do Monte SJH. 'First Report on the Antibody Verification of HLA-DR, HLA-DQ and HLA-DP Epitopes Recorded in the HLA Epitope Registry' *Human Immunology* 75:1097-1103, 2014
  - 24 HLA-DRB1/3/4/5, 15 DQB, 3 DQA and 8 DPB antibody-verified epitopes
- Duquesnoy RJ, Marrari M, **Mosteckí J**, Da Silva AS, da Mata Sousa LCD, do Monte SJH. 'First Report on the Antibody Verification of MICA Epitopes Recorded in the HLA Epitope Registry' *Int J Immunogenetics* 41: 370-377, 2014
  - MICA eplets were defined in comparison with MICB polymorphisms
  - 21 MICA epitopes verified with antibodies which have primarily been tested in Luminex assays with single alleles

# Allosera specific for MICA epitopes defined by 129M and 173K

	GP MFI		GP MFI		GP MFI		GP MFI
Positive control	23684	Positive control	15784	Positive control	16097	Positive control	11089
Negative control	185	Negative control	255	Negative control	209	Negative control	50
129M+ MICA*001	7606	129M+ MICA*001	11219	173K+ MICA*001	3719	173K+ MICA*001	1717
129M+ MICA*002	3472	129M+ MICA*002	8270	173K+ MICA*002	6068	173K+ MICA*002	2416
129M+ MICA*007	10112	129M+ MICA*007	12955	173K+ MICA*005	4953	173K+ MICA*005	1935
129M+ MICA*011	1780	129M+ MICA*011	6890	173K+ MICA*007	5456	173K+ MICA*007	2239
129M+ MICA*012	8258	129M+ MICA*012	11666	173K+ MICA*011	4523	173K+ MICA*011	1848
129M+ MICA*015	3393	129M+ MICA*015	8013	173K+ MICA*012	4588	173K+ MICA*012	1770
129M+ MICA*017	3443	129M+ MICA*017	6982	173K+ MICA*015	5312	173K+ MICA*015	1885
129M+ MICA*018	8500	129M+ MICA*018	11478	173K+ MICA*017	4784	173K+ MICA*017	1794
129M+ MICA*029	8011	129M+ MICA*029	13010	173K+ MICA*018	5341	173K+ MICA*018	2007
129M+ MICA*030	3744	129M+ MICA*030	6859	173K+ MICA*029	4974	173K+ MICA*029	1951
129M+ MICA*036	2297	129M+ MICA*036	6777	173K+ MICA*030	4816	173K+ MICA*030	1869
129M+ MICA*037	8227	129M+ MICA*037	15031	173K+ MICA*037	4978	173K+ MICA*037	1801
129M+ MICA*041	1862	129M+ MICA*041	5206	173K+ MICA*041	3342	173K+ MICA*041	1353
129M+ MICA*042	1768	129M+ MICA*042	1446	173K+ MICA*042	4693	173K+ MICA*042	1957
129M+ MICA*043	9214	129M+ MICA*043	12422	173K+ MICA*043	5012	173K+ MICA*043	1815
129M+ MICA*046	4453	129M+ MICA*046	9962	173K+ MICA*046	5213	173K+ MICA*046	1962
129M+ MICA*050	3576	129M+ MICA*050	6982	173K+ MICA*050	5410	173K+ MICA*050	2053
129M+ MICA*051	2097	129M+ MICA*051	2046	173K+ MICA*051	5156	173K+ MICA*051	2044
129M-negative alleles	563 ± 225	129M-negative alleles	408 ± 153	173K-negative alleles	310 ± 56	173K-negative alleles	190 ± 83