HLA Epitopes-Special Seminar

Sydney, Australia

March 5-6, 2015

HLA Antibodies in Transplantation

- HLA antibodies cause allograft rejection and transplant failure
- HLA antibodies recognize epitopes

Therefore

HLA epitopes are important in transplantation

Antibody Responses to HLA Affect Transplant Outcome

- Class I HLA antigens
  - HLA-A and HLA-B
  - HLA-C
  - MICA
- Class II HLA antigens
  - DRB1
  - DRB3, 4, 5
  - DQB and DQA
  - DPB and DPA

HLA Antigens Have Multiple Epitopes

- “Private” epitopes and “public” epitopes shared between cross-reacting antigens (CREGs)
- Epitopes defined by polymorphic amino acid residues
- Structurally based matching with HLAMatchmaker
  - Original version (2002): “triplets”, i.e. linear three-residue sequences
  - New version (2006): “eplets”, i.e. patches of residues within a 3 Angstrom radius of a polymorphic residue on the molecular surface.

Duquesnoy: “A Structurally Based Approach to Determine HLA Compatibility at the Humoral Immune Level”, Human immunology, 67: 847-862, 2006

What Do We Need to Know about HLA Epitopes?

- Structure and topography of epitopes on mismatched HLA antigens
- Structural interactions of HLA antigen-antibody complexes
- Epitope antigenicity: reactivity with antibody *in vivo* and in different assays
- Epitope immunogenicity: ability to induce specific antibodies

Objectives

- Structural concepts of HLA epitopes
  - Triplets, eplets, HLAMatchmaker, structural epitopes
  - Website-based International Registry of HLA-A,B,C, DR, DQ, DP and MICA epitopes
- HLA epitope antigenicity
  - HLAMatchmaker analysis of sera with antibodies
  - Epitope specificities of HLA antibodies tested in different assays
  - Mismatch acceptability for sensitized patients

Objectives (continued)

- HLA epitope immunogenicity
  - Epitope loads and HLA antibody responses
  - Physiochemical characteristics of HLA epitopes
  - Nonself-self paradigm
  - Mismatch permissibility for non-sensitized patients
- Clinical relevance of HLA matching at the epitope level
  - Solid organ transplantation
  - Platelet transfusion
  - Stem cell transplantation
- Future plans for HLA epitopes

Duquesnoy's Presentations

- More than 350 Powerpoint slides
- References to almost 100 publications about HLA epitopes since 2002 (List will be provided)

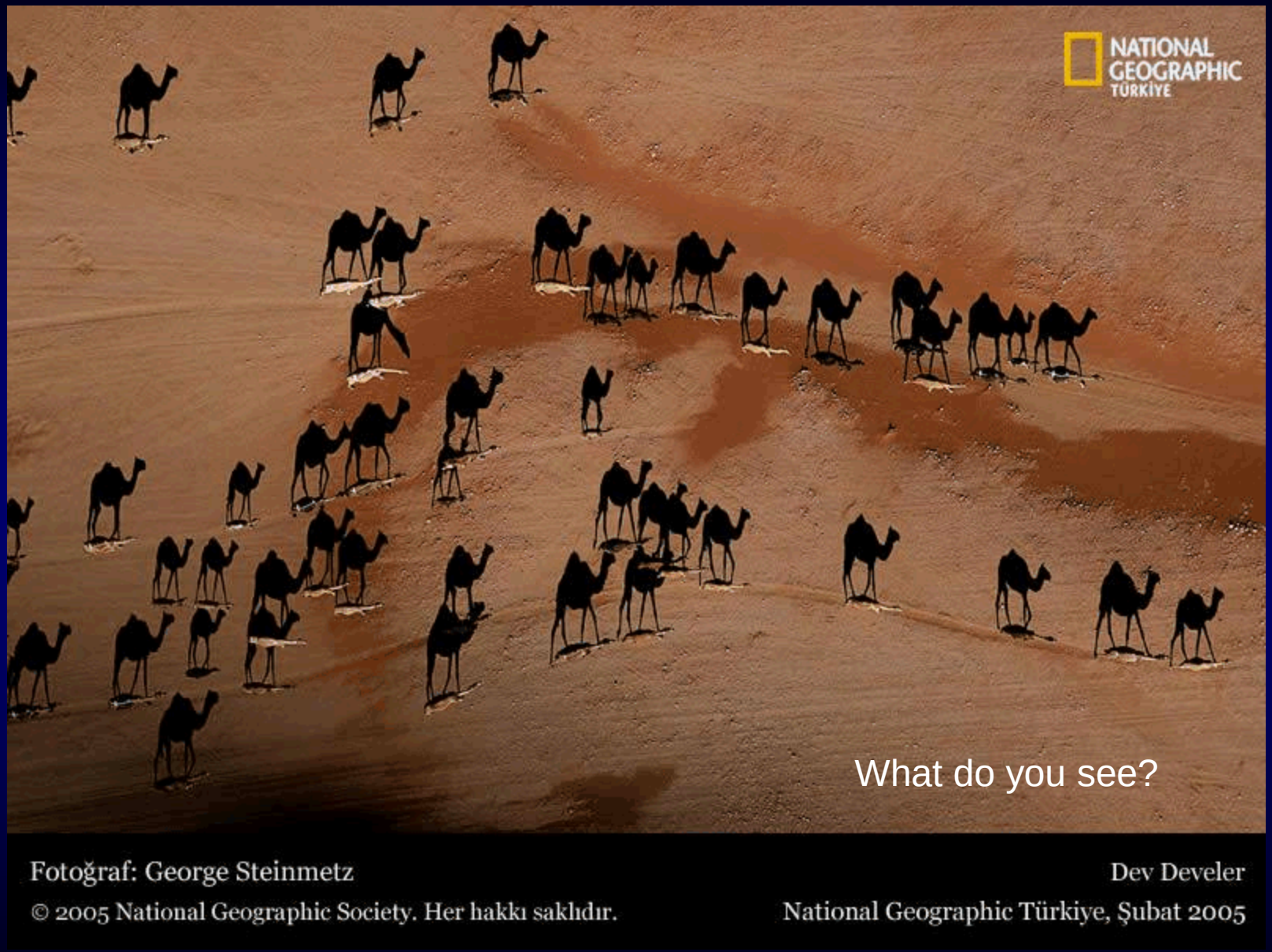
Lecture 1

Basic Aspects of HLA Epitope Structure

How Do Antibodies React with HLA Molecules?

Consider the structure of

- HLA molecules
- Antibodies
- Epitope-Paratope Interphase

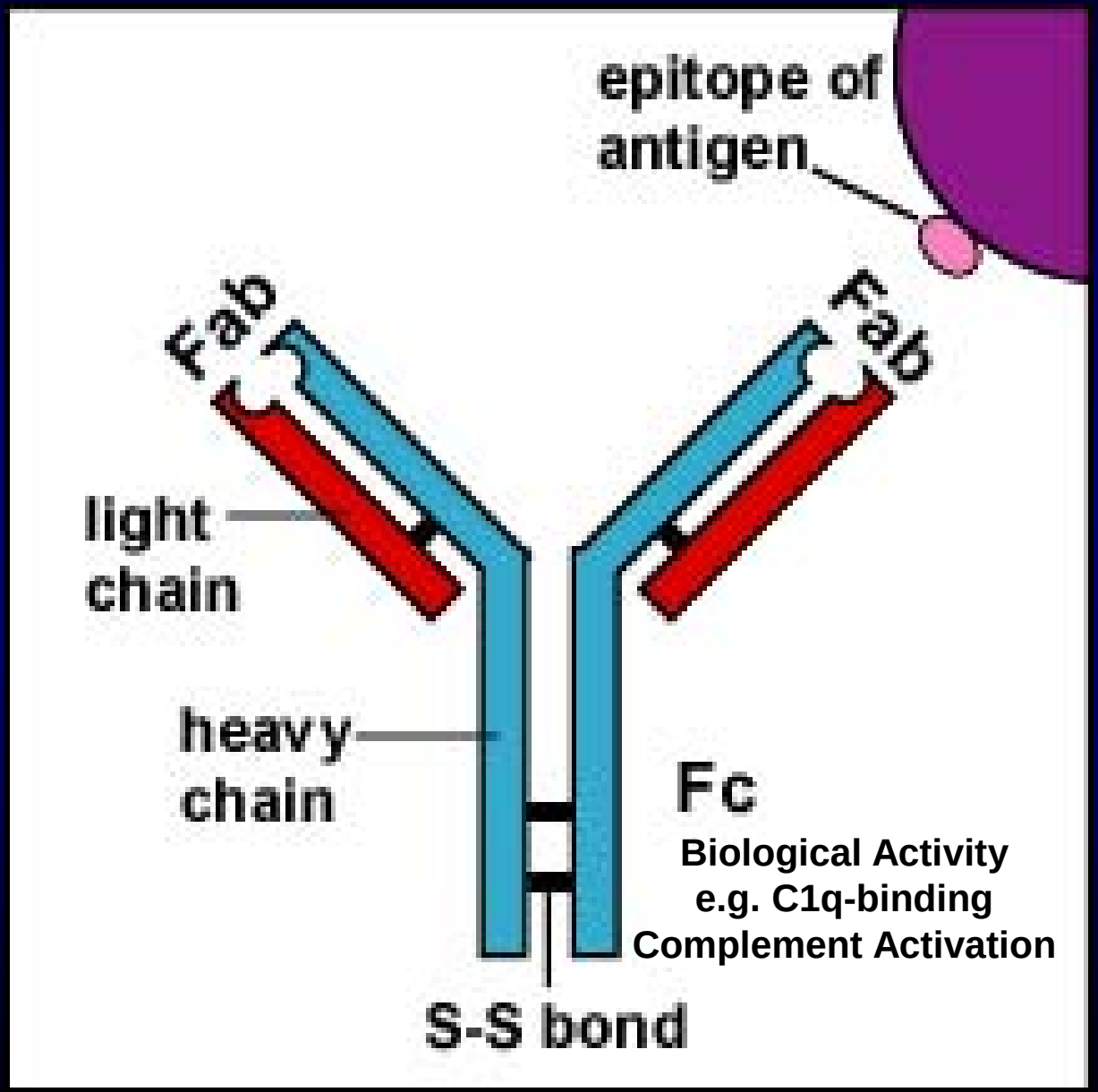


Fotoğraf: George Steinmetz

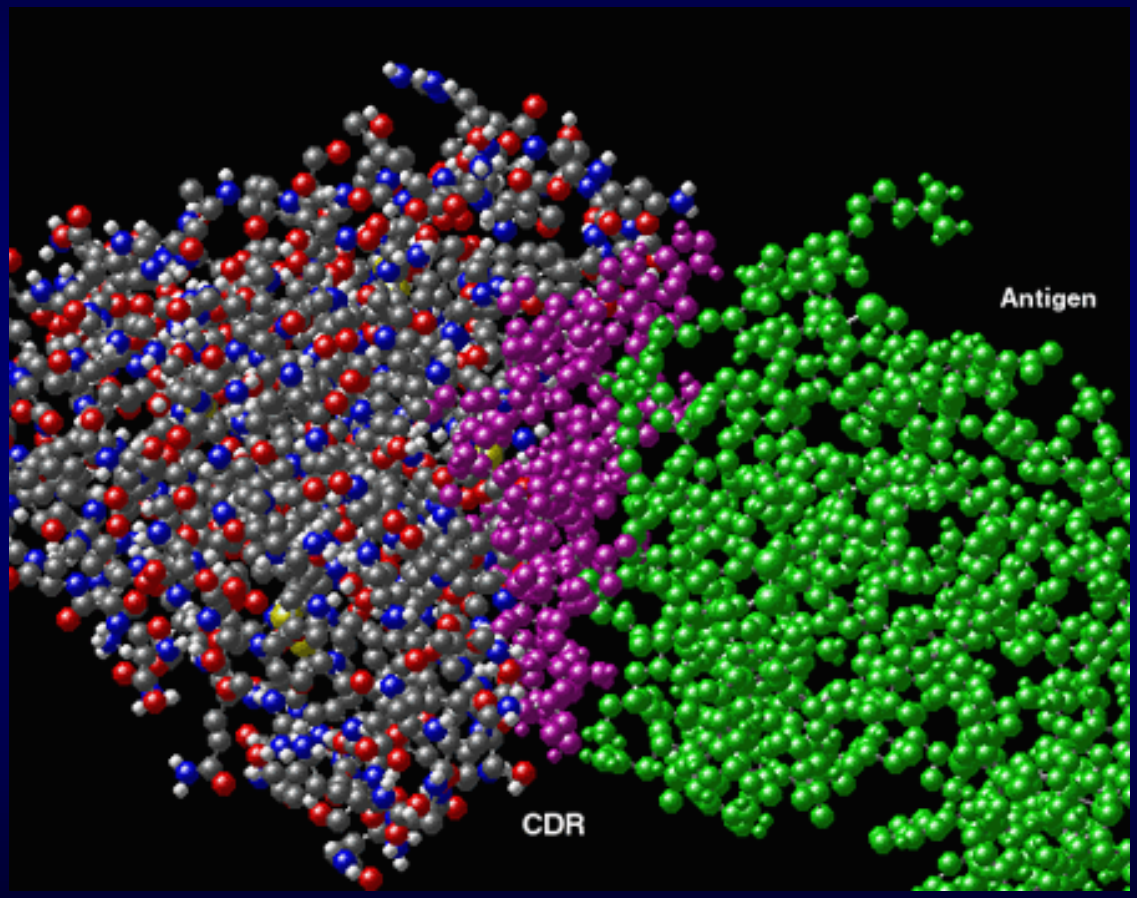
Dev Develer

National Geographic Türkiye, Şubat 2005

Binding of Antibody to an Epitope

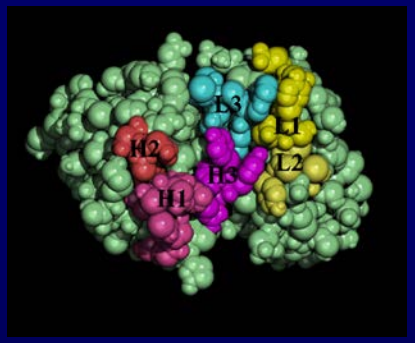


Antigen-Antibody Complex

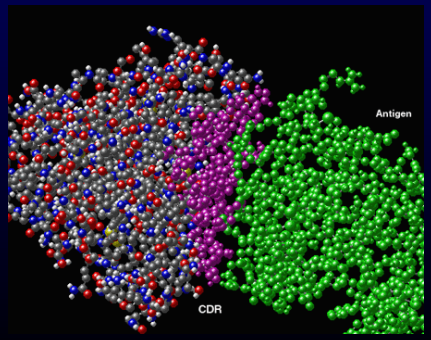


How Do Antibodies Bind to Epitopes on Proteins?

CDRs of Antibody



Antigen-Antibody Complex



- Complementarity Determining Regions H1, H2, H3, on heavy chain and L1, L2 and L3 on light chain represent the “binding face” or “paratope” of an antibody
- The antibody specificity is often determined by a centrally located loop (CDR-H3) that binds to a short amino acid sequence of the protein antigen
- Other CDRs serve as contact sites to stabilize binding to antigen (they play a role in the affinity of antibody)

MMDB Database

http://www.ncbi.nih.gov/Structure

- Large database of crystal structures
  - Complexes of protein antigens and antibody domains
  - Class I and class II HLA molecules
  - MHC-T-Cell Receptor complexes
- **Cn3D molecular viewer**
  - "Space-fill" command identifies surface-exposed amino acid residues
  - "Select by distance" command permits an assessment of epitope size and the distance between residues
- Basic Local Alignment Search Tool (BLAST)
  - Identifies sequence differences between antigen proteins and homologous self-proteins of the antibody producer

Commonly Studied Protein Antigens

- Hen Egg Lysozyme (HEL)
- Horse Cytochrome C
- Human Interferon-γ Receptor

With HLAMatchmaker on my mind I have analyzed data in more than 100 publications

Mouse Monoclonal Antibodies Against Hen Egg Lysozyme

What are the differences between mouse and hen egg lysozyme?

Sequence Differences between HEL and Mouse Lysozymes

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
intestinal	K	V	Y	N	R	C	E	L	A	R	I	L	K	R	N	G	M	D	G	Y	R	G	V	K	L
milk	K	V	Y	E	R	C	E	F	A	R	T	L	K	R	N	G	M	A	G	Y	Y	G	V	S	L
HEL	-	-	F	G	-	-	-	-	-	A	A	M	-	-	H	-	L	-	N	-	-	-	Y	S	-

	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
intestinal	A	D	W	V	C	L	A	Q	H	E	S	N	Y	N	T	R	A	T	N	Y	N	R	D	R	S
milk	A	D	W	V	C	L	A	Q	H	E	S	N	Y	N	T	R	A	T	N	Y	N	R	D	Q	S
HEL	G	N	-	-	-	A	-	K	F	-	-	-	F	-	-	Q	-	-	-	R	-	T	-	G	-

	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
intestinal	T	D	Y	G	I	F	Q	I	N	S	R	Y	W	C	N	D	G	K	T	P	R	S	K	N	A
milk	T	D	Y	G	I	F	Q	I	N	S	R	Y	W	C	N	D	G	K	T	P	R	A	V	N	A
HEL	-	-	-	-	-	L	-	-	-	-	-	W	-	-	-	-	-	R	-	-	G	-	R	-	L

	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
intestinal	C	G	I	N	C	S	A	L	L	Q	D	D	I	T	A	A	I	Q	C	A	K	R	V	V	R
milk	C	G	I	N	C	S	A	L	L	Q	D	D	I	T	A	A	I	Q	C	A	K	R	V	V	R
HEL	-	N	-	P	-	-	-	-	-	S	S	-	-	-	-	S	V	N	-	-	-	K	I	-	S

	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126
intestinal	D	P	Q	G	I	R	A	W	V	A	W	R	T	Q	C	Q	N	R	D	L	S	Q	Y	I	R	N
milk	D	P	Q	G	I	R	A	W	V	A	W	R	A	H	C	Q	N	R	D	L	S	Q	Y	I	R	N
HEL	-	G	N	-	M	N	-	-	-	-	-	-	N	R	-	K	G	T	-	V	Q	A	W	-	-	G

Which Residues are Critical for Antigen-Antibody Contact?

- Antibody reactivity with natural variants

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Mouse intest	K	V	Y	N	R	C	E	L	A	R	I	L	K	R	N	G	M	D	G	Y	R	G	V	K	L	A	D
Mouse milk	K	V	Y	E	R	C	E	F	A	R	T	L	K	R	N	G	M	A	G	Y	Y	G	V	S	L	A	D
HEL	-	-	F	G	-	-	-	-	-	A	A	M	-	-	H	-	L	-	N	-	-	-	Y	S	-	G	N
Cal Quail	-	-	F	G	-	-	-	-	-	A	A	M	-	-	H	-	L	-	N	-	-	-	Y	S	-	G	N
BW Quail	-	-	F	G	-	-	-	-	-	A	A	M	-	-	H	-	L	-	N	-	-	-	Y	S	-	G	N
Jap Quail	-	-	-	G	-	-	-	-	-	A	A	M	-	-	H	-	L	-	K	-	Q	-	Y	S	-	G	N
Turkey	-	-	-	G	-	-	-	-	-	A	A	M	-	-	L	-	L	-	N	-	-	-	Y	S	-	G	N
RN Pheasant	G	-	-	G	-	-	-	-	-	A	A	M	-	-	M	-	L	-	N	-	-	-	Y	S	-	G	N
Chachalaca	-	I	-	K	-	-	-	-	-	A	A	M	-	-	Y	-	L	-	N	-	-	-	Y	S	-	G	N
Duck A	-	-	-	-	-	-	-	-	-	A	A	M	-	-	L	-	L	-	N	-	-	-	Y	S	-	G	N
Guinea Fowl	-	-	F	G	-	-	-	-	-	A	A	M	-	-	H	-	L	-	N	-	-	-	Y	S	-	G	N

	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70
Mouse intest	N	Y	N	R	D	R	S	T	D	Y	G	I	F	Q	I	N	S	R	Y	W	C	N	D	G	K	T	P
Mouse milk	N	Y	N	R	D	Q	S	T	D	Y	G	I	F	Q	I	N	S	R	Y	W	C	N	D	G	K	T	P
HEL	-	R	-	T	-	G	-	-	-	-	-	-	L	-	-	-	-	-	W	-	-	-	-	-	R	-	-
Cal Quail	-	R	-	T	-	G	-	-	-	-	-	V	L	-	-	-	-	-	W	-	-	-	-	-	R	-	-
BW Quail	-	R	-	T	-	G	-	-	-	-	-	V	L	-	-	-	-	-	W	-	-	-	-	-	-	-	-
Jap Quail	-	R	-	T	-	G	-	-	-	-	-	-	L	-	-	-	-	-	W	-	-	-	-	-	R	-	-
Turkey	-	R	-	T	-	G	-	-	-	-	-	-	L	-	-	-	-	-	W	-	-	-	-	-	R	-	-
RN Pheasant	-	R	-	T	-	G	-	-	-	-	-	-	L	-	-	-	-	-	W	-	-	-	-	-	R	-	-
Chachalaca	-	R	S	N	-	G	-	-	-	-	-	-	E	-	-	-	-	-	W	-	-	-	-	-	R	-	-
Duck A	-	R	-	T	-	G	-	-	-	-	-	V	L	-	-	-	-	-	W	-	-	-	-	-	-	-	-
Guinea Fowl	-	R	-	T	-	G	-	-	-	-	-	-	L	-	-	-	-	-	W	-	-	-	-	-	R	-	-

	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113
Mouse intest	D	I	T	A	A	I	Q	C	A	K	R	V	V	R	D	P	Q	G	I	R	A	W	V	A	W	R	T
Mouse milk	D	I	T	A	A	I	Q	C	A	K	R	V	V	R	D	P	Q	G	I	R	A	W	V	A	W	R	A
HEL	-	-	-	-	S	V	N	-	-	-	K	I	-	S	-	G	N	-	M	N	-	-	-	-	-	-	N
Cal Quail	-	-	-	-	T	V	N	-	-	-	K	I	-	S	-	G	N	-	M	N	-	-	-	-	-	-	N
BW Quail	-	-	-	-	T	V	N	-	-	-	K	I	-	S	-	G	N	-	M	N	-	-	-	-	-	-	N
Jap Quail	-	-	-	-	S	V	N	-	-	-	K	I	-	S	-	V	H	-	M	N	-	-	-	-	-	-	N
Turkey	-	-	-	-	S	V	N	-	-	-	K	I	A	S	G	G	N	-	M	N	-	-	-	-	-	-	N
RN Pheasant	-	-	-	-	S	V	N	-	-	-	K	I	-	S	-	G	N	-	M	N	-	-	-	-	-	K	H
Chachalaca	-	A	P	-	S	V	R	-	-	-	-	I	-	S	-	G	N	-	M	N	-	-	-	-	-	K	H
Duck A	-	-	E	-	-	V	R	-	-	-	-	I	-	S	-	G	N	-	M	N	-	-	-	-	-	-	N
Guinea Fowl	-	-	-	T	T	A	N	-	-	-	K	I	-	S	-	G	N	-	M	N	-	-	-	-	-	K	H

3D molecular analysis of the paratope-epitope interface

A “Structural Epitope” consists of amino acid residues that contact all six CDRs of antibody

It involves 15-25 residues

Which Residues are Critical for Antigen-Antibody Contact?

- Antibody reactivity with natural variants
- Effect of residue substitutions on antigen-antibody binding
  - Site mutagenesis (alanine scanning)
  - Identification of “energetic” contact residues (“hot spots”)
  - Which energetic residues are non-self?

This analysis was done on data published in the literature

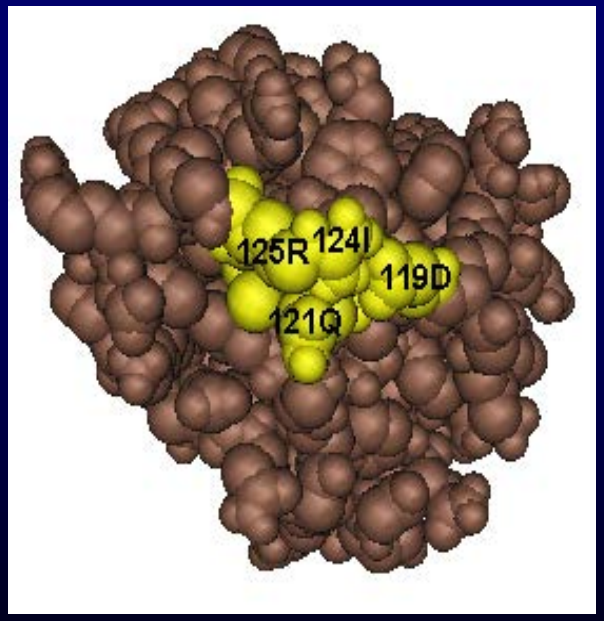
Two Types of Residues in a Structural Epitope

Most residues of a structural epitope can be replaced by other residues without impairing its binding with antibody

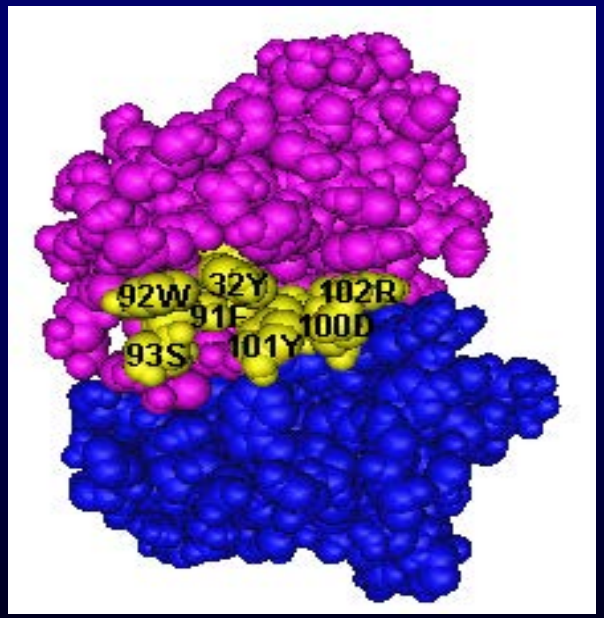
Some residues cannot replaced without causing a major loss in binding activity, they constitute the “hot spot” or “functional” part of the epitope.

Energetic Residues of Functional HEL-D1.3 Epitope, the Corresponding Functional Paratope and in the Epitope-Paratope Interface

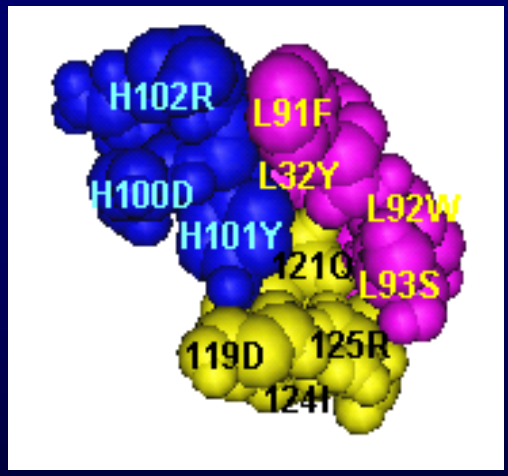
HEL-D1.3 Ep



HyHEL-D1.3 CDR



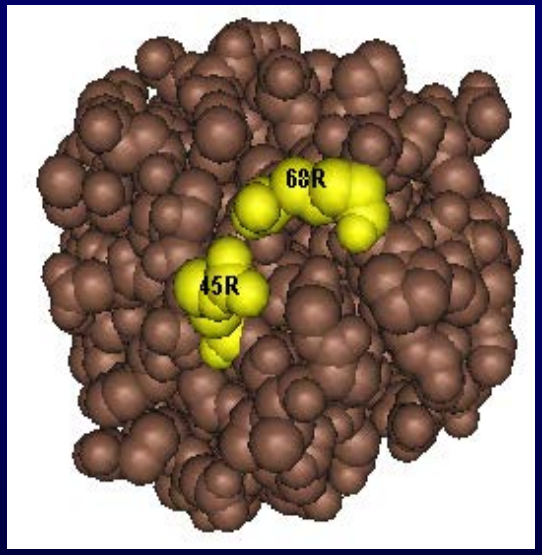
Interface



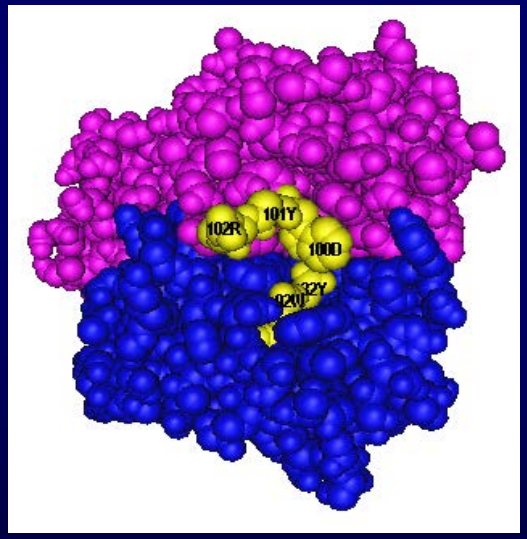
119D, 124I and 125R are within 3.0 Angstroms from $121Q^{NS}$

Functional HEL-5 Epitope

HEL-5 Ep



HyHEL-5 CDR



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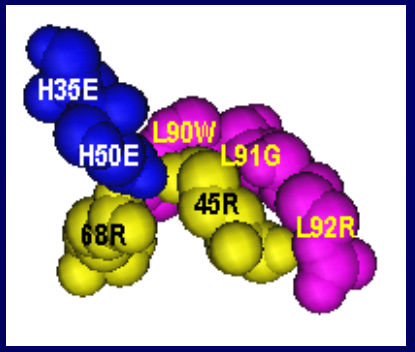
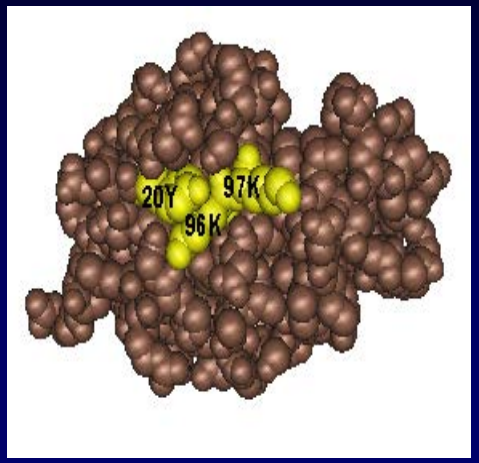
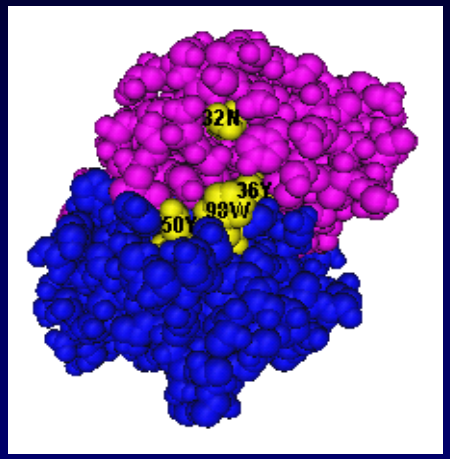


$45R^{NS}$ and $68R^{NS}$ are 3.5 Angstroms apart

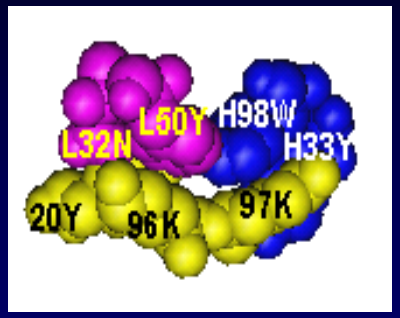
HEL-63 Ep



HyHEL-63 CDR

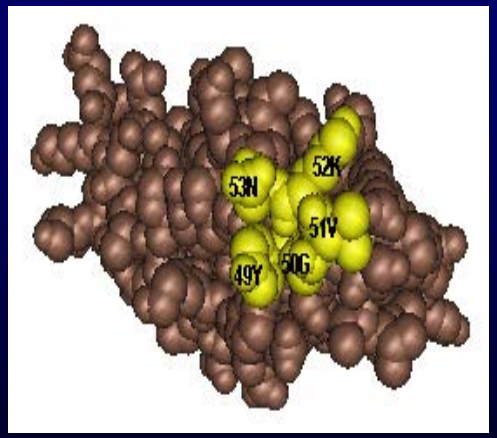


Interface

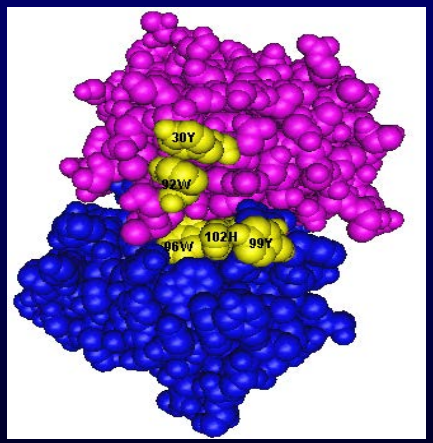


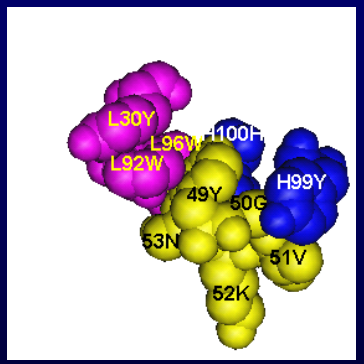
20Y is 3.5 Angstroms away from 97K^{NS}

INFγR-A6 Ep



INFγR-A6 CDR





All functional epitope residues are within 3.0-3.5 Angstroms from each other

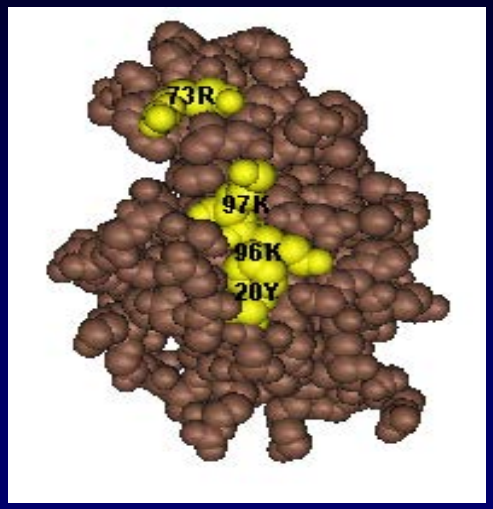
These functional epitopes (“hot spots”) are represented by patches of few residues that are 3.0-3.5 Angstroms apart

Residues are in linear or discontinuous sequences

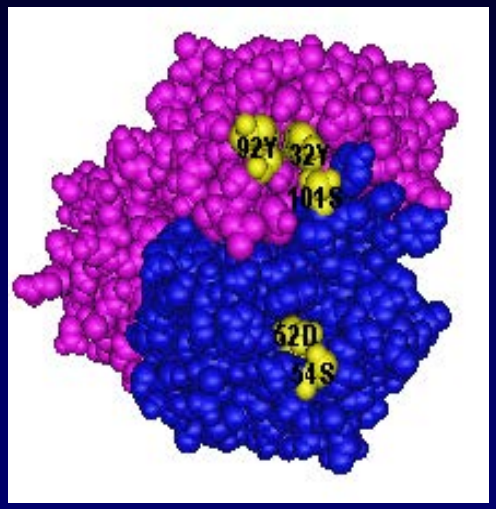
Other epitopes are represented by two distinct patches

Examples of HEL Epitopes Defined by a Pair of Patches

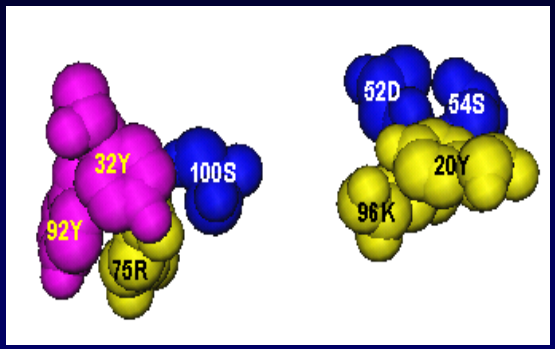
HEL-F9.13.7 Ep



HEL-F9.13.7 CDRs

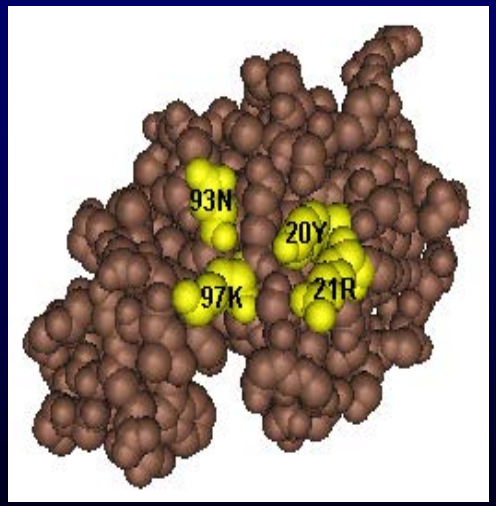


Interface

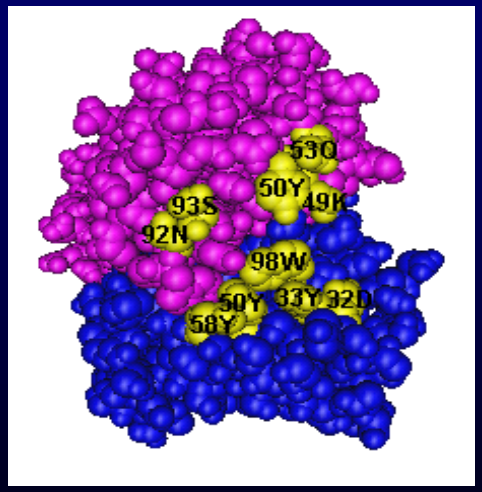


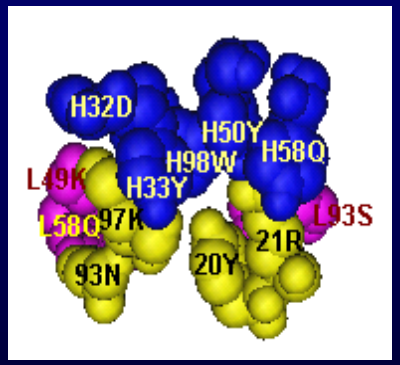
Patch 1: $73R^{ns}$
Patch 2: 20Y and $96K^{ns}$
Distance: 13 Angstroms

HyHEL-10 Ep



HyHEL-10 CDRs

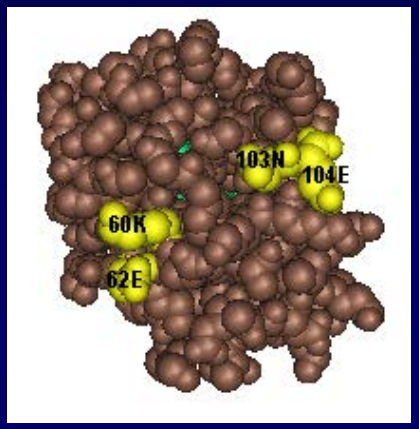




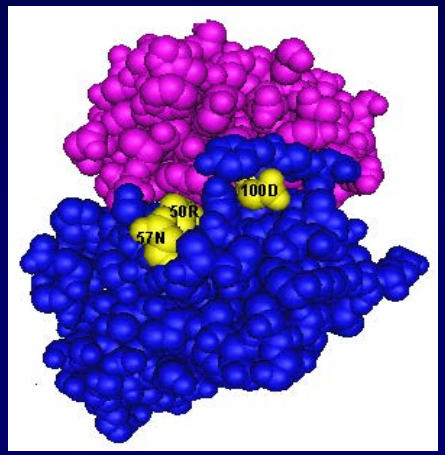
Patch 1: $93N^{NS}$ and $97K^{NS}$
Patch 2: 20Y and 21R (self)
Distance: 7.5 Angstroms

Two-Patch Shape of an Epitope on Horse Cytochrome C Recognized by a Mouse Monoclonal Antibody

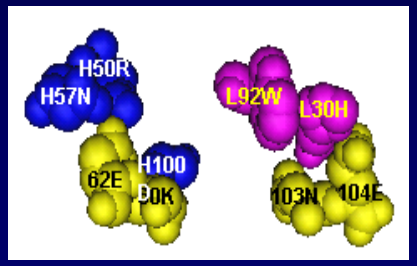
hCytC-E8 Ep



hCYTC-E8 CDR



Interface



Patch 1: 60K^{NS} and 62E^{NS}
Patch 2: 103N and 104E (self)
Patches are 11.5 Angstroms apa

Only Six Non-Self Residues on Horse Cytochrome C

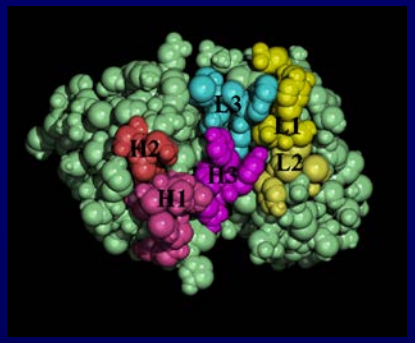
Horse:
2 GDVEKGKKIFVQKCAQCHTVEKGGKHKTGPNLHGLFGRKTGQA**P**GF**T**YTDANKNKGITW**K** 61
Mouse:
2 GDVEKGKKIFVQKCAQCHTVEKGGKHKTGPNLHGLFGRKTGQAAGFSYTDANKNKGITWG 61

Horse
62 E**E**TLMEYLENPKKYIPGTKMIFAGIKKK**T**ER**E**DLIAYLKKATNE 105
Mouse
62 EDTLMEYLENPKKYIPGTKMIFAGIKKKGERADLIAYLKKATNE 105

How Do Antibodies Bind to Epitopes?

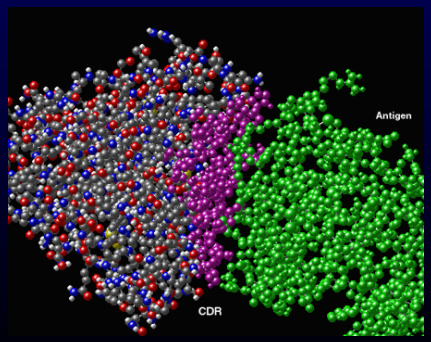
- So-called "structural" epitopes on protein antigens have 15-25 surface residues that can make contact with all six CDRs of antibody
- These residues cover an area of 700-900 square Ångstroms
- Within each lies a centrally located "functional" epitope with a few residues and which binds to CDR-H3 plays a dominant role in specific recognition

CDRs of Antibody



- Complementarity Determining Regions H1, H2, H3, on heavy chain and L1, L2 and L3 on light chain represent the "binding face" or "paratope" of an antibody

Antigen-Antibody Complex

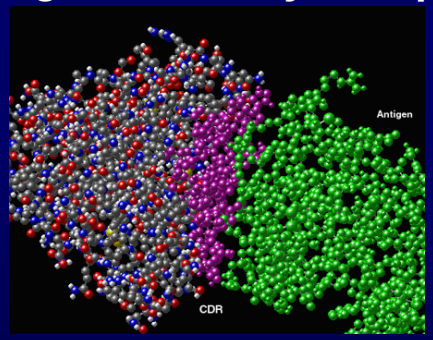


- The antibody specificity is often determined by a centrally located loop (CDR-H3) that binds to a short amino acid sequence of the protein antigen
- Other CDRs serve as contact sites to stabilize binding to antigen (they play a role in the affinity of antibody)

How Do Antibodies Bind to Epitopes on Proteins?

- So-called "structural" epitopes on protein antigens have 15-25 surface residues that can make contact with all six CDRs of antibody
- These residues cover an area of 700-900 square Ångstroms
- Within each lies a centrally located "functional" epitope with a few residues and which binds to CDR-H3 plays a dominant role in specific recognition

Antigen-Antibody Complex



CDRs of Antibody

HEL antibodies

Hen Egg Lysozyme Epitopes

Antibody CDR loops

L1: light blue

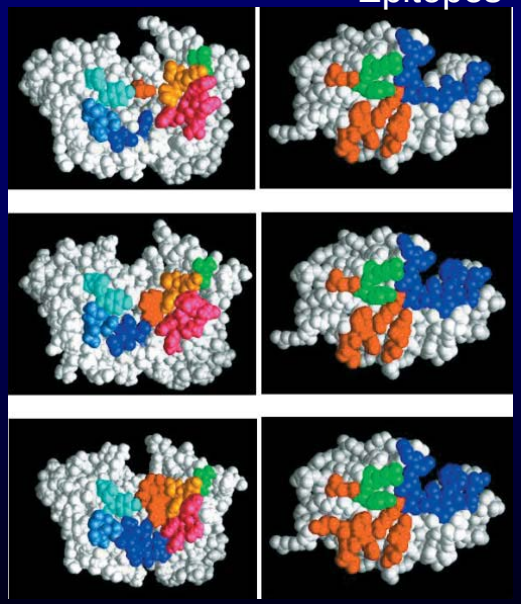
L2: cyan

L3: dark blue

H1: orange

H2: magenta

H3: red



Antigen residues contacted by

Light chains: orange

Heavy chains: blue

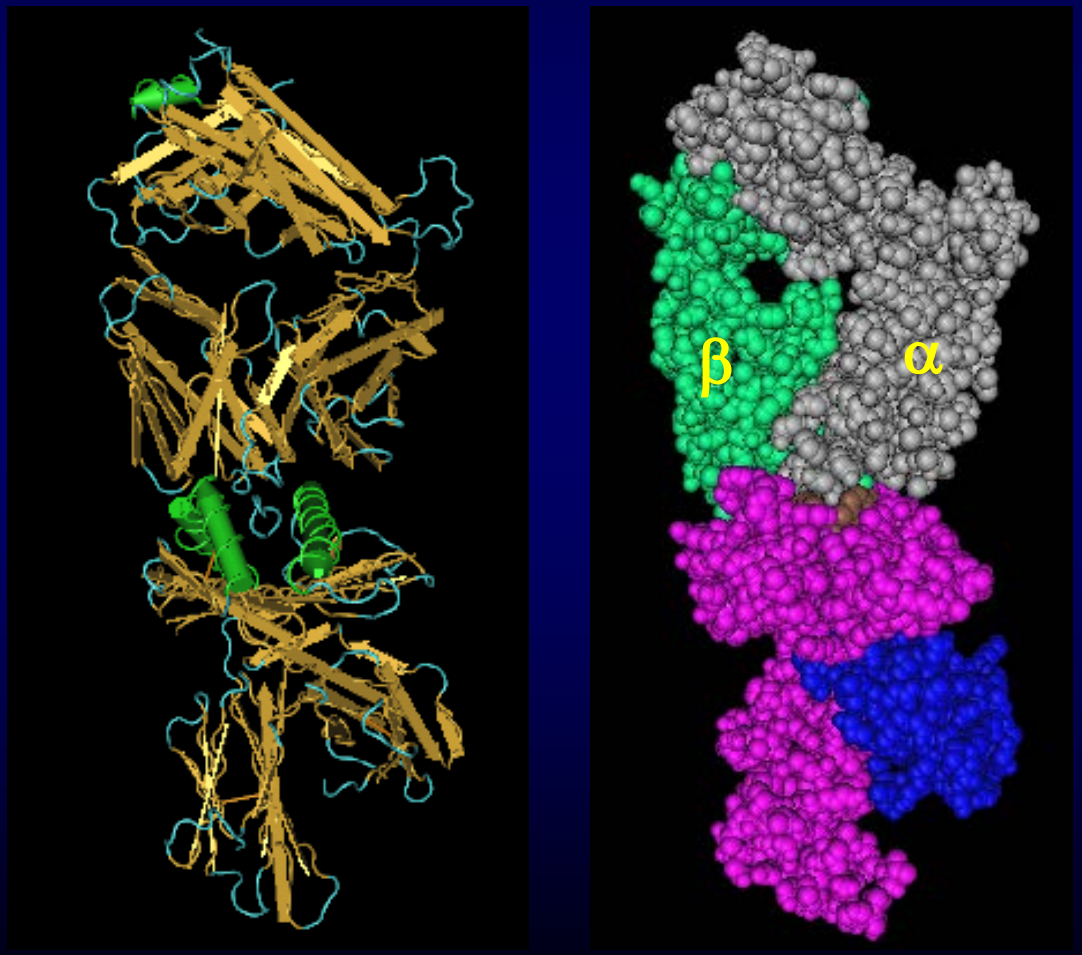
Both chains: green

Mohan et al. Biophys J. 85:3221, 2003

Structural Aspects of Complexes with HLA Molecules

- Crystal models of MHC-TCR complexes
- Crystal structure of antibody-HLA + peptide complex

T-Cell Receptor-HLA Complex



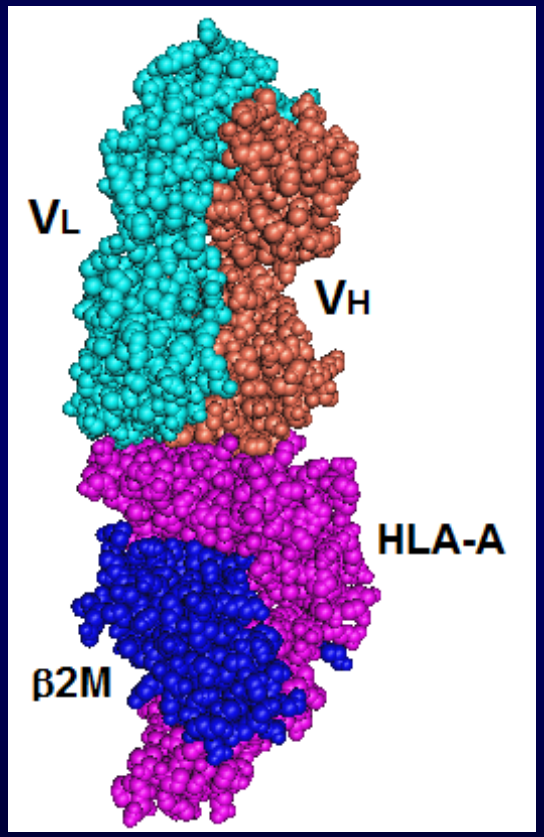
Crystal Structure of HLA-A1-MAGE-A1 Complex with Antibody Fab-Hyb3

- Structural epitope: 4 peptide residues + 9 HLA-A1 residues in α helices
- Paratope: 11 VH and 4 VL residues
- Predominant role of CDR-H2 and H3 in peptide contact

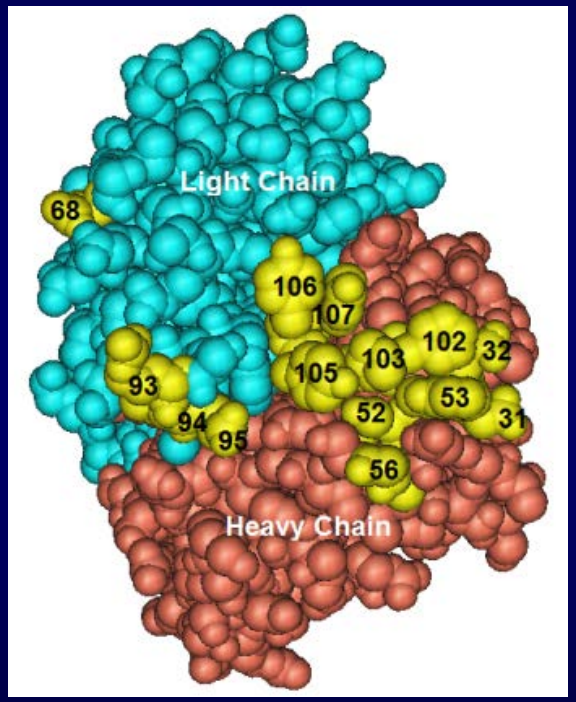
Hulsmeyer et al J. Biol. Chem. 280: 2972-2790, 2005
(Andrew Ziegler's lab at Humboldt University, Berlin)

Molecular modeling of the crystalline structure of human monoclonal antibody Hyb3 complexed with HLA-A1-MAGE-A1 as reported by Ziegler's group*

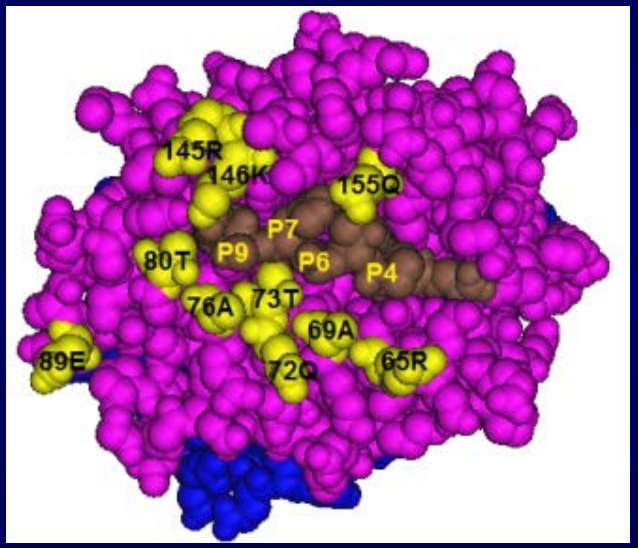
Antigen-Antibody Complex



Structural paratope of Hyb3



Structural epitope of HLA-A1-MAGE-A1



Contact residues are within a molecular surface of about 900 Å^2

*Hulsmeyer et al. J Biol. Chem. 280:2972-2980, 2005

Crystal Structure of HLA-A1-MAGE-A1 Complex with Antibody Fab-Hyb3

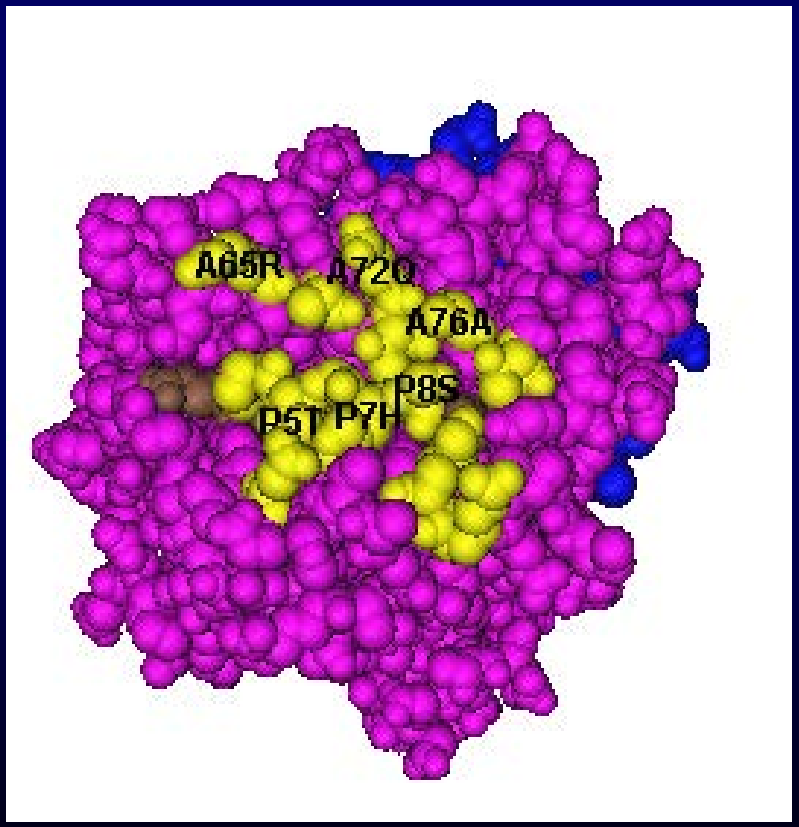
- Structural epitope: 4 peptide residues + 9 HLA-A1 residues in α helices
- Paratope: 11 VH and 4 VL residues
- Predominant role of CDR-H2 and H3 in peptide contact
- Functional epitope:
  - Contact sites: 65R and 72Q of HLA-A1
  - Antibody does not react with MAGE-A1 presented by HLA-A3 (only difference is contact residue 76A)
  - Antibody does not react with MAGE-A3 which has three residue differences
  - No mutagenesis information

Hulsmeyer et al J. Biol. Chem. 280: 2972-2790, 2005
(Andrew Ziegler's lab at Humboldt University, Berlin)

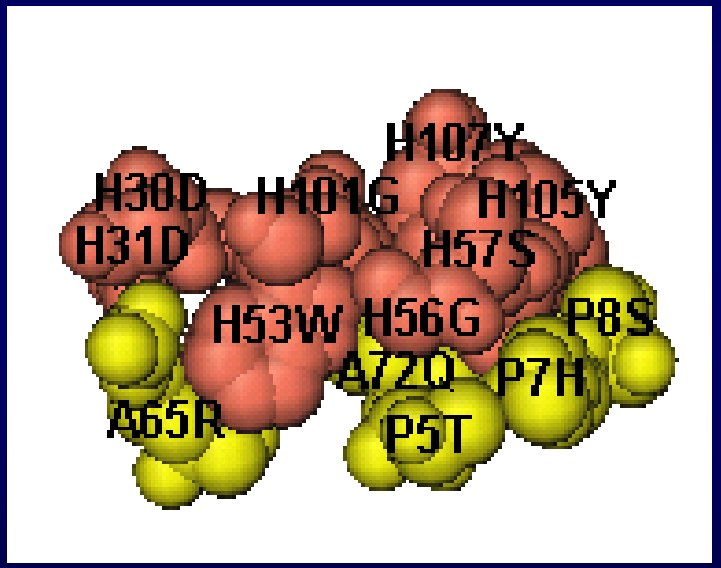
Prominent residues of the structural MAGE-A1-HLA-A1-Hyb3 epitope and their interface with the Hyb3 paratope

(adapted from Hulsmeyer et al, 2005)

epitope



paratope



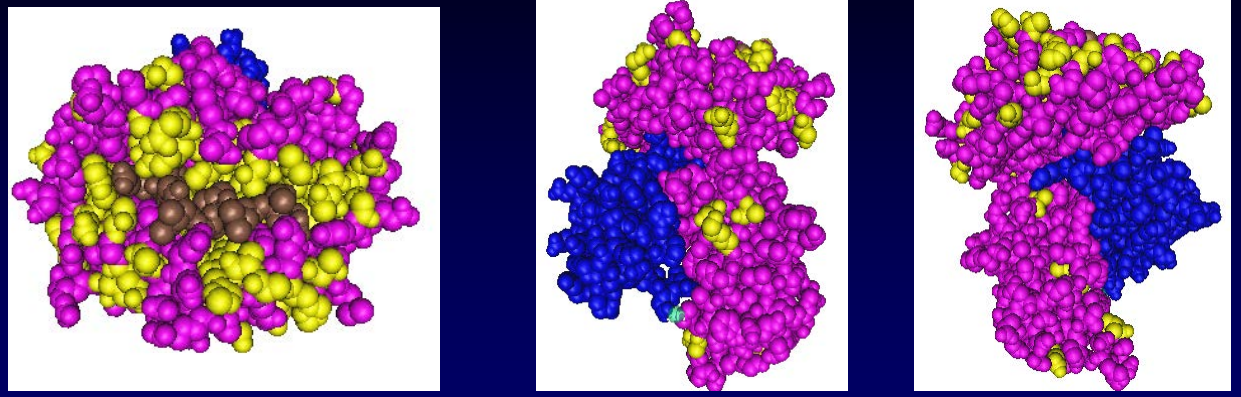
Patches are 6-8 Angstroms away

How do we describe HLA epitopes?

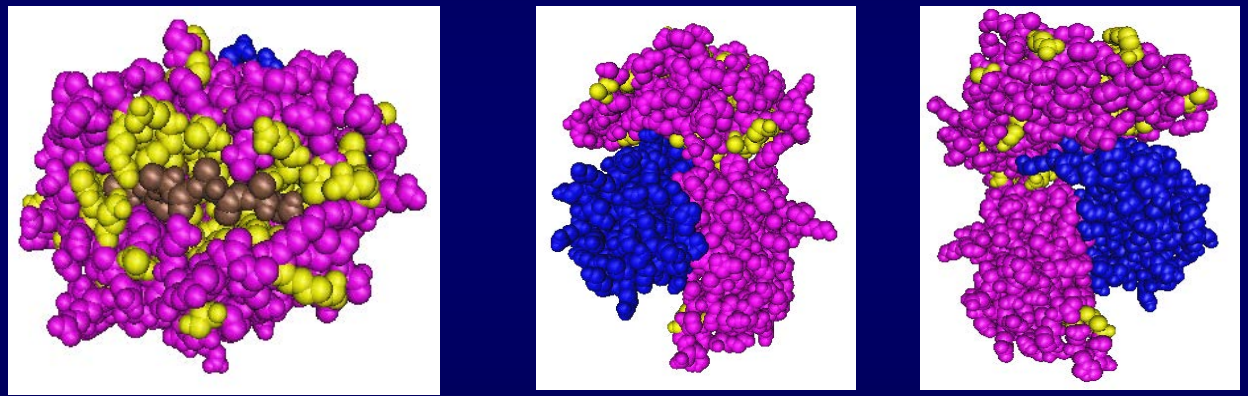
- Public and private HLA determinants (Cross-Reacting Groups, CREG)

Polymorphic Residues on Class I Antigens

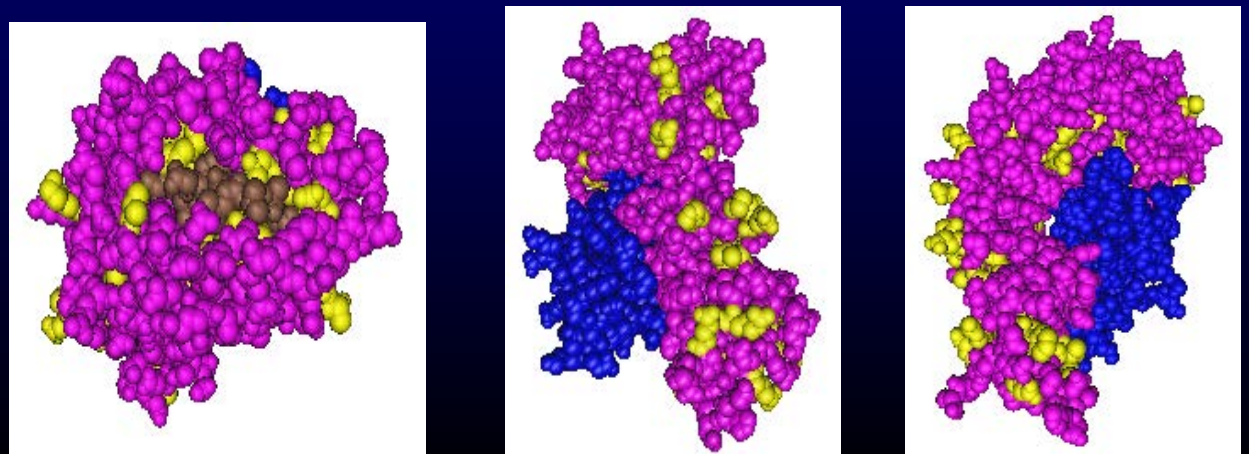
HLA-A2



HLA-B27



HLA-Cw3



How do we describe HLA epitopes?

- Public and private HLA determinants (Cross-Reacting Groups, CREG)
- Single amino acid residues
- HLAMatchmaker: Triplets (2002)
- HLAMatchmaker: Eplets (2006)

Concepts about Antibody-Antigen Interactions

- Contact areas of CDR loops of antibody and epitopes on protein antigens (paratope-epitope interface)
- Determination of “structural” epitopes
- Identification of “functional” epitopes
- Determine the sizes and shapes of functional epitopes

Apply concepts to eplet version of HLAMatchmaker

Functional epitopes are represented by patches of few residues that are 3.0-3.5 Angstroms apart

In HLAMatchmaker:
Eplets are patches consisting of a polymorphic residue and residues within a 3.0-3.5 Angstrom radius

Recent Update:
Designing Epitope Repertoires for HLAMatchmaker and the HLA Epitope Registry

Reference:

Update of the HLA Class I Eplet Database in the Website Based Registry of Antibody-Defined HLA Epitopes, Tissue Antigens, 83:382-390, 2014

Eplets

- Essential components of HLA epitopes recognized by antibody
- Amino acid configurations within a 3 Angstrom radius of surface-exposed polymorphic residues
- Parts of “structural” HLA epitopes that contact the CDRs of antibody

Design

1. With Cn3D modelling of HLA molecules identify all combinations of polymorphic residues within a 3 Ångstrom radius

There are 116 configurations for exposed residues and 21 configurations solely defined by hidden polymorphic residues

exposed	seq	seq	seq
1	1		
14	14	17	
16	16		
19	19		
41	41		
44	44	45	46
45	45	46	
56	56		
62	62	63	
62	62	63	65
62	62	63	66
62	62	65	
62	62	65	66
62	62	66	
62	62		
63	63	65	
63	63	66	
63	63	65	66
65	65	66	
65	65	66	67
65	65	66	69
65	65	69	
65	65		
66	66	67	
66	66	67	69
66	66	69	
66	66	69	70
66	66		
69	69	70	71
69	69	70	
69	69	70	73
69	69	71	
69	69	73	
69	69	73	77
69	69		
70	70	73	
70	70		
71	71	73	77
73	73	74	

exposed	seq	seq	seq
73	73	74	76
73	73	76	
73	73	76	77
73	73		
76	76	77	
76	76	77	79
76	76	79	
76	76	77	80
76	76	80	
76	76	79	80
76	76		
77	77	79	80
80	80	81	82
80	80		
80	80	81	
80	80	82	83
82	81	82	83
82	82	83	
82	82		
90	90	91	
102	102	103	
105	105		
107	107		
109	109		
127	127		
131	131		
138	138		
138	138	142	
142	142	143	144
142	142	145	
144	144	145	
144	144	149	
144	144		
145	145	149	
145	145		
147	147		
149	149	150	151
149	149	150	
149	149		

exposed	seq	seq	seq
149	149	151	
150	150	151	152
150	150	151	
150	150		
151	151		
151	151	152	
156	156		
156	156	158	
158	158		
161	161		
162	162	163	167
163	163		
163	163	166	
163	163	166	167
166	166	167	
166	166		
170	170	171	
173	173		
177	177		
177	177	178	
180	180		
182	182	183	184
186	186		
193	193	194	
193	193		
194	194		
207	207		
211	211		
219	219		
245	245	246	
247	247		
248	248		
249	249		
253	253		
261	261	270	
211	211	212	
273	273		
275	275	276	

hidden	seq	seq
4	4	
6	6	
9	9	
11	11	12
21	21	
24	24	
30	30	
32	32	
35	35	
49	49	
67	67	
70	70	
74	74	
94	94	95
97	97	
99	99	
113	113	114
116	116	
152	152	
156	156	
199	199	

Configurations of polymorphic sequence positions on class I HLA molecules

Design

1. With Cn3D modelling of HLA molecules identify all combinations of polymorphic residues within a 3 Ångstrom radius
There are 116 configurations for exposed residues and 21 configurations solely defined by hidden polymorphic residues

2. With HLAPatch Generator (developed by Dr. Grzegorz Dudek (now at Czestchowa University of Technology, Poland). determine polymorphic residue compositions of 3 Ångstrom patches from amino acid sequences of 107 HLA alleles in commercially available Luminex panels

Results: 616 patches were identified, 82 of them are monomorphic for one or two ABC loci and they were ruled out as potential epitopes. Many of the remaining 534 patches are shared by identical groups of Luminex alleles.

Multiple patches shared between the same group of Luminex alleles

Patch	Luminex Alleles						
73TAN	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
73TA	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76ANG	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76AGT	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76AG	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76AT	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76A	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76AN	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76ANT	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01

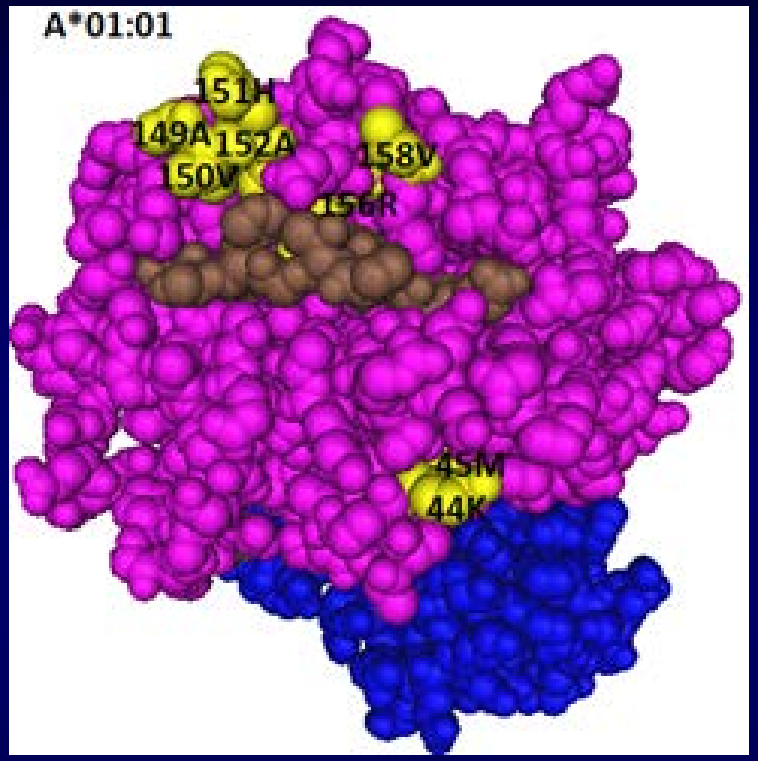
Patches shared between the same group of Luminex alleles

Patch	Luminex Alleles						
73TAN	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
73TA	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76ANG	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76AGT	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76AG	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76AT	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76A	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76AN	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01
76ANT	A*01:01	A*26:01	A*29:01	A*29:02	A*36:01	A*43:01	A*80:01

Eplet: 76ANT Residue description: 73T76A77N79G80T

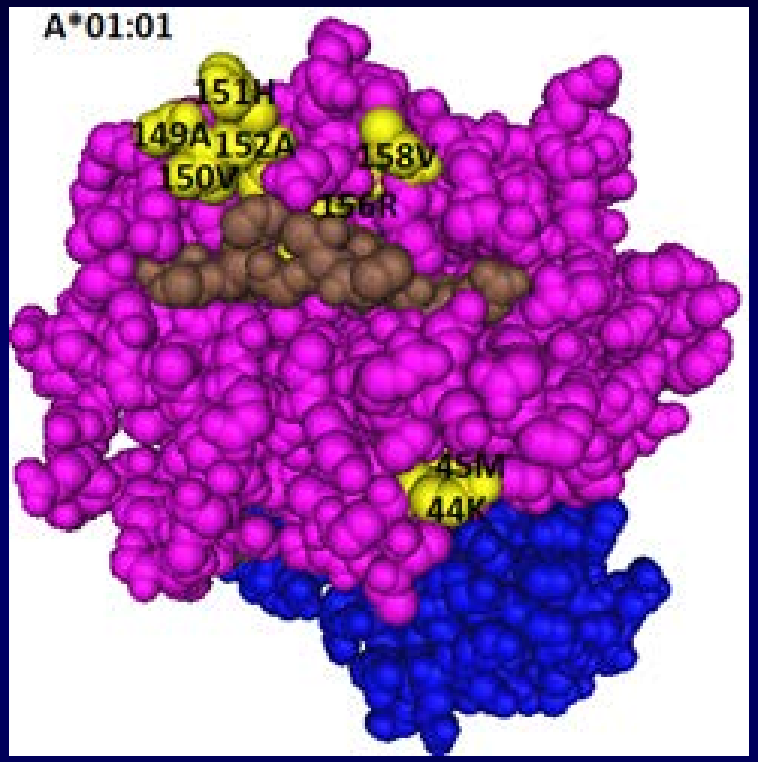
Patches shared between A*01:01 and A*36:01

Patch	Luminex Alleles	
44KM	A*01:01	A*36:01
149AVH	A*01:01	A*36:01
149AV	A*01:01	A*36:01
150V	A*01:01	A*36:01
150VH	A*01:01	A*36:01
150VHA	A*01:01	A*36:01
156RV	A*01:01	A*36:01
158V	A*01:01	A*36:01



Patches shared between A*01:01 and A*36:01

Patch	Luminex Alleles	
44KM	A*01:01	A*36:01
149AVH	A*01:01	A*36:01
149AV	A*01:01	A*36:01
150V	A*01:01	A*36:01
150VH	A*01:01	A*36:01
150VHA	A*01:01	A*36:01
156RV	A*01:01	A*36:01
158V	A*01:01	A*36:01



Eplet: $44KM_3$ Description: 44K45M(149A150V151H152A)(156R158V)

Overlapping eplets that are shared between similar but not identical groups of Luminex alleles

Example: six 62R-related eplets on Luminex alleles

Eplet	Residues	A*25:01	A*26:01	A*33:01	A*33:03	A*34:01	A*34:02	A*66:01	A*66:02	A*68:01	A*68:02	A*69:01	B*15:16	Other
62RR	62R65R	+	+	+	+	+	+	+	+	+	+	+	+	
62RNR	62R63N65R	+	+	+	+	+	+	+	+	+	+	+	has 63E	
62RN	62R63N	+	+	+	+	+	+	+	+	+	+	+	has 63E	32 HLA-B alleles
62RRN	62R65R66N	+	+	+	+	has 66K	+	+	+	+	+	+	+	
62RTN	62R64T66N	+	+	+	+	has 66K	+	+	+	+	+	+	+	C*07:01, C*15:02
63NN	62R63N65R66N	+	+	+	+	has 66K	+	+	+	+	+	+	has 63E	

Design

1. With Cn3D modelling of HLA molecules identify all combinations of polymorphic residues within a 3 Ångstrom radius
There are 116 configurations for exposed residues and 21 configurations solely defined by hidden polymorphic residues

2. With HLAPatch Generator (developed by Dr. Grzegorz Dudek, Czestchowa University of Technology, Poland) determine polymorphic residue compositions of 3 Ångstrom patches from amino acid sequences of 107 HLA alleles in commercially available Luminex panels

Results: 616 patches were identified, 82 of them are monomorphic for one or two class I loci and were ruled out as potential epitopes. Many of the remaining 534 patches are shared by identical groups of Luminex alleles

3. Annotations of 219 eplets defined by polymorphic residues on the molecular surface and 51 eplets defined solely by polymorphic residues in antibody-inaccessible positions

219 Eplets in antibody-accessible positions on the molecular surface of HLA-A, B, C antigens

Eplet	#A	#B	#C	Total	Eplet	#A	#B	#C	Total	Eplet	#A	#B	#C	Total
1C	0	0	17	17	71ATN	0	4	4	8	145KHA	7	0	0	7
16S	0	0	3	3	71ATS	0	11	8	19	145RT	7	0	0	7
17RS	2	0	0	2	71HS	22	0	0	22	147L	0	3	4	7
17WR	0	0	1	1	71KA	0	4	0	4	149AH	14	0	0	14
19K	1	0	0	1	71QS	13	0	0	13	149TAH	8	0	0	8
41T	0	13	0	13	71SA	0	4	0	4	150AAH	12	0	0	12
43RRM	2	0	0	2	71STN	12	0	0	12	150AH	20	0	0	20
44KM$_3$	2	0	0	2	71TTD	0	2	0	2	151AHA	2	0	0	2
44RM	33	12	0	45	71TTN	0	12	0	12	151AHE	9	0	0	9
44RMA	0	11	0	11	71TTS	0	27	0	27	151AHV	9	0	0	9
44RME	33	0	0	33	72QTD	25	22	12	59	151ARV	9	35	0	44
44RT	0	10	0	10	73ID	3	0	0	3	151H	22	0	0	22
45EE	0	26	0	26	73AN	0	0	6	6	152HA	4	0	0	4
45KE	0	11	0	11	73AS	0	0	5	5	152RA	0	0	4	4
56E$_4$	1	0	0	1	73TD	17	4	0	21	152RE	0	24	18	42
56R	3	0	0	3	73TDA	6	0	0	6	152RR	2	0	0	2
62EE	5	0	0	5	73TDE	7	20	0	27	152RT	0	0	1	1
62GE	5	3	0	8	73TDV	12	2	12	26	152RW	1	0	0	1
62GK$_2$	5	0	0	5	73TN	12	16	4	32	156DA	0	8	1	9
62GRN	0	3	0	3	73TS	2	37	9	48	156LA	16	40	8	64
62LQ	3	0	0	3	73TV	17	2	12	31	156QA	4	0	1	5
62QE	10	0	0	10	73TVD	17	0	0	17	156RA	0	3	8	11
62REN	0	1	2	3	73TVN	0	0	4	4	156WA	12	4	5	21
62RER	0	1	0	1	73TVS	0	1	8	9	158T	0	4	0	4
62RK	1	1	21	23	73TY	0	37	0	37	161D	1	0	0	1
62RN	11	32	0	43	76ANT	7	0	0	7	162DLS	0	1	0	1
62RNQ	0	32	0	32	76ED	0	4	0	4	162GLS	0	4	0	4
62RNR	11	0	0	11	76EG	1	0	0	1	163E	2	14	3	19
62RR	11	1	0	12	76EN	5	16	0	21	163EW	1	14	3	18
62RRN	10	1	0	11	76ENI	4	12	0	16	163L	0	29	3	32
62RTN	10	1	2	13	76ENR	4	16	0	20	163LE	0	28	3	31
63EI	0	22	0	22	76ENT	1	4	0	5	163LG	0	1	0	1
63EK	9	1	21	31	76ES	2	37	0	39	163LW	0	23	3	26
63EN	11	4	2	17	76ESI	2	0	0	2	163R	7	0	0	7
63ER	16	4	0	20	76ESN	0	37	0	37	163RG	1	0	0	1
63ERN	12	4	0	16	76ET	1	8	0	9	163RW	6	0	0	6

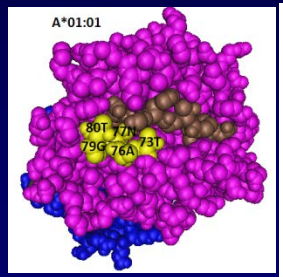
Eplet	#A	#B	#C	Total	Eplet	#A	#B	#C	Total	Eplet	#A	#B	#C	Total
63NN	10	0	0	10	76VDT	20	0	0	20	163T	25	16	17	58
65GK	4	0	0	4	76VRN	0	2	13	15	163TEW	22	16	17	55
65QIA	0	13	0	13	76VS	0	1	13	14	163TG	3	0	0	3
65QKR	0	1	21	22	77NGT	8	0	0	8	166DG	5	1	0	6
65QNR	0	0	2	2	77SRN	0	38	13	51	166ES	0	5	0	5
65RA	30	4	0	34	79GT	28	0	0	28	170RH	1	9	0	10
65RK	6	0	0	6	80I	6	12	0	18	170RY	35	50	16	99
65RNA	24	4	0	28	80K	0	0	10	10	173K	0	0	3	3
66I	0	54	0	54	80N	0	39	13	52	177DK	0	5	0	5
66IC	0	13	0	13	80T	28	8	0	36	177DT	0	3	0	3
66ICT	0	9	0	9	80TA	0	4	0	4	177KT	0	0	4	4
66IF	0	8	0	8	80TL	28	4	0	32	180E	0	8	0	8
66IS	0	22	0	22	80TLR	0	8	0	8	182TDP	15	0	0	15
66IY	0	11	0	11	81ALR	6	16	0	22	184A	19	0	0	19
66IYT	0	2	0	2	82LR	6	20	0	26	184H	0	0	19	19
66K	10	1	21	32	90AR	0	0	1	1	184R$_3$	0	0	1	1
66KA	10	0	0	10	90D	11	1	8	20	186R	1	0	0	1
66KAH	9	0	0	9	102DV	33	42	3	78	193AV	23	0	0	23
66N	24	4	2	30	102HV	1	0	0	1	193LV	0	0	1	1
66NAH	12	0	0	12	103L	0	16	20	36	193PI	12	51	0	63
66NAQ	12	0	0	12	103M$_2$	0	1	0	1	193PL$_3$	0	0	3	3
66NM	2	4	0	6	105S	21	0	0	21	193PV	0	8	19	27
66NV	23	0	0	23	107W	6	0	0	6	194V	22	8	20	50
66RKQ	1	0	0	1	109F	32	0	0	32	207S	23	0	0	23
69AA	0	17	0	17	109FE	31	0	0	31	211T	0	0	1	1
69AQT	13	9	0	22	127K	12	0	0	12	219W	0	0	9	9
69AT	31	17	0	48	131S	0	47	0	47	245AS	14	0	0	14
69ATD	17	2	0	19	138K	0	0	2	2	245TA	0	2	0	2
69ATN	12	4	0	16	138MI	26	0	0	26	245VA	2	0	0	2
69ATS	2	10	0	12	142ITQ	18	56	22	96	248M	0	0	1	1
69RA	0	0	11	11	143S	0	3	1	4	253Q	22	1	4	27
69RT	0	1	12	13	144K	16	0	0	16	267PE	1	0	19	20
69TNT	0	41	0	41	144KA	15	0	0	15	267QE	0	1	4	5
70HT	18	0	0	18	144QL	0	2	0	2	270C	0	1	1	2
70IAQ	0	9	0	9	144TKH	8	0	0	8	275EL	7	0	0	7
70QT	13	10	12	35	144KR	8	0	0	8	275G	0	0	3	3
71ATD	0	2	0	2	145HT	1	0	0	1	275K	0	1	5	6

51 Eplets based solely on residue polymorphisms in antibody-inaccessible sequence positions

Eplet	#A	#B	#C	Total	Eplet	#A	#B	#C	Total	Eplet	#A	#B	#C	Total
9D	0	1	6	7	94IL	0	0	1	1	113HD	0	16	1	17
9F	10	0	1	11	94TF	0	0	1	1	113HN	0	26	0	26
9H	0	14	0	14	94TI	25	1	1	27	113YD	0	4	14	18
9S	6	0	2	8	94TL	6	31	17	54	113YH	11	4	0	15
9T	5	0	0	5	94TV	4	0	0	4	113YN	0	9	8	17
9Y	14	44	14	72	94TW	0	16	0	16	113YQ	12	0	0	12
11AM	0	37	0	37	97I	9	0	0	9	113YR	10	0	0	10
11AV	0	3	20	23	97M	13	0	0	13	116D	22	6	0	28
11SM	1	0	0	1	97N	0	3	0	3	116F	0	10	9	19
11SV	34	19	3	56	97R	13	32	18	63	116L	0	11	1	12
21H	0	0	7	7	97S	0	9	0	9	116S	0	15	10	25
24S	0	21	7	28	97T	0	11	0	11	116Y	11	17	3	31
24T	0	17	0	17	97V	0	2	0	2	152A	4	0	4	8
30G	0	1	0	1	97W	0	2	5	7	152E	9	24	18	51
32L	0	14	0	14	99F	4	3	5	12	152V	19	35	0	54
35Q	1	0	3	4	99S	0	1	1	2	156L	17	44	8	69
94II	0	11	3	14	99Y	31	55	13	99	156R	2	3	8	13
										199V	0	2	0	2

Eplets on Non-Luminex Alleles

Comparisons of relevant amino acid configurations between 76ANT-carrying Luminex alleles and non-Luminex alleles



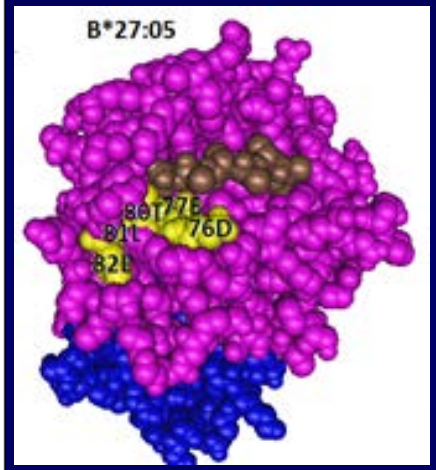
Selected Alleles		73	76	77	79	80	Selected Alleles	73	76	77	79	80	Selected Alleles		73	76	77	79	80
A*01:01	LUM	T	A	N	G	T	A*26:06	T	V	D	G	T	A*26:37		T	A	N	G	T
A*01:02		T	A	N	G	T	A*26:07	T	A	N	G	T	A*29:01	LUM	T	A	N	G	T
A*01:03		T	A	N	G	T	A*26:08	T	A	N	G	T	A*29:02	LUM	T	A	N	G	T
A*01:06		T	A	N	G	T	A*26:09	T	A	N	G	T	A*29:03		T	A	N	G	T
A*01:07		T	E	N	G	T	A*26:10	T	A	N	G	T	A*29:04		T	A	N	G	T
A*01:08		T	A	N	G	T	A*26:12	T	A	N	G	T	A*29:05		T	A	N	G	T
A*01:09		T	A	N	G	T	A*26:13	T	A	N	G	T	A*29:06		T	A	N	G	T
A*01:10		T	A	N	G	T	A*26:14	T	A	N	G	T	A*29:07		T	A	N	G	T
A*01:12		T	A	N	G	T	A*26:15	T	A	N	G	T	A*29:09		T	A	N	G	T
A*01:13		T	V	D	G	T	A*26:16	T	A	N	G	T	A*29:10		T	A	N	G	T
A*01:14		T	A	N	G	T	A*26:17	T	A	N	G	T	A*29:11		T	A	N	G	T
A*01:17		T	A	N	G	T	A*26:18	T	A	N	G	T	A*29:12		T	A	N	G	T
A*01:19		T	A	N	G	T	A*26:19	T	A	N	G	T	A*29:13		T	E	S	R	I
A*01:20		T	A	N	G	T	A*26:20	T	A	N	G	T	A*29:14		T	A	N	G	T
A*01:21		T	A	N	G	T	A*26:21	T	V	D	G	T	A*29:15		T	A	N	G	T
A*01:23		T	A	N	G	T	A*26:22	T	A	N	G	T	A*29:16		T	A	N	G	T
A*01:24		T	A	N	G	T	A*26:23	T	A	N	G	T	A*29:17		T	A	N	G	T
A*01:25		T	A	N	G	T	A*26:24	T	A	N	G	T	A*29:18		T	A	N	G	T
A*01:26		T	A	N	G	T	A*26:26	T	A	N	G	T	A*29:19		T	V	D	G	T
A*01:28		T	V	D	G	T	A*26:27	T	A	N	G	T	A*36:01	LUM	T	A	N	G	T
A*01:29		T	A	N	G	T	A*26:28	T	A	N	G	T	A*36:02		T	A	N	G	T
A*01:30		T	A	N	G	T	A*26:29	T	A	N	G	T	A*36:03		T	A	N	G	T
A*01:32		T	A	N	G	T	A*26:30	T	V	D	G	T	A*36:04		T	A	N	G	T
A*01:33		T	A	N	G	T	A*26:31	T	A	N	G	T	A*43:01	LUM	T	A	N	G	T
A*26:01	LUM	T	A	N	G	T	A*26:32	T	A	N	G	T	A*80:01	LUM	T	A	N	G	T
A*26:02		T	A	N	G	T	A*26:33	I	A	N	G	T	A*11:17		T	A	N	G	T
A*26:03		T	V	D	G	T	A*26:34	T	A	N	G	T	A*11:40		T	A	N	G	T
A*26:04		T	A	N	G	T	A*26:35	T	A	N	G	T	A*24:04		T	A	N	G	T
A*26:05		T	E	N	G	T	A*26:36	T	A	N	G	T	A*74:10		T	A	N	G	T

Residue configurations of non-Luminex alleles in relation to an eplet annotated as 44KM$_3$ and shared between A*01:01 and A*36:01

Alleles		44	45	149	150	151	152	156	158
A*01:01 LUM	44KM$_3$	K	M	A	V	H	A	R	V
A*36:01 LUM	44KM$_3$	K	M	A	V	H	A	R	V
A*01:02	44KM$_3$	K	M	A	V	H	A	R	V
A*01:03	44KM$_3$	K	M	A	V	H	A	R	V
A*01:06		K	M	A	V	H	A	L	A
A*01:07	44KM$_3$	K	M	A	V	H	A	R	V
A*01:08	44KM$_3$	K	M	A	V	H	A	R	V
A*01:09	44KM$_3$	K	M	A	V	H	A	R	V
A*01:10		K	M	A	A	R	R	R	V
A*01:12		K	M	A	A	H	V	Q	A
A*01:13	44KM$_3$	K	M	A	V	H	A	R	V
A*01:14	44KM$_3$	K	M	A	V	H	A	R	V
A*01:17	44KM$_3$	K	M	A	V	H	A	R	V
A*01:19		K	M	A	A	H	V	Q	A
A*01:20	44KM$_3$	K	M	A	V	H	A	R	V
A*01:21		K	M	A	A	H	V	R	V
A*01:23	44KM$_3$	K	M	A	V	H	A	R	V
A*01:24	44KM$_3$	K	M	A	V	H	A	R	V
A*01:25		K	M	A	V	H	A	Q	V
A*01:26		K	M	A	A	H	A	R	V
A*01:28	44KM$_3$	K	M	A	V	H	A	R	V
A*01:29	44KM$_3$	K	M	A	V	H	A	R	V
A*01:30	44KM$_3$	K	M	A	V	H	A	R	V
A*01:32	44KM$_3$	K	M	A	V	H	A	R	V
A*01:33	44KM$_3$	K	M	A	V	H	A	R	V
A*01:35	44KM$_3$	K	M	A	V	H	A	R	V
A*36:02		K	M	A	V	H	A	R	A
A*36:03	44KM$_3$	K	M	A	V	H	A	R	V
A*36:04	44KM$_3$	K	M	A	V	H	A	R	V
A*03:18		R	M	A	V	H	A	R	V
A*68:41		R	M	A	V	H	V	W	A

Residue configurations of non-Luminex alleles in relation to the 76ED and 80TLL eplets shared between Luminex alleles B*27:03, B*27:05, B*37:01 and B*47:01

Alleles	76ED/80TLL Eplet	76	77	80	81	82
B*27:03 LUM	76ED and/or 80TLL	E	D	T	L	L
B*27:05 LUM	76ED and/or 80TLL	E	D	T	L	L
B*37:01 LUM	76ED and/or 80TLL	E	D	T	L	L
B*47:01 LUM	76ED and/or 80TLL	E	D	T	L	L
B*27:07/09/10/13/14/16/17/19/27/28/	76ED and/or 80TLL	E	D	T	L	L
29/32/34/35/37/38/39/41/43/45/46/50	76ED and/or 80TLL	E	D	T	L	L
B*37:02/04/06/07/08/09/12/13, B*47:05	76ED and/or 80TLL	E	D	T	L	L
B*07:27, B*15:43,B*38:17, B*53:03	76ED and/or 80TLL	E	D	T	L	L
B*27:42, B*37:05	76ED only	E	D	N	L	R
B*27:04/06/11/15/20/21/23/24/25/31/36	80TLL only	E	S	T	L	L
B*27:08 LUM	not76ED/80TLL	E	S	N	L	R
B*27:12/18/26/33/40/44, B*37:11, B*47:02	not76ED/80TLL	E	S	N	L	R
B*37:34, B*47:03	not76ED/80TLL	E	S	N	L	L
B*27:01/02/30, B*37:10, B*47:04	not 76ED/80TLL	E	N	T	A	L



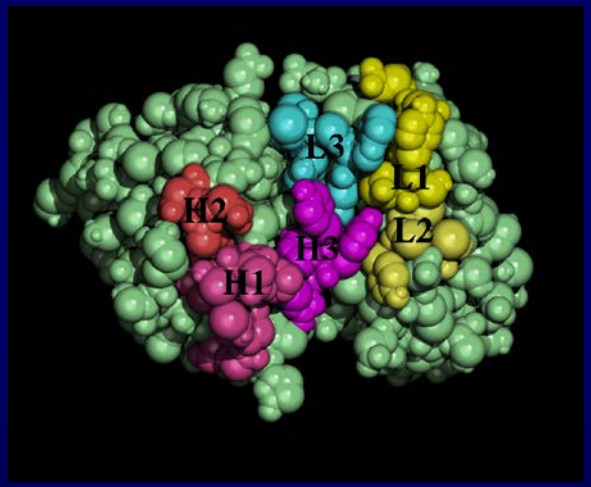
Distance between 76D and 82L is 7 Ångstroms

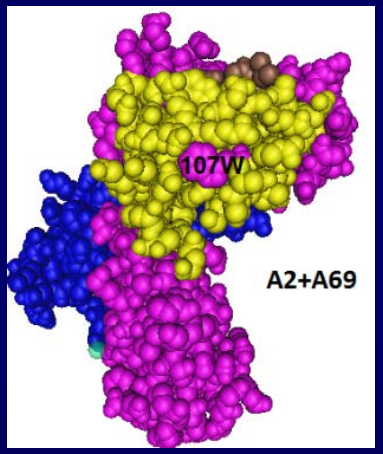
HLAMatchmaker: Examples of Eplets in Class I Sequence Positions

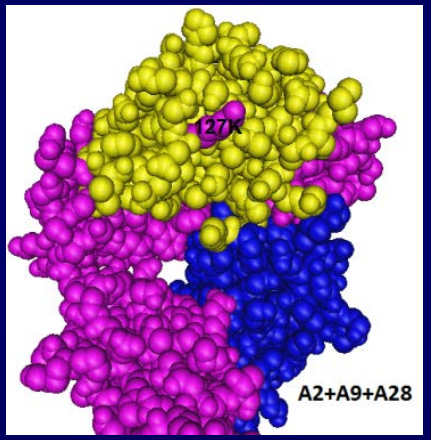
	44	62	65	69	77	….	163	184	193	248
A*0101	44KM	62QE	65RNM	66RNH	77ANT		163RG	184P	193PI	248VK
A*0201	44RM	62GE	65RKV	70KAH	77VDT		163TW	184A	193AV	248VK
A*0202	44RM	62GE	65RKV	70KAH	77VDT		163TW	184A	193AV	248VK
A*0203	44RM	62GE	65RKV	70KAH	77VDT		163TW	184A	193AV	248VK
A*0205	44RM	62GE	65RKV	70KAH	77VDT		163TW	184A	193AV	248VK
A*0301	44RM	62QE	65RNV	66RNQ	77VDT		163TW	184P	193PI	248VK
A*0302	44RM	62QE	65RNV	66RNQ	77VDT		163TW	184P	193PI	248VK
A*1101	44RM	62QE	65RNV	66RNQ	77VDT		163RW	184P	193PI	248VK
A*1102	44RM	62QE	65RNV	66RNQ	77VDT		163RW	184P	193PI	248VK
A*2301	44RM	62EE	66GKH	70KAH	77ENI		163TG	184P	193PI	248VK
A*2402	44RM	62EE	66GKH	70KAH	77ENI		163TG	184P	193PI	248VK
A*2403	44RM	62EE	66GKH	70KAH	77ENI		163TW	184P	193PI	248VK
A*2501	44RM	62RN	65RNV	66RNH	77ESI		163RW	184A	193AV	248VK
A*2601	44RM	62RN	65RNV	66RNH	77ANT		163RW	184A	193AV	248VK
A*2901	44RM	62LQ	65RNV	66RNQ	77ANT		163TW	184A	193AV	248VK
A*2902	44RM	62LQ	65RNV	66RNQ	77ANT		163TW	184A	193AV	248VK
A*3001	44RM	62QE	65RNV	66RNQ	77VDT		163TW	184P	193PI	248VK
A*3002	44RM	62QE	65RNV	66RNH	77ENT		163TW	184P	193PI	248VK
A*3101	44RM	62QE	65RNV	66RNH	77VDT		163TW	184P	193AV	248VK

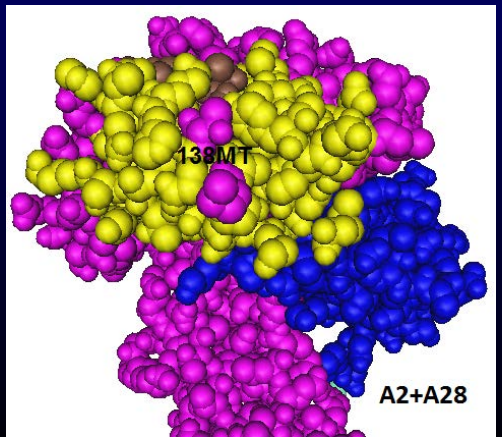
HLA Epitopes

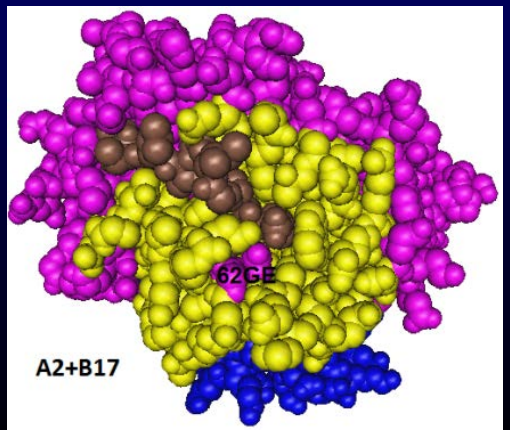
- HLAMatchmaker considers eplets as equivalents to functional epitopes
- Amino acid residues within a 15 Ångstrom radius of eplets can contribute structural HLA epitopes











Four Examples on HLA-A2 molecules

Two strategies to determine the antibody-verified HLA epitope repertoire

1. HLAMatchmaker is a theoretical algorithm that predicts HLA epitopes on the HLA molecular surface from stereochemical modeling of epitope-paratope interfaces of antigen-antibody complexes

2. An empirical method to analyze antibody reactivity with HLA-typed panels and the identification of amino acid configurations shared between reactive alleles

Two strategies to determine the HLA epitope repertoire

1. HLAMatchmaker is a theoretical algorithm that predicts HLA epitopes on the HLA molecular surface from stereochemical modeling of epitope-paratope interfaces of antigen-antibody complexes

2. An empirical method to analyze antibody reactivity with HLA-typed panels and the identification of amino acid configurations shared between reactive alleles

Terasaki and his group used single allele Luminex panels tested with mouse monoclonal antibodies and (absorbed/eluted) alloantibodies

They reported 103 HLA-A,B.C epitopes annotated with a numbering system (El-Awar et al. : *Transplantation* 84:532-540, 2007)

How do “Terasaki’s” epitopes correspond to eplets ?

Tissue Antigens, 74:117-133, 2009; 74:134-146, 2009

Results

50/103 Terasaki's class I epitopes correspond to eplets

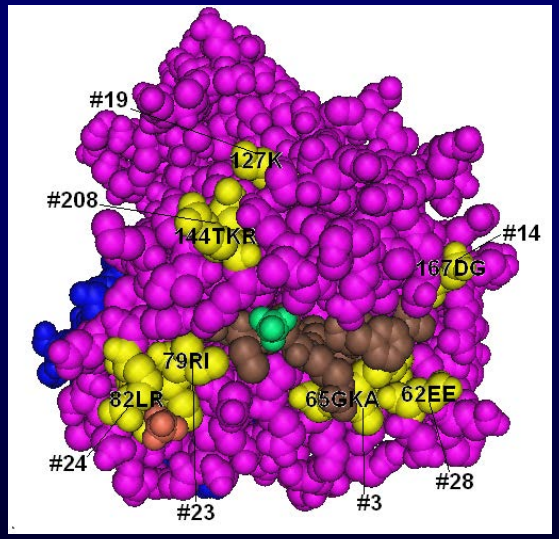
Examples of Terasaki's epitopes that correspond to eplets [a]

TerEp	Antibody Reactive Antigens	Residue Description of TerEp	Eplet
#19	A2,23,24,68,69	127K	127K
#3	A23, 24	65G	65GKA
#28	A23,24,80	62E	62EE
#14	A1,23,2402,80; B76	166D/ 167G	167DG
#24	A23,24,25,32; B13,2705,37,38, 44,47,49,51,52,53,57,58,59,63,77	82L/ 83R	82LR
#23	A23,24,25,32; B38,49,51, 52,53,57,58,59,63,77	80I	79RI
#217	B13,2705,37,44,47	76E+80T/ 79R+80T/ 80T+82L/ 80T+83R	79RT
#245	B35,4005,46,49,50,51,52,53,56, 57,58,62,63,71,72,75, 77,78; Cw9,w10	163L+167W	163LW
#35	B18,35,37,51,52,53,58,78	45T	44RT
#222	A6602; B7,13,27,47,48,60,61,73,81; Cw2,w17	163E+166E/ 163E+167W	163EW
#39	Cw2,w9,w10,w15	21H	21H
#246	B46,73; Cw1,w7,w8,w9,w10,w12,w14,w16	76V+80N/ 73T+76V+79R	80VRN
#421	B46; Cw1,w8,w9,w10,w14,w16	(73T)+76V+80N+90A	77TVS

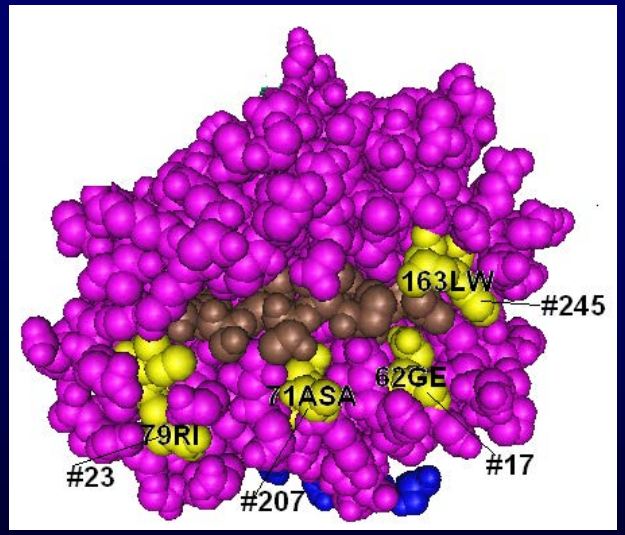
[a] These epitopes are on A24, B57 and Cw3

Molecular locations of Terasaki's epitopes and corresponding eplets

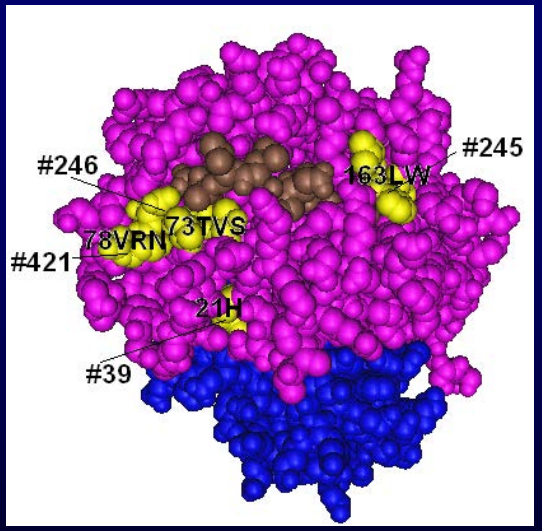
A*2402



B*5701



Cw*0303



Epitopes Defined by Eplet Pairs

- Duquesnoy, Mulder, et al: *HLAMatchmaker-Based Analysis of Human Monoclonal Antibody Reactivity Demonstrates the Importance of an Additional Contact Site for Specific Recognition of Triplet-Defined Epitopes* *Human Immunology 66: 749-761 2005*

Antibody Producer: A2,A68; B7,B27; Cw2,Cw7
Mismatched triplets on immunizing A3:
62QE,142MI,144TKR,151AHE,163DT

Hu mAb	Specific epitope
OK2H12	62QE + self 56G
OK4F9 and OKF10	142MI + self 79GTLRG
OK5A3	144TKR + self 151H

Reactivity of mAb OK2H12 with 62Qe-carrying alleles

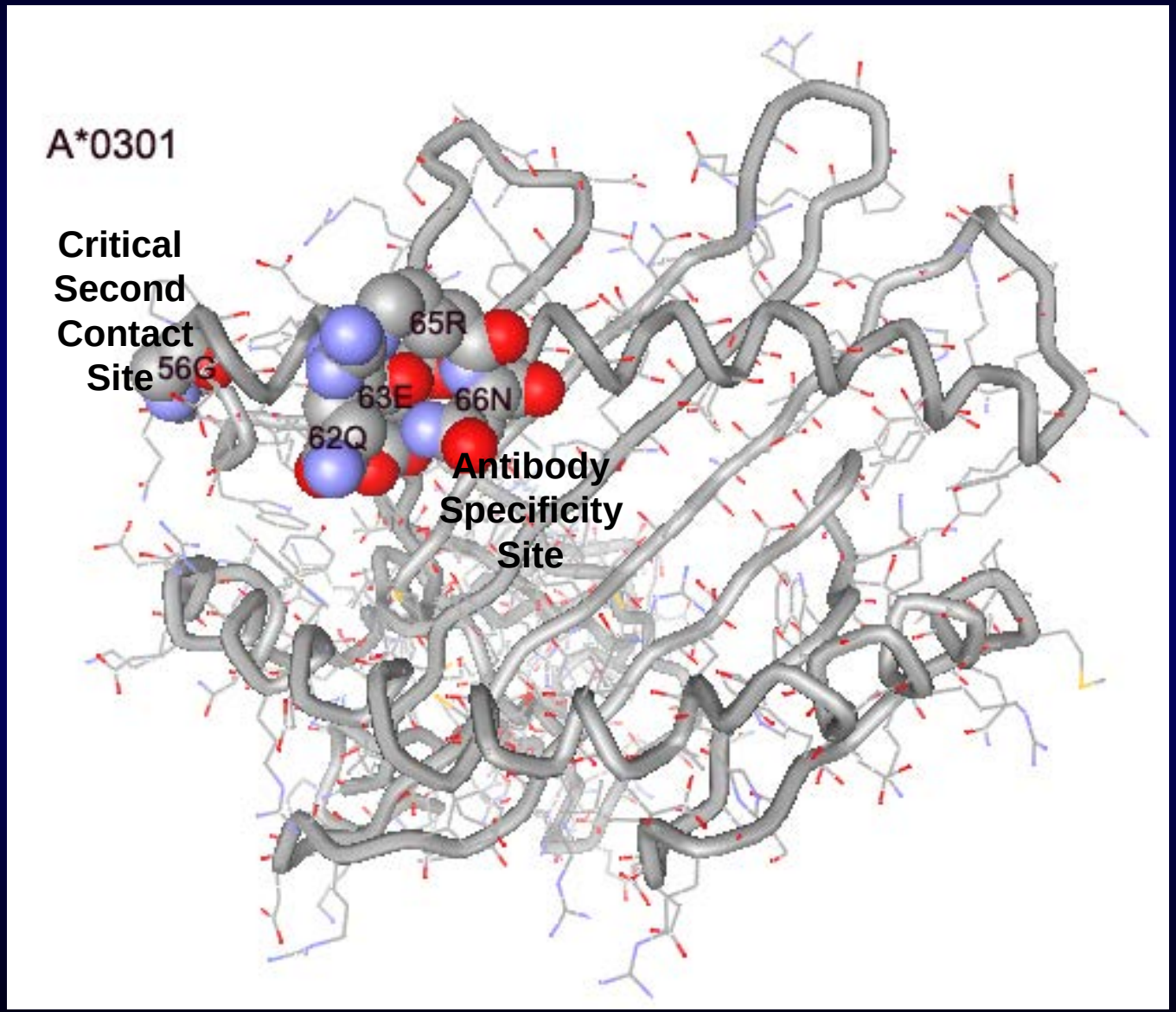
			Lymphocytotoxicity		Antigen-Binding	
Allele	Triplets shared with HLA-A3	N	%Pos Rx	Rx Strength	Flow Beads	Elisa
A*0301	**62Qe**,142ml,144tKr,151aHe,163dT	103	99%	7.6	++	++
A*0101	**62Qe**,142ml,144tKr	61	100%	7.6	++	+
A*0103	**62Qe**,142ml,144tKr	4	75%	6.3	nd	nd
A*1101	**62Qe**,142ml,144tKr	83	96%	7.7	++	++
A*3201	**62Qe**,142ml	29	100%	7.8	++	++
A*3601	**62Qe**,142ml,144tKr	6	83%	6.5	nd	+
A*7401	**62Qe**,142ml	8	100%	7.5	nd	++
A*3001	**62Qe**,142ml	12	0%	1.0	Neg	Neg
A*3002	**62Qe**,142ml	16	0%	1.0	nd	nd
A*3101	**62Qe**,142ml	38	8%	1.6	Neg	Neg
A*2301	142ml	31	0%	1.0	Neg	Neg
A*2402	142ml,144tKr	107	4%	1.2	nd	nd
A*2403	142ml,144tKr	4	0%	1.0	Neg	nd
A*2407	142ml,144tKr	6	0%	1.0	nd	nd
A*2501	142ml,151aHe	11	0%	1.0	Neg	Neg
A*2601	142ml,151aHe	36	6%	1.3	Neg	Neg
A*2901	142ml	24	4%	1.2	Neg	Neg
A*3301	142ml	15	0%	1.0	Neg	Neg
A*3303	142ml	16	0%	1.0	nd	Neg
A*3401	142ml,151aHe	22	3%	1.2	nd	Neg
A*3402	142ml,151aHe	11	9%	1.7	Neg	nd
A*6601	142ml,151aHe	14	0%	1.0	Neg	Neg
A*8001	142ml,144tKr	6	0%	1.0	Neg	Neg
A2/A28	none	67	1%	1.1	Neg	Neg

56G is the only residue shared between HLA-A3 and 62Qe-carrying alleles that react with OK2H12

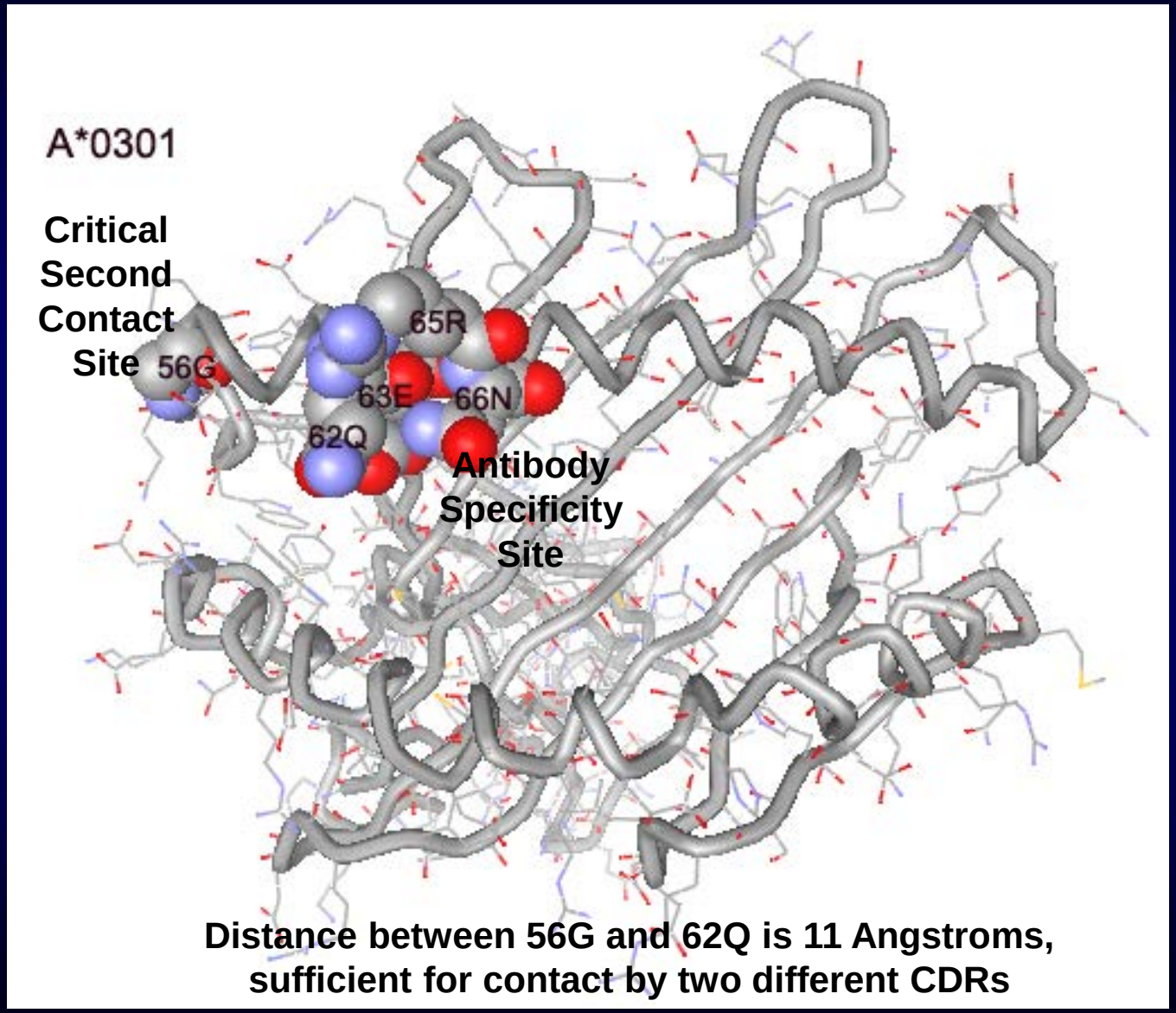
	Rx	9	17	35	44	45	*56*	**62**	**63**	65	66	67	70	73	74	76	77	79	80	81	82	83	90	95	97	99	105	107
A*0301	Pos	F	R	R	R	M	*G*	**Q**	**E**	R	N	V	Q	T	D	V	D	G	T	L	R	G	A	I	I	Y	S	G
A*0101	Pos	F	R	R	K	M	*G*	**Q**	**E**	R	N	M	H	T	D	A	N	G	T	L	R	G	D	I	I	Y	P	G
A*0103	Pos	F	R	R	K	M	*G*	**Q**	**E**	R	N	M	H	T	D	A	N	G	T	L	R	G	D	I	M	Y	P	G
A*1101	Pos	Y	R	R	R	M	*G*	**Q**	**E**	R	N	V	Q	T	D	V	D	G	T	L	R	G	D	I	I	Y	P	G
A*3201	Pos	F	R	R	R	M	*G*	**Q**	**E**	R	N	V	H	T	D	E	S	R	I	A	L	R	A	I	M	Y	P	G
A*3601	Pos	F	R	R	K	M	*G*	**Q**	**E**	R	N	M	H	T	D	A	N	G	T	L	R	G	D	I	I	Y	P	G
A*7401	Pos	F	R	R	R	M	*G*	**Q**	**E**	R	N	V	H	T	D	V	D	G	T	L	R	G	A	I	M	Y	P	G
A*3001	Neg	S	S	R	R	M	*R*	**Q**	**E**	R	N	V	Q	T	D	V	D	G	T	L	R	G	A	I	I	Y	S	G
A*3002	Neg	S	S	R	R	M	*R*	**Q**	**E**	R	N	V	H	T	D	E	N	G	T	L	R	G	A	I	I	Y	S	G
A*3101	Neg	T	R	R	R	M	*R*	**Q**	**E**	R	N	V	H	I	D	V	D	G	T	L	R	G	A	I	M	Y	S	G

		109	114	116	127	138	142	143	144	145	149	150	151	152	156	158	161	163	166	167	171	184	186	193	194	207	246	253
A*0301	Pos	F	R	D	N	M	I	T	K	R	A	A	H	E	L	A	D	T	E	W	Y	P	K	P	I	G	A	E
A*0101	Pos	F	R	D	N	M	I	T	K	R	A	V	H	A	R	V	E	R	D	G	Y	P	K	P	I	G	A	E
A*0103	Pos	F	R	D	N	M	I	T	K	R	A	V	H	A	R	V	E	R	D	G	Y	P	K	P	I	G	A	E
A*1101	Pos	F	R	D	N	M	I	T	K	R	A	A	H	A	Q	A	E	R	E	W	Y	P	K	P	I	G	A	E
A*3201	Pos	L	Q	D	N	M	I	T	Q	R	A	A	R	V	L	A	E	T	E	W	Y	A	K	A	V	S	S	Q
A*3601	Pos	F	R	D	N	M	I	T	K	R	A	V	H	A	R	V	E	T	E	W	Y	P	K	P	I	G	A	E
A*7401	Pos	L	Q	D	N	M	I	T	Q	R	A	A	R	V	L	A	E	T	E	W	Y	A	K	A	V	S	S	Q
A*3001	Neg	F	E	H	N	M	I	T	Q	R	A	A	R	W	L	A	E	T	E	W	Y	P	K	P	I	G	A	E
A*3002	Neg	F	E	H	N	M	I	T	Q	R	A	A	R	R	L	A	E	T	E	W	Y	P	K	P	I	G	A	E
A*3101	Neg	F	Q	D	N	M	I	T	Q	R	A	A	R	V	L	A	E	T	E	W	Y	P	K	A	V	S	S	Q

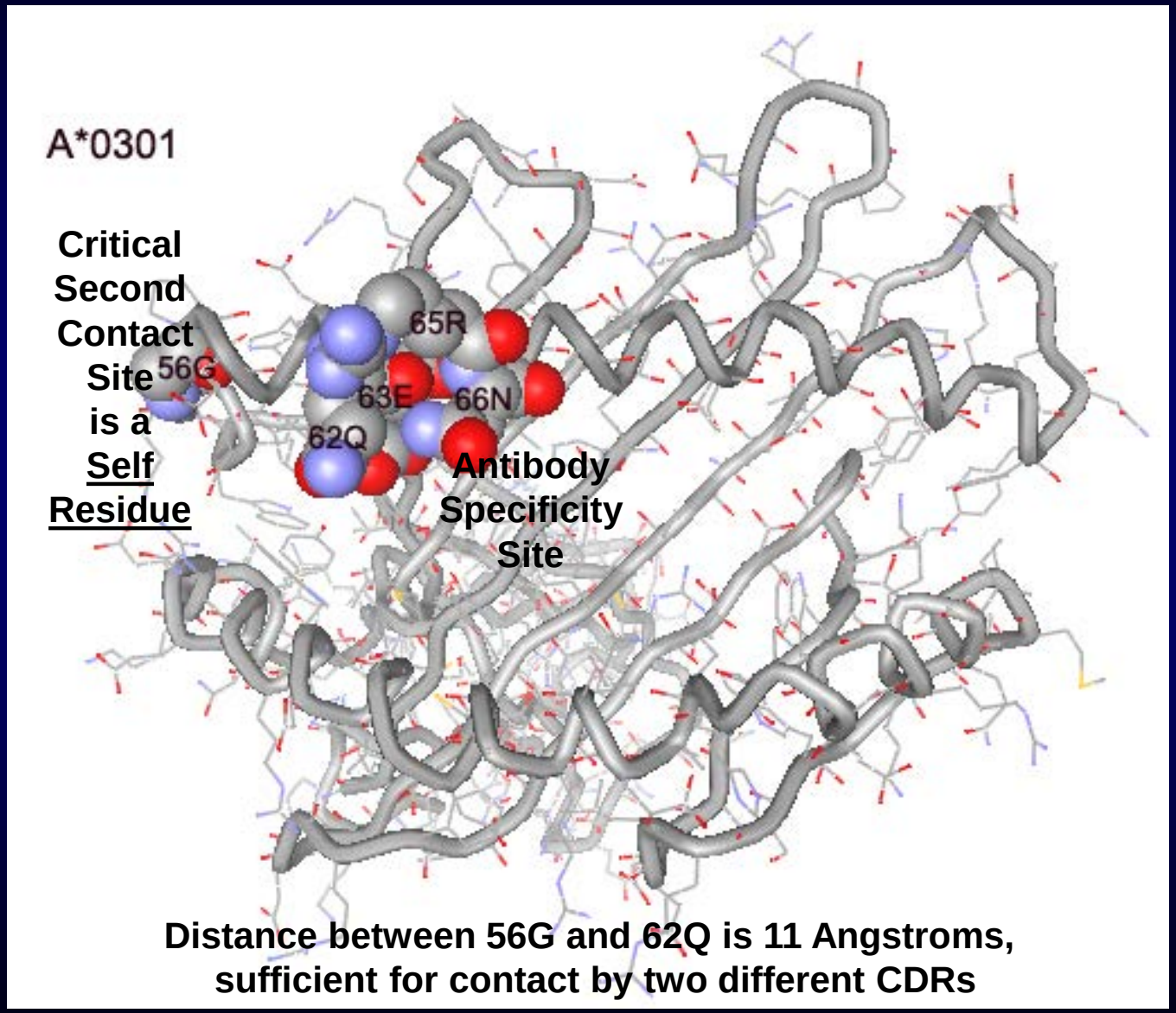
Shared Polymorphic Amino Acids in the 62Qe-Defined Specificity Site



Shared Polymorphic Amino Acids in the 62Qe-Defined Specificity Site



Shared Polymorphic Amino Acids in the 62Qe-Defined Specificity Site



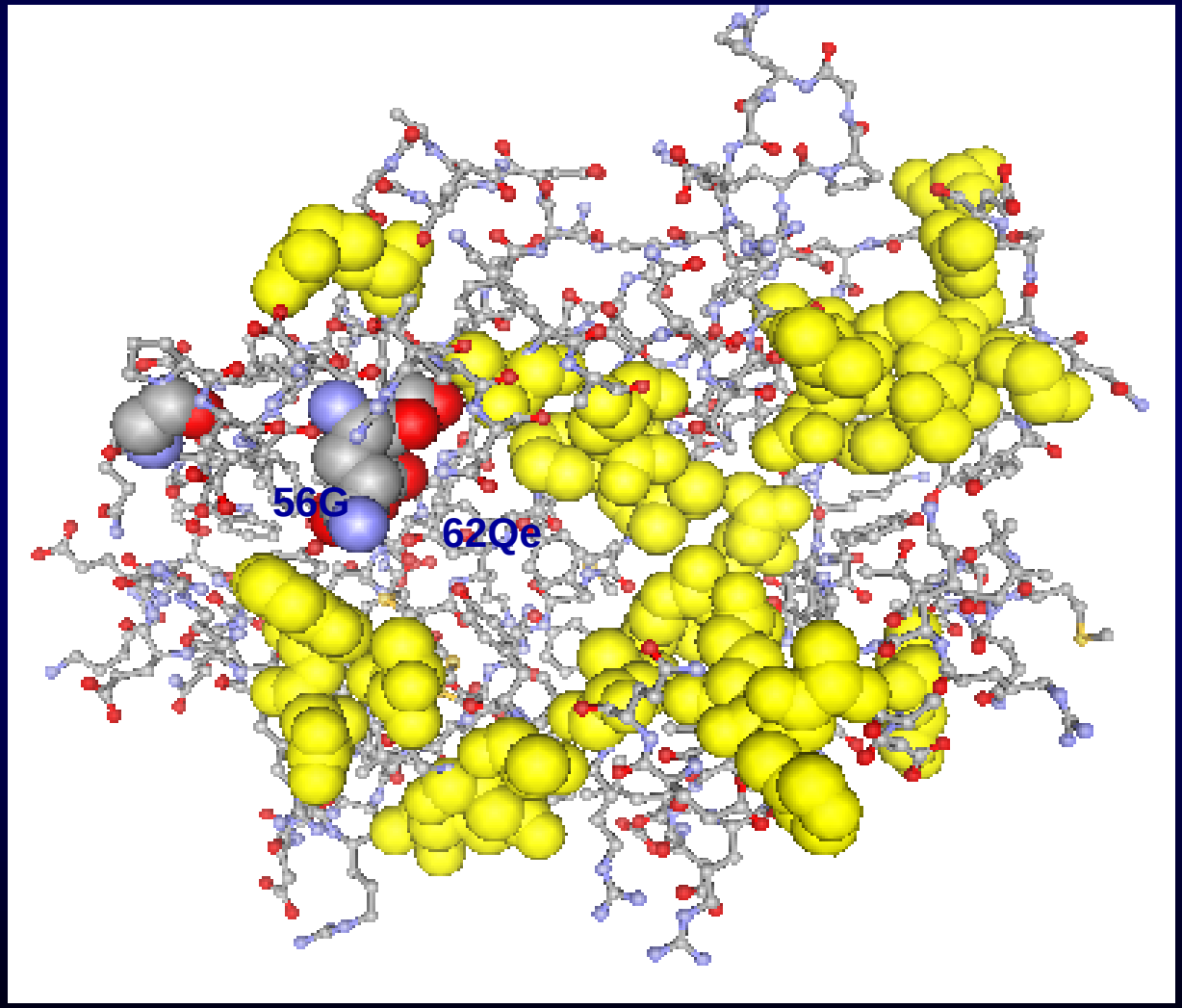
Many Residue Substitutions on HLA do not affect Reactivity with Alloantibody

Permissive Residue Substitutions that Do Not Inhibit Reactivity of the 62Qe+56G-Specific mAb

	Rx	9	17	35	44	45	*56*	**62**	**63**	**65**	**66**	67	70	73	74	76	77	79	80	81	82	83
A*0301	Pos	F	R	R	R	M	*G*	**Q**	**E**	**R**	**N**	V	Q	T	D	V	D	G	T	L	R	G
A*0101	Pos	F	R	R	K	M	*G*	**Q**	**E**	**R**	**N**	M	H	T	D	A	N	G	T	L	R	G
A*0103	Pos	F	R	R	K	M	*G*	**Q**	**E**	**R**	**N**	M	H	T	D	A	N	G	T	L	R	G
A*1101	Pos	Y	R	R	R	M	*G*	**Q**	**E**	**R**	**N**	V	Q	T	D	V	D	G	T	L	R	G
A*3201	Pos	F	R	R	R	M	*G*	**Q**	**E**	**R**	**N**	V	H	T	D	E	S	R	I	A	L	R
A*3601	Pos	F	R	R	K	M	*G*	**Q**	**E**	**R**	**N**	M	H	T	D	A	N	G	T	L	R	G
A*7401	Pos	F	R	R	R	M	*G*	**Q**	**E**	**R**	**N**	V	H	T	D	V	D	G	T	L	R	G
A*3001	Neg	S	S	R	R	M	*R*	**Q**	**E**	**R**	**N**	V	Q	T	D	V	D	G	T	L	R	G
A*3002	Neg	S	S	R	R	M	*R*	**Q**	**E**	**R**	**N**	V	H	T	D	E	N	G	T	L	R	G
A*3101	Neg	T	R	R	R	M	*R*	**Q**	**E**	**R**	**N**	V	H	I	D	V	D	G	T	L	R	G

		109	114	116	127	138	142	143	144	145	149	150	151	152	156	158	161	163	166	167	171	184
A*0301	Pos	F	R	D	N	M	I	T	K	R	A	A	H	E	L	A	D	T	E	W	Y	P
A*0101	Pos	F	R	D	N	M	I	T	K	R	A	V	H	A	R	V	E	R	D	G	Y	P
A*0103	Pos	F	R	D	N	M	I	T	K	R	A	V	H	A	R	V	E	R	D	G	Y	P
A*1101	Pos	F	R	D	N	M	I	T	K	R	A	A	H	A	Q	A	E	R	E	W	Y	P
A*3201	Pos	L	Q	D	N	M	I	T	Q	R	A	A	R	V	L	A	E	T	E	W	Y	A
A*3601	Pos	F	R	D	N	M	I	T	K	R	A	V	H	A	R	V	E	T	E	W	Y	P
A*7401	Pos	L	Q	D	N	M	I	T	Q	R	A	A	R	V	L	A	E	T	E	W	Y	A
A*3001	Neg	F	E	H	N	M	I	T	Q	R	A	A	R	W	L	A	E	T	E	W	Y	P
A*3002	Neg	F	E	H	N	M	I	T	Q	R	A	A	R	R	L	A	E	T	E	W	Y	P
A*3101	Neg	F	Q	D	N	M	I	T	Q	R	A	A	R	V	L	A	E	T	E	W	Y	P

Locations of Permissive Polymorphic Residues for 62Qe+s56G-Specific mAb Reactivity



Human Monoclonal Antibody Reactivity with HLA Class I Epitopes Defined by Pairs of Mismatched Eplets and Self Eplets

Rene Duquesnoy[1] Marilyn Marrari[1], Justin Mostecki[2], Arend Mulder[3], Frans Claas[3] and Ivan Balazs[2]

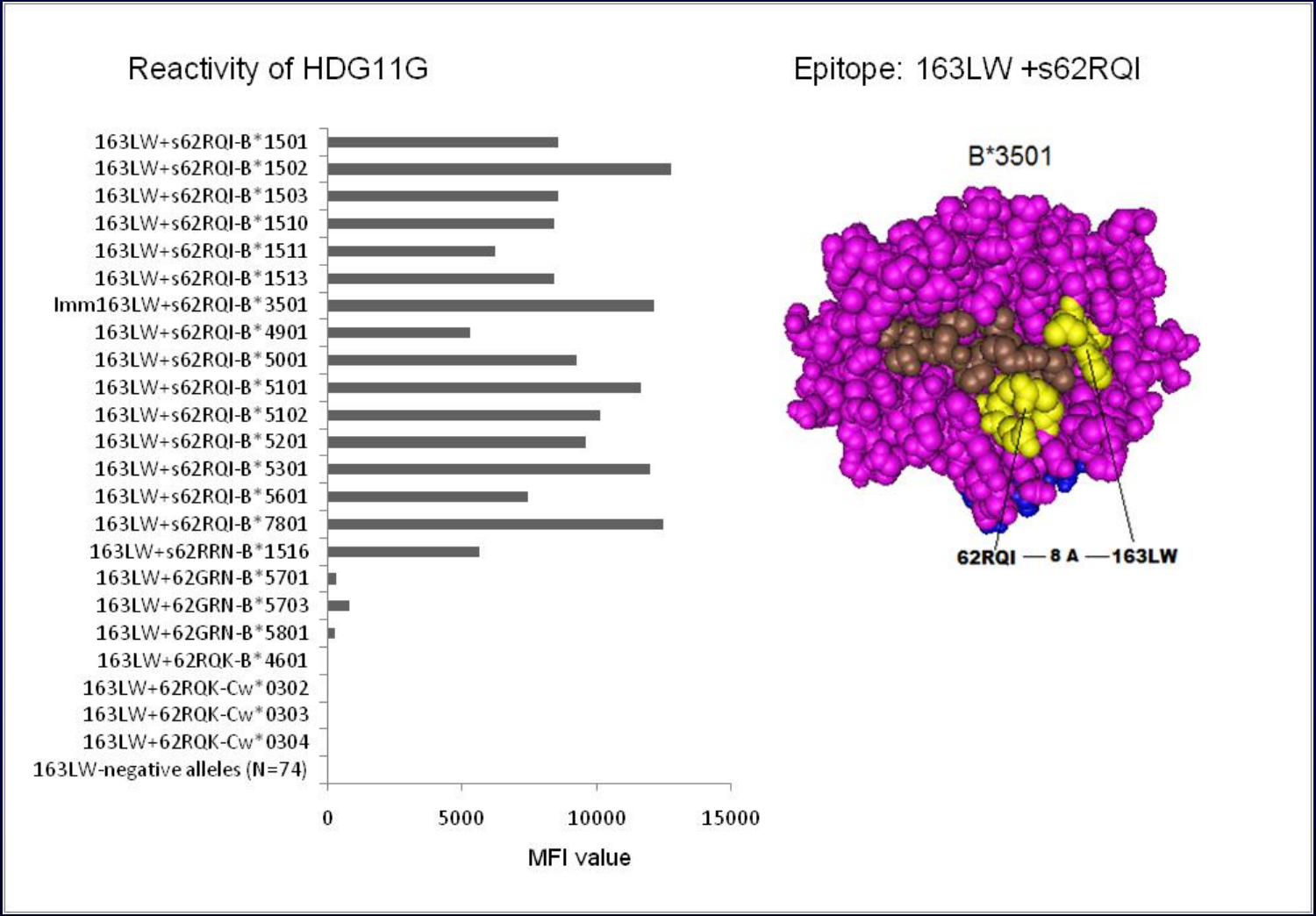
[1] University of Pittsburgh Medical Center

[2]Gen-Probe Transplant Diagnostics, Stamford, CT

[3] Leiden University Medical Center, The Netherlands

Transplantation. 2010; 90:1468-72

Monoclonal: HDG11G Antibody Producer: A2, 24; B7,40;Cw7,-
Immunizer: B35 Mismatched eplets:
44RT,63NQIN,65QIF,131S,**163LW**,193PV



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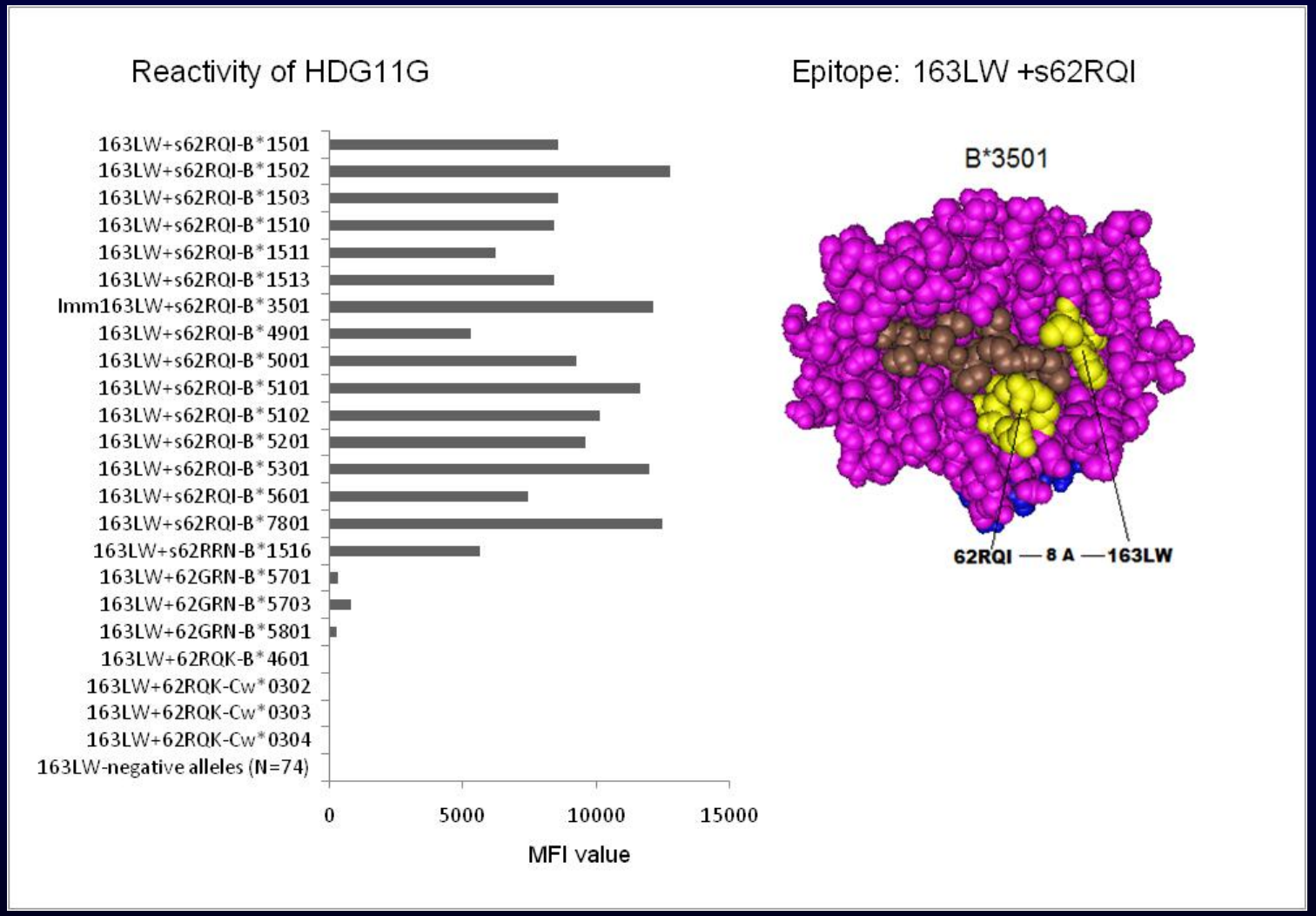
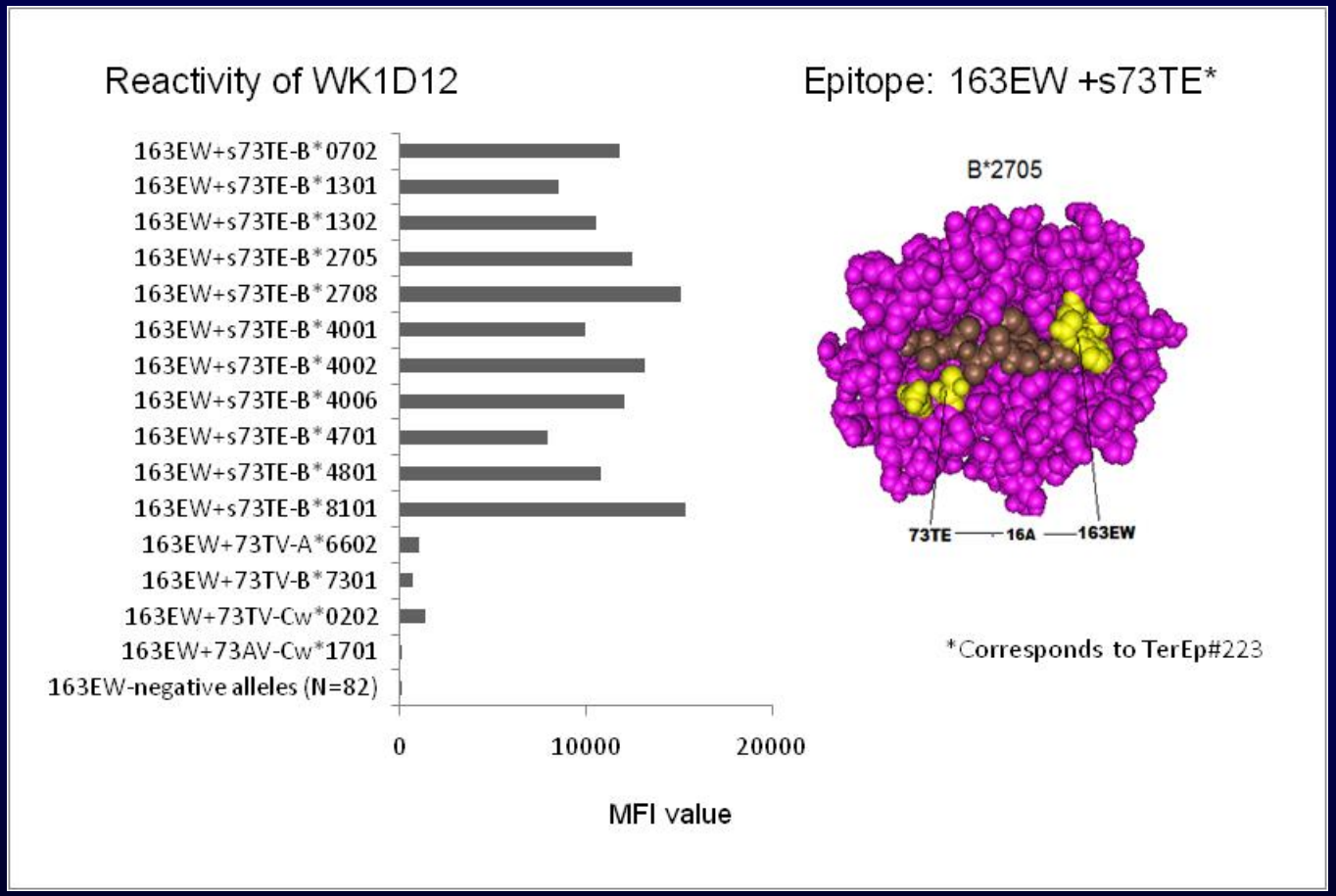


62QRI is a self eplet on B7 and B40 of the antibody producer

Monoclonal: WK1D12 Antibody producer A1,-; B8,-;Cw7,-
Immunizer: B27 Mismatched eplets:
32L,62REI,65QIA,71KA,76EDT,79ERT,82LR,131S,**163EW**



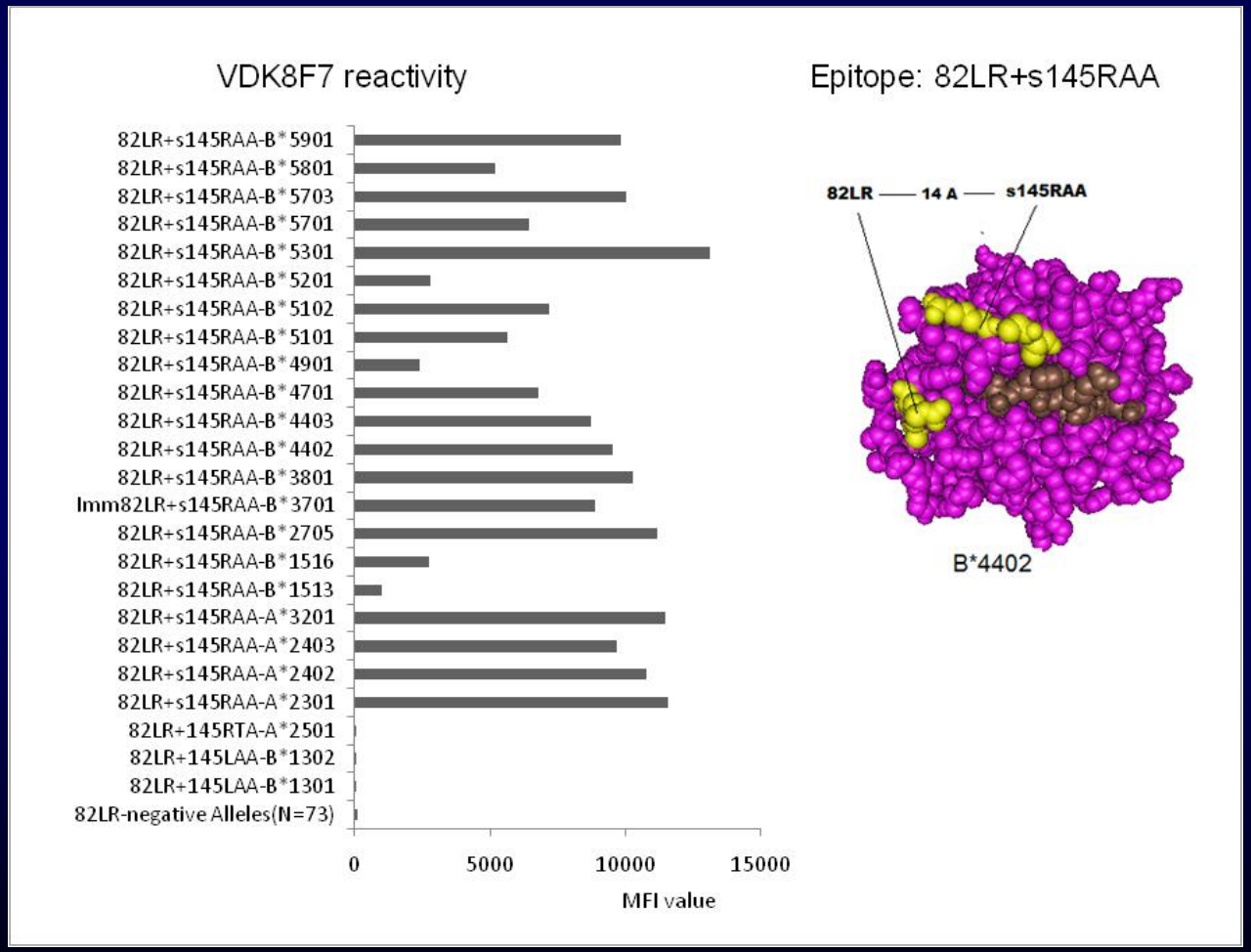
73TE is a self eplet on B8 of the antibody producer

This antibody reacts with all Bw4 antigens except A25 and B13

Monoclonal VDK8F7 Antibody producer: A3, 31; B35,-;Cw4,-

Immunizer: B37 Mismatched eplets:

62REI,66QIN,65QIS,73TTD,76EDT,79ERT,**82LR**,156DA



145RAA is a self eplet on the alleles of the antibody producer

Marrari M, Mostecki J, Mulder A, Balazs I, Claas F, Duquesnoy R. Human Monoclonal Antibody Reactivity with HLA Class I Epitopes Defined by Pairs of Mismatched Eplets and Self Eplets. Transplantation 2010; 90:1468-72

Antibody	HLA type Antibody producer	Imm	Eplet Specificity	Distance	Reactive Antigens
HDG4B1	A2, 24; B7, 60; Cw7, w10	A32	65RNA+s79ERI	11 Å	A25/A32/B17/B63
MUL9E11	A2, 25; B18, 51; Cw12, 15	B55	69AA-s76E	8 Å	B7/B*27/B42/B54/B55/B56/B57/B58/B63/B67/B81/B82
MUL9F4	A2, 25; B18, 51; Cw12, 15	B55	65QIA-s76ES	7 Å	B7/B*2708/B42/B54/B55/B56/B67/B81/B82
MUL4C8	A2, 25; B18, 51; Cw12, 15	A11	144KR+s151H	7 Å	A1/A3/A11/A24/A36
BRO11F6	A26, 68; B38, 44; Cw7	??	144KR+s151H	7 Å	A1/A3/A11/A24/A36
OK2H12	A2, 28; B7, 27; Cw2, w7	A3	62QE-s56G	11 Å	A1/A3/A11/A32/A36/A74
OK4F10	A2, 28; B7, 27; Cw2, w7	A3	142MI+s79GT	13 Å	A1/A3/A11/A26/A29/A30/A31/A33/A34/A36/A43/A66/A74/A80
OK 4F9	A2, 28; B7, 27; Cw2, w7	A3	142MI+s79GT	13 Å	A1/A3/A11/A26/A29/A30/A31/A33/A34/A36/A43/A66/A74/A80
VDK8C7	A3, 31; B35,-; Cw4,-	B37	82LR-s145RAA	14 Å	All Bw4 except A25 and B13
WK1D12	A1,-; B8,-; Cw7,-	?	163EW+s73TE	16 Å	B7/B13/B27/B47/B48/B40/B81
HA5C2	A1, 32; B8, 61; Cw2, w7	A2?	l42TKH-149AAH	3 Å	A2 (not A*0203)/A28
HDG11G12	A2, 24; B7, 60; Cw7, w10	B35	163LW+s62RQI	8 Å	B15/B35/B49/B50/B51/B52/B53/B56/B78
			Average	10 Å	

Summary of Terasaki's epitopes and their corresponding eplets

Locus	TerEps	Eplets	Eplet pairs	Eplets with substitutions	No eplets
ABC[a]	103	50	31	12	10
DR[b]	60	45	3	12	2
DQ[b]	18	13	1	3	1

[a] Duquesnoy RJ and Marrari M, *Tissue Antigens*, 74:117-133**, 2009**.
[b] Marrari M and Duquesnoy RJ, *Tissue Antigens,* 74:134-146**, 2009**.

16th International Workshop Project

International Registry of HLA Epitopes

http://www.epregistry.com.br

Duquesnoy RJ, Marrari M, **da M Sousa LCD**, de M. Barroso JRP, de SE Aita KM, da Silva AS and **do Monte SJH** : 16th IHIW: a website for the antibody-defined HLA epitope registry. Int J. Immunogenetics, 40: 54-59, 2013

Databases for HLA-ABC, HLADRB1/3/4/5, HLA-DQA-B, HLA-DPA-B and MICA

The HLA Epitope Registry has now the updated class I eplet repertoire

Recording Antibody-Defined Epitopes in Registry

- Each database in the HLA Epitope Registry comprises a list of potential epitopes that can be predicted to serve as recognition sites for specific antibodies
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- For each antibody-defined epitope a link on the webpage leads to detailed information about antibody reactivity patterns

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, Mostecki 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

Antibody-verified HLA-ABC Epitopes corresponding to Eplets and Recorded in the Registry (October 2014)

Eplet	Antibody Verified	Eplet	Antibody Verified	Eplet	Antibody Verified	Eplet	Antibody Verified	Eplet	Antibody Verified	Eplet	Antibody Verified	Eplet	Antibody Verified
9D		62RN		69ATS		76ES		102HV		151AHE		173K	Confirmed
9F		62RNQ		69RA		76ESI	Confirmed	103L		151AHV		177DK	
9H		62RNR	Provisional	69RT		76ESN	Confirmed	$103M_2$		151ARV		177DT	
9S		62RR	Provisional	69TNT	Provisional	76ET		105S		151H		177KT	Provisional
9T		62RRN		70HT		76VDT		107W	Confirmed	152A		180E	Confirmed
9Y		62RTN		70IAQ	Provisional	76VRN	Provisional	109F		152E		182TDP	
11AM		63EI		70QT		76VS		109FE		152HA		184A	
11AV		63EK		71ATD	Provisional	77NGT		113HD		152RA		184H	
11SM		63EN		71ATN		77SRN		113HN		152RE		$184R_3$	
16S		63ER		71ATS		79GT		113YD		151RR	Provisional	186R	
17RS	Provisional	63ERN		71HS		80I	Confirmed	113YH		152RT		193AV	
17WR		63NN		71KA		80K	Provisional	113YN		152RW	Provisional	193LV	
19K		65GK	Confirmed	71QS		80N	Confirmed	113YQ		152V		193PI	
1C		65QIA	Confirmed	71SA	Confirmed	80T		113YR		156DA	Provisional	$193PL_3$	Confirmed
21H	Confirmed	65QKR		71STN		80TA		116D		156L		193PV	Provisional
24S		65QNR		71TTD		80TL		116F		156LA		194V	
24T		65RA		71TTN		80TLR	Provisional	116L		156QA		199V	
30G		65RK		71TTS	Provisional	81ALR		116S		156R		207S	
32L		65RNA		72QTD		82LR	Confirmed	116Y		156RA		211T	
35Q		66I		73AN		90AR		11SV		156WA		219W	Confirmed
41T	Confirmed	66IC		73AS		90D	Provisional	127K	Confirmed	158T	Confirmed	245AS	
43RRM		66ICT		73ID		94II		131S		161D	Confirmed	245TA	
$44KM_3$	Confirmed	66IF		73TD		94IL		138K	Provisional	162DLS		245VA	
44RG		66IS		73TDA		94TF		138MI		162GLS		248M	
44RM		66IY		73TDE		94TI		142ITQ		163E		253Q	Provisional
44RMA	Confirmed	66IYT		73TDV		94TL		143S		163EW	Confirmed	267PE	
44RME		66K		73TN		94TV		144K	Provisional	163L		267QE	Provisional
44RT	Provisional	66KA		73TS		94TW		144KA		163LE		270C	
45EE		66KAH		73TV		97I		144KR	Confirmed	163LG		275EL	
45KE		66N		73TVD		97M		144QL	Confirmed	163LW	Confirmed	275G	
56R	Confirmed	66NAH		73TVN		97N		144TKH		163R	Provisional	275K	
62EE	Provisional	66NAQ		73TVS	Provisional	97R		145HT		163RG	Confirmed		
62GE	Confirmed	66NM		73TY		97S		145KHA	Provisional	163RW	Confirmed		
$62GK_2$	Confirmed	66NV		76ANT	Confirmed	97T		145RT	Provisional	163T			
62GRN	Confirmed	66RKQ		76ED		97V		145L	Confirmed	163TEW			
62LQ	Confirmed	69AA	Confirmed	76EG		97W		149AH		163TG			
62QE		69AQT		76EN		99F		149TAH	Confirmed	166DG	Confirmed		
62REN		69AT		76ENI		99S		149AAH	Confirmed	166ES			
62RER		69ATD		76ENR		99Y		150AH		170RH			
62RK		69ATN		76ENT		102DV		151AHA	Confirmed	170RY			

Antibody-verified HLA-ABC Epitopes corresponding to Eplet Pairs and Recorded in the Registry (October 2014)

HLA-A,B,C Epitope	Polymorphic Residue Description	HLA-A,B,C Epitope	Polymorphic Residue Description
44RT+69TNT	44R45T46E paired with 69T70N71T	82LR+138T	79R82L83R paired with 138T
62QE+56G	62Q63E65R66N paired with self56G	82LR+144Q	79R82L83R paired with 144Q
62QE+151H	62Q63E65R66N paired with self151H	82LR+144QR	79R82L83R paired with 144Q145R
62RN+163TW	62R63N paired with 163T166W	82LR+145R	79R82L83Rpaired with 145R
65QIA+76ESN	65Q66I69A paired with 76E77S80N	82LR+145RA	79R82L83R paired with145R149A
65QK+76VS	62R65Q66K67Y69R paired with self 76VS	90D+138M	90D91G paired with 138M
65RNA+ 80I	65R66N69A paired with self80I82L83R	131S+163LW	131S paired with 163L166W
66IF+163TW	66I67F69T paired with 163T166W	131S+163T	131S paired with 163T166W
69AA+ 80N	69A71A paired with self80N	138MI+79GT	138M142I143T145R paired with 79G80T
69TNT+80N	69T70N71T paired with 80N	143S+76ESN	142I143S144Q145R paired with self 76ESN
76ENI+62RE	76E77N79R80I paired with 62R62E	144K+76DVT	144K paired with 76DVT
79GT+19E	79G80T81L82R83G paired with 19E	144KR+151H	142I143T144K145R paired with self151H
80I+65QI	79R80I81A82L83R paired with 65Q66I	163EW+73TE	162G163E166E167W paired with 73T76E
80I+90A	79R80I81A82L83R paired with 90A or 145T	163LW+65QI	162G163L166E167Wpaired with self 65Q66I
80I+151RE	79R80I81A82L83R paired with 151R152E	163TEW+65QI	163T166E167W paired with 65Q66i
82LR+90A	79R82L83R paired with 90A	163TEW+103L	163T166E167Wpaired with 103L
82LR+138M	79R82L83R paired with 138M		

All have provisional status

Example of a record of an antibody-verified HLA-A epitope

PANEL		OL MFI	GP MFI
Positive control		9935	15973
Negative control		31	127
Immunizing allele:	144TKH+ A*02:01	16165	17273
	144TKH+ A*02:02	nt	17579
	144TKH+ A*02:03	21200	17606
	144TKH+ A*02:05	nt	18235
	144TKH+ A*02:06	22505	nt
	144TKH+ A*68:01	17886	18358
	144TKH+ A*68:02	18034	17741
	144TKH+ A*69:01	19158	12210
Self Alleles		35 ± 16	70 ± 21
Other 144TKH-negative Alleles		31 ± 10	89 ± 30

Example of a record of an antibody-verifiedHLA-B epitope

Epitope: 41T (confirmed) on B13,B40,B41,B44,B45,B47,B49,B50

Report Date: 21Feb2012

Duquesnoy et al *Human Immunology* 73: 267-77, 2012

Pregnancy induced human monoclonal antibody ROU9A6 (IgG) from Arend Mulder (Leiden):

Antibody producer: HLA-A1, A25; B8, B18; Cw7.

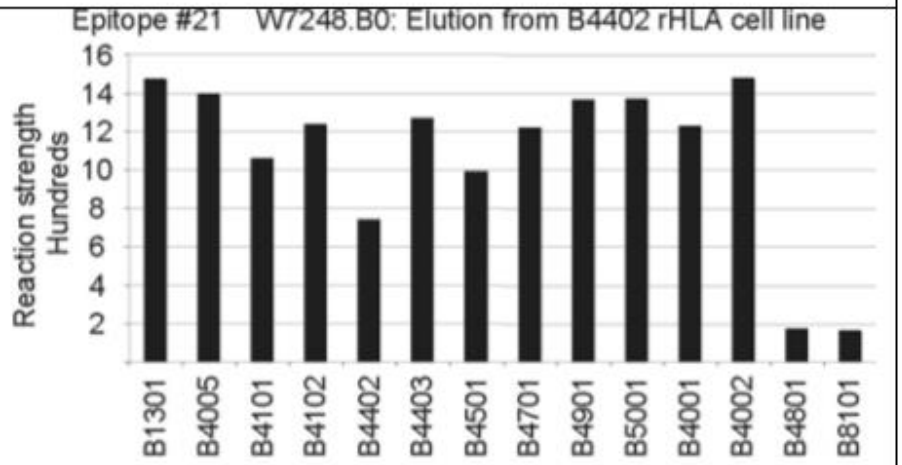
Immunizer:HLA-A2; B44, B60; Cw5, w10.

PANEL	OL MFI	GP MFI
Positive control	9488	nt
Negative control	9	nt
41T+ B*13:01	15174	nt
41T+ B*13:02	19404	18245
41T+ B*40:01	10610	22974
41T+ B*40:02	18522	21031
41T+ B*40:06	16626	nt
41T+ B*41:01	20733	21957
41T+ B*44:02	18295	7464
Immunizing allele: 41T+ B*44:03	18305	14695
41T+ B*45:01	21450	19061
41T+ B*47:01	17208	20014
41T+ B*49:01	13830	15192
41T+ B*50:01	17934	12216
Self Alleles	11 ± 2	68 ± 12
Other 41T-negative Alleles	76 ±232	81 ± 41

El-Awar et al, *Human Immunology* 68: 170-180, 2007:

Serum eluate W7248.BO (absorbed with B*44:02 cells, no information on HLA types of antibody producer/immunizer)

Equivalent to TerEp #21



Fernandez-Vina et al, in Immunobiology of the Human MHC vol. I, J.A. Hansen (Ed), pp 890-931, 2006:

ROU9A6 (13-WS 0120) positive with HLA-B*13:01, *13:02, *13:04, *40:01, *40:02, *40:04, *40:06, *40:11, *40:19, *40:30, *41:01, *41:02, *44:02, *44:03, *44:04, *44:21, *45:01, *47:01, *47:02, *47:03, *49:01, *50:01, *50:02 in CDC assays in 13th IHWS.

Example of a record of an antibody-verified HLA-C epitope

	PANEL	OL MFI	GP MFI
	Positive control	nt	nt
	Negative control	10	1
	219W+ C*01:02	13483	7444
	219W+ C*03:02	11733	nt
Immunizing allele:	219W+ C*03:03	13204	9740
	219W+ C*03:04	14135	10896
	219W+ C*04:01	7073	4549
	219W+ C*04:03	nt	7939
	219W+ C*14:02	12176	10036
	219W+ C*18:01	nt	10985
	219W+ C*18:02	12659	nt
	Self Alleles	83 ± 110	11 ± 1
	Other 219W-negative Alleles	109 ± 259	9 ± 3

Newly Antibody-Verified Epitopes

- For a successful registry we need contributions by HLA professionals who have identified antibodies specific for new and not so well described epitopes
- From the website one can download instructions how to submit information about epitope-specific antibody reactivity