

16th International Workshop Project

Development of HLA Epitope Database

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16th Workshop Project Goals

- Develop a nomenclature for HLA epitopes
- Determination of the repertoire of clinically relevant epitopes
 - Develop a database of HLA epitopes that have already been defined by antibodies
 - Identify new antibody-defined HLA epitopes

HLA Nomenclature

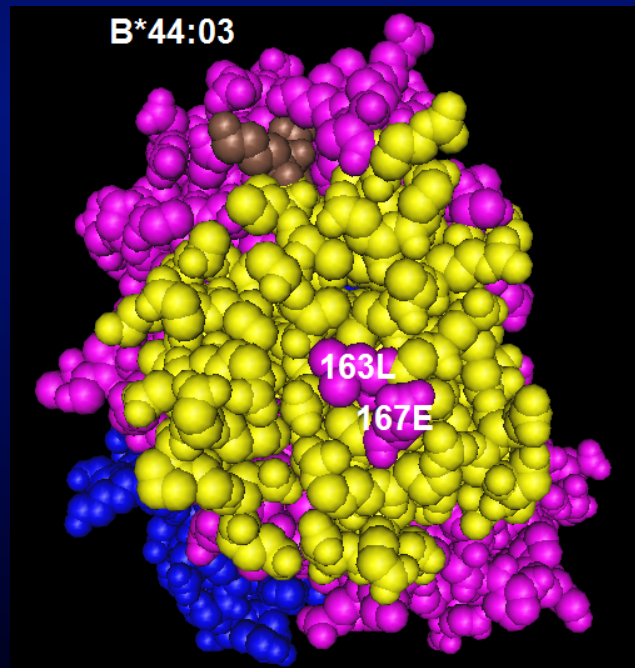
- For HLA alleles: based on DNA and amino acid sequence differences (chemical method)
- For HLA epitopes: based on reactivity patterns of antibodies with allele panels (biophysicochemical method)
 - Different methodologies available
 - Structural basis of the antigen-antibody complex

Structure of Antigen-Antibody Complex

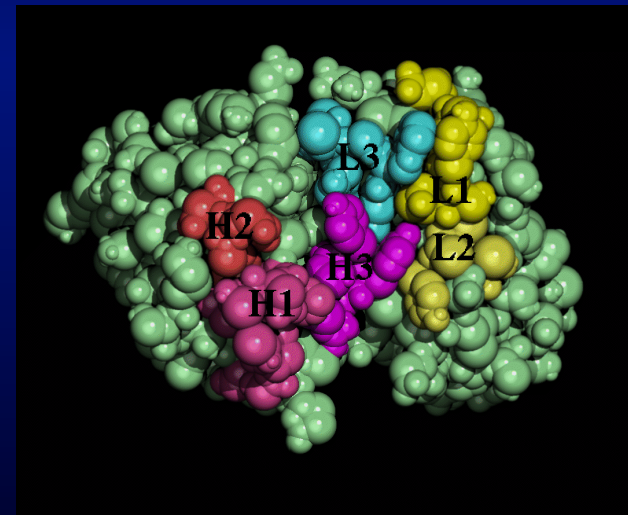
A “**structural**” epitope is that part of the antigen that makes contact with all six CDRs of antibody. A structural epitope has about 15-25 surface residues and within its surface of about 700-900 square Ångstroms lies a centrally located “**functional**” epitope consisting of a few residues that dominate the determination of epitope specificity.

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Residues within a 15 Å radius of an eplet



Six CDRs of antibody

Human Monoclonal Antibody* OK6H12 Defined 163LW Epitope

<u>OK6H12</u>	Eplet:	163LW		Polymorphic surface residues within 15 Å of 163LW								
Sequence position		163	167	62	65	66	107	109	151	173	177	180
IMM	B*15:03	L	W	R	Q	I	G	L	R	E	E	Q

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OK6H12	Eplet:	163LW		Polymorphic surface residues within 15 Å of 163LW								
		163	167	62	65	66	107	109	151	173	177	180
IMM	B*15:03	L	W	R	Q	I	G	L	R	E	E	Q
OL MFI	PANEL											
8125	B*15:01	L	W	-	-	-	-	-	-	-	-	-
8972	B*15:02	L	W	-	-	-	-	-	-	-	-	-
7638	B*15:03	L	W	-	-	-	-	-	-	-	-	-
8241	B*15:10	L	W	-	-	-	-	-	-	-	-	-
7300	B*15:13	L	W	-	-	-	-	-	-	-	-	-
8625	B*35:01	L	W	-	-	-	-	-	-	-	-	-
6438	B*49:01	L	W	-	-	-	-	-	-	-	-	-
9862	B*50:01	L	W	-	-	-	-	-	-	-	-	-
7292	B*53:01	L	W	-	-	-	-	-	-	-	-	-
6252	B*56:01	L	W	-	-	-	-	-	-	-	-	-
1903	B*51:01	L	W	-	-	-	-	-	-	-	-	-
8339	B*51:02	L	W	-	-	-	-	-	-	-	-	-
3899	B*52:01	L	W	-	-	-	-	-	-	-	-	-
3620	B*78:01	L	W	-	-	-	-	-	-	-	-	-
3702	B*57:01	L	W	G	R	N	-	-	-	-	-	-
3087	B*57:03	L	W	G	R	N	-	-	-	-	-	-
3554	B*58:01	L	W	G	R	N	-	-	-	-	-	-
2055	B*15:16	L	W	-	-	N	-	-	-	-	-	-
111	B*46:01	L	W	-	-	K	-	-	-	-	-	-
796	C*03:02	L	W	-	-	K	-	-	-	K	-	-
438	C*03:03	L	W	-	-	K	-	-	-	K	-	-
554	C*03:04	L	W	-	-	K	-	-	-	K	-	-
10 ± 4	Self alleles											
10 ± 5	163LW-negative alleles											

* All human mAbs were generated by Arend Mulder, Leiden, The Netherlands)

OK6H12 and VD1F11 Defined 163LW Epitopes

<u>VD1F11</u>	<u>OK6H12</u>	Eplet:	163LW		Polymorphic surface residues within 15 Å of 163LW								
	Sequence position		163	167	62	65	66	107	109	151	173	177	180
Unknown	IMM	B*15:03	L	W	R	Q	I	G	L	R	E	E	Q
OL MFI	OL MFI	PANEL											
8362	8125	B*15:01	L	W	-	-	-	-	-	-	-	-	-
7226	8972	B*15:02	L	W	-	-	-	-	-	-	-	-	-
6474	7638	B*15:03	L	W	-	-	-	-	-	-	-	-	-
8361	8241	B*15:10	L	W	-	-	-	-	-	-	-	-	-
5416	7300	B*15:13	L	W	-	-	-	-	-	-	-	-	-
7244	8625	B*35:01	L	W	-	-	-	-	-	-	-	-	-
4577	6438	B*49:01	L	W	-	-	-	-	-	-	-	-	-
7812	9862	B*50:01	L	W	-	-	-	-	-	-	-	-	-
6010	7292	B*53:01	L	W	-	-	-	-	-	-	-	-	-
5275	6252	B*56:01	L	W	-	-	-	-	-	-	-	-	-
5586	1903	B*51:01	L	W	-	-	-	-	-	-	-	-	-
7879	8339	B*51:02	L	W	-	-	-	-	-	-	-	-	-
2049	3899	B*52:01	L	W	-	-	-	-	-	-	-	-	-
5547	3620	B*78:01	L	W	-	-	-	-	-	-	-	-	-
3749	3702	B*57:01	L	W	G	R	N	-	-	-	-	-	-
3749	3087	B*57:03	L	W	G	R	N	-	-	-	-	-	-
2783	3554	B*58:01	L	W	G	R	N	-	-	-	-	-	-
5480	2055	B*15:16	L	W	-	-	N	-	-	-	-	-	-
5172	111	B*46:01	L	W	-	-	K	-	-	-	-	-	-
8056	796	C*03:02	L	W	-	-	K	-	-	-	K	-	-
7453	438	C*03:03	L	W	-	-	K	-	-	-	K	-	-
7532	554	C*03:04	L	W	-	-	K	-	-	-	K	-	-
19 ± 10	10 ± 4	Self alleles											
101 ± 262*	10 ± 5	163LW-negative alleles											

* Includes A*11:02=416, A*66:01=1299, C*06:02=566, C*08:01=1127, C*12:03=1195 and C*14:02=455

Reactivity of four 62GE-specific mAbs with OneLambda and Gen-Probe Luminex panels

Panel	SN510G11		ROU2D3	
	OL	GP	OL	GP
62GE pos A*02:01	15679	13400	13596	9071
62GE pos A*02:02	nt	14029	nt	9084
62GE pos A*02:03	21265	15787	14529	10191
62GE pos A*02:05	nt	16994	nt	12339
62GE pos A*02:06	21981	nt	13571	
62GE pos B*57:01	14267	10580	13670	6319
62GE pos B*57:03	18374	nt	13741	
62GE pos B*58:01	11813	14699	12256	9813
62GE neg alleles	25 (13-50)	108 (58-194)	9 (4-14)	13 (1-56)
Panel	VN2F1		WIM1B3	
	OL	GP	OL	GP
62GE pos A*02:01	3614	165	9663	6457
62GE pos A*02:02	nt	1350	nt	6190
62GE pos A*02:03	6610	223	13865	9888
62GE pos A*02:05	nt	7979	nt	10173
62GE pos A*02:06	7254	nt	14387	nt
62GE pos B*57:01	7850	3398	4184	159
62GE pos B*57:03	11309	nt	8187	nt
62GE pos B*58:01	6682	8652	2324	3107
62GE neg alleles	19 (2-81)	10 (4-20)	9 (1-258)	19 (3-34)

Antibody Assays for HLA Epitopes

- Binding assays on informative antibodies with single allele panels (Luminex)
- Use kits from two or more manufacturers
- Quantitative assessments of MFI values, cut-off points for positive reactions
- Interpretations of unexpected reactivities including false-positive and false-negative values

Interpretations of Antibody Reactivity

- HLA Epitope Antigenicity: Its reactivity with specific antibody
 - Structural epitopes and functional epitopes (eplets)
 - Effects of surface residue differences on antibody binding
 - Effects of hidden residues and bound peptides
 - How do antibodies react in other assays (CDC, C1q binding, etc.)?

Interpretations of Antibody Reactivity

- HLA Epitope Antigenicity: Its reactivity with specific antibody
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 - Effects of surface residue differences on antibody binding
 - Effects of hidden residues and bound peptides
 - How do antibodies react in other assays (CDC, C1q binding, etc.)?
- HLA Epitope Immunogenicity: Its ability to induce specific antibodies
 - HLA type information of immunizer and antibody producer
 - Nonself-self paradigm of HLA epitope immunogenicity

HLA Epitope Nomenclature

- Eplets or other residue defined configurations in sequence positions provide the basis for HLA epitopes
- Define epitopes from antibody reactivity patterns in context with specific eplets on immunizing alleles and residue differences with panel alleles in sequence locations corresponding to structural epitopes

Participants are invited to identify new antibody-defined HLA epitopes

Criteria:

- Detection method: antigen-binding with single alleles vs complement-dependent cytotoxicity
- Selection of informative antibodies
 - Monoclonals (human), isolated B-cell supernatants
 - Eluates from sera absorbed with informative alleles
- Reporting and verification of new epitopes

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16th Workshop Goals

- Develop criteria for a clinically useful HLA epitope nomenclature
- Design a database of residue configurations (eplets) from amino acid sequences of HLA alleles
- Characterize eplet-specific and eplet-associated epitopes from antibody reactivity patterns with informative panels
- Participants submit data on monospecific antibodies