Correlations between Terasaki's HLA class II epitopes and HLAMatchmaker-defined eplets on HLA-DR and -DQ antigens

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Key words
epitope; eplet; HLAMatchmaker; human leukocyte antigen-DR, -DQ; human leukocyte antigen antibody

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Received 31 January 2009; accepted 22 March 2009

doi: 10.1111/j.1399-0039.2009.01272.x

Abstract

Human leukocyte antigen (HLA) class II-specific antibodies increase the risk of transplant failure, and their characterization must consider epitopes rather than antigens. There are two strategies to determine HLA epitope structure. Terasaki's group has analyzed antibody reactivity patterns with single antigen panels with a computer program based on shared amino acid residues of reactive alleles. 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. Our epitope repertoire is based on so-called 'eplets' representing 3-Å patches of at least one polymorphic residue on the molecular surface. This report describes how 49 of 53 Terasaki's HLA-DR epitopes correspond to HLAMatchmaker-defined eplets. Most of them are equivalent to single eplets ($n = 33$) or two or more possible eplets ($n = 10$), but six had corresponding eplet pairs. There were 10 cases whereby eplets have permissible residue combinations, and in 5 cases, we found that eplet specificity might be influenced by nearby hidden residues. We could assign corresponding eplets to 17 of 18 Terasaki's HLA-DQ epitopes. This study demonstrates how the HLAMatchmaker interpretation of amino acid residues shared between antibody-reactive antigens can increase our understanding of the structural basis of HLA epitopes.

Introduction

Human leukocyte antigen (HLA) class II-specific antibodies increase the risk of transplant failure because they lead to a higher incidence of acute and chronic rejection (1–6). Antibodies react with epitopes on antigenic molecules, and a characterization of the antibody response to class II epitopes rather than antigens seems important for the management of sensitized patients considered for transplantation. Sensitive antibody detection methods such as the Luminex assays with single antigen panels are commonly used to assess humoral sensitization against HLA-DR and -DQ mismatches. The interpretation of antibody reactivity patterns requires an understanding of the repertoire of class II epitopes that can now be structurally defined with amino acid polymorphisms.

There are two strategies to determine the structural basis of HLA epitopes. One is based on the analysis of antibody reactivity patterns with HLA panels and a determination of amino acid residues exclusively shared by antibody-reactive alleles. Terasaki's group has recently published a series of papers that describe the amino acid configurations of HLA class II epitopes (7–12). Another strategy is a theoretical algorithm called HLAMatchmaker that predicts epitope structure on HLA molecules from stereochemical models of protein antigen–antibody complexes (13–15). The annotation 'eplet' refers to a patch of amino acids within a 3 Å radius of polymorphic residues on the molecular surface. The Web site http://HLAMatchmaker.net has more details about this algorithm.

An accompanying paper in this issue describes our strategy in more detail and addresses a comparative analysis of Terasaki's epitopes (TerEps) and HLAMatchmaker-defined eplets on HLA-A, -B and -C antigens (16). This report describes a similar analysis of 60 HLA-DR and 18 HLA-DQ epitopes reported by Terasaki's group (7–11).

Methods

TerEps on class II antigens are described by amino acid residues shared between alleles that react with mouse

monoclonal antibodies (mAbs) or human allosera from renal transplant recipients. Antibody testing was performed with Luminex-based binding assays, and Table 1 shows the composition of a typical panel of 28 single DRB alleles and 18 DQ heterodimers. This report addresses 60 TerEps on DRB (#1001–1043, #1401–1411 and #1601–1606), 15 on DQB (#2001–2015) and 3 on DQA (#2017–2019). Their residue compositions have the standard notation system of sequence positions and single letter amino acid codes. Combinations of two or more residues are separated by a + sign, i.e. 98E+104A; hidden residues under the molecular surface are shown between parentheses, i.e. (9W) and (10Q), and two or more possible amino acid combinations are separated by a slash, i.e. 70G+71A/116I/125S.

For each TerEp, we searched HLAMatchmaker for one or more eplets present on the same group of antigens and/or alleles of the Luminex panel and with similar amino acid residue compositions. We conducted this analysis in a stepwise manner as previously described (16). We searched for TerEps that are equivalent to single eplets with comparable amino acid compositions or correspond to pairs of eplets on the molecular surface locations separated far enough for contact by two different complementarity-determining regions (CDRs) of antibody. The locations of these eplet pairs were determined on crystallographic structures of DRB and DQ molecules downloaded from the http://www.ncbi.nlm.nih.gov/Structure Web site and viewed with the CN3D structure and sequence alignment software program (17). This program has a 'select by distance' (in Ångstroms) command that permits an assessment of the distances between eplets. From the 700–900 $Å^2$ surface area of a structural epitope, one can estimate that two eplets contacted by two different CDRs cannot be further apart than about 15 Å.

We have also considered polymorphic residues in unexposed locations below the molecular surface. Although such hidden residues cannot make direct contact with antibody, they may alter the conformation of a nearby epitope (18–22). Current definitions of eplets include hidden residues within a 3 Å radius, but it is also possible that hidden residues somewhat further away have an effect. Therefore, we have also considered hidden residues up to 6 Å away from an eplet.

Conversely, two or more eplets in the same molecular location may define the same epitope because their amino acid differences do not significantly influence the binding with specific antibody. This permissible residue variability reflects the structural type of cross-reactivity, and such eplet configuration has surface residues that dominate epitope specificity. Our eplet notations use an asterisk to indicate permissible residue combinations. For instance, residues in positions 73, 77 and 78 define the 77ATY and 77GTV eplets recognized by the same antibody. An epitope shared between these eplets has permissible differences between 73A and 73G and between 78V and 78Y, whereas its specificity is dominated by 77T. We annotate this epitope as 77T*, whereby asterisk represents the permissible 73A/G and 78V/Y combinations.

Table 1 High-resolution types of DR and DQ antigens in the Luminex panel[a]

DRB alleles				DQ heterodimers	
DR1	DRB1*0101	DRB1*0102	DRB1*0103	DQB1*0201	DQA1*0201
DR4	DRB1*0401	DRB1*0404	DRB1*0405	DQB1*0201	DQA1*0301
DR7	DRB1*0701			DQB1*0201	DQA1*0501
DR8	DRB1*0801			DQB1*0202	DQA1*0201
DR9	DRB1*0901			DQB1*0401	DQA1*0201
DR10	DRB1*1001			DQB1*0402	DQA1*0201
DR11	DRB1*1101			DQB1*0402	DQA1*0401
DR12	DRB1*1201	DRB1*1202		DQB1*0501	DQA1*0101
DR13	DRB1*1301	DRB1*1303		DQB1*0502	DQA1*0102
DR14	DRB1*1401			DQB1*0601	DQA1*0103
DR15	DRB1*1501	DRB1*1502		DQB1*0602	DQA1*0102
DR16	DRB1*1601			DQB1*0301	DQA1*0301
DR17	DRB1*0301			DQB1*0301	DQA1*0505
DR18	DRB1*0302			DQB1*0301	DQA1*0601
DR51	DRB5*0101	DRB5*0202		DQB1*0302	DQA1*0101
DR52	DRB3*0101	DRB3*0202	DRB3*0301	DQB1*0302	DQA1*0301
DR53	DRB4*0101	DRB4*0103		DQB1*0303	DQA1*0301
				DQB1*0303	DQA1*0201

[a] Composition of the single class II allele kit, lot 6 by One Lambda Inc., Canoga Park, CA, USA.

Results

DRB TerEps with surface residue descriptions and their equivalent eplets

There are 60 DRB TerEps described by residues on and/or below the molecular surface. Our analysis separated TerEps described exclusively by hidden residues (see below). Table 2 summarizes how 30 DRB TerEps with surface residue descriptions correspond to eplets, and Figure 1A–F depicts their locations on informative DRB molecules.

Residues in sequence position 96 in the β2 domain describe four TerEps. Each one corresponds to an eplet that has a polymorphic residue at that position and another polymorphic residue in position 180 about 3 Å away: #1031 is 96EV (Figure 1F), #1032 is 96HV (Figure 1C,E) and #1033 is 96QK (Figure 1D). The Luminex panel in Table 1 has three DR4 alleles, DRB1*0401, *0404 and *0405. Although their residue descriptions are different, the DR4-specific #1034 seems to be the same as the DR4-specific #1042 and #1605. This TerEp corresponds to 96YL and two adjoining eplets, 98EN and 180LT, as well as 32FYH on the α-helix (Figure 1A,B).

The DR52-specific #1036 is equivalent to 96QV in the β2 domain (Figure 1E). DR17, DR18 and DR52 share #1027 with the 77N description and is equivalent to 77GNY on the α-helix (Figure 1C,E). The DR17- and DR18 (or

Table 2 Thirty DRB TerEps with surface residue descriptions and their corresponding eplets

TerEp	Defined by	Antibody-reactive antigens	Residue description[a]	Comments	Eplets[b]	Model
#1031	Allo	DR1; DR51	96E		96EV	Figure 1F
#1032	Allo	DR7, 8, 9, 10, 11, 12, 13, 14, 17, 18; DR52	96H	96H not on DR10	96HV	Figure 1C,E
#1033	Allo	DR10, 15, 16; DR53	96Q		96QK	Figure 1D
#1034	Allo	DR4	96Y		32FYH/96YL/98EN/180LT	Figure 1A,B
#1042	Allo	DR4	180L		32FYH/96YL/98EN/180LT	Same as #1034
#1605	mAb, Allo	DR4	(13H), 33H, 96Y, 180L		32FYH/96YL/98EN 180LT	Same as #1034
#1036	mAb, Allo	DR52	98Q		98QS	Figure 1E
#1027	mAb, Allo	DR17, 18; DR52	77N		77GNY	Figure 1C,E
#1026	mAb, Allo	DR17, 18	74R	74R also on DRB3*0101	71QKGR + 60Y	Figure 1C (eplets are 14 Å apart)
#1040	Allo	DR4, 8, 10, 11, 12, 13, 14, 17, 18	140T	140T also on DRB3*0301	140TV	Figure 1B,C
#1041	Allo	DR8, 11, 12, 13, 14, 17, 18	149H	149H also on DRB3*0301	149H	Figure 1C
#1603	Allo	DR15, 16	(11P), (13R), 133L, 142M		133L/140AM	Figure 1D
#1020	mAb, Allo	DR15	71A	71A also on DRB5*0202	71QAA	Figure 1D
#1402	mAb	DR51	(9Q), 108T		108T	Figure 1F
#1017	mAb	DR11	58E		57DE	
#1008	Allo	DR7	25Q		26QKF/71DRG	
#1602	mAb	DR7	(11G), 14K, 25Q, (30L)		26QKF/71DRG	Same as #1008
#1001	mAb, Allo	DR7, 9; DR53	4Q		4Q	
#1019	mAb, Allo	DR9, 10, 14; DR53	70R		71RRA	
#1029	Allo	DR7, 9	78V		77TV/98ES	
#1043	Allo	DR7, 9, 10	181M	181M also on DR53	180VM	
#1410	mAb, Allo	DR7, 9, 12	(57V), 60S	both also on DRB3*0101 and *0301	60S+71R	11 Å apart
#1606	mAb	DR14	(57A), 60H, 112Y		57AA/60H/112Y	
#1409	Allo	DR13	(57S)+71K	71K only on DRB1*1303	71DKA	
#1030	Allo	DR1, 12	85A	85A only on DRB1*0102 also on DRB5*0202	85AV	
#1407	mAb, Allo	DR8, 12	(13G), 16Y		14GEY/71DRA-(13G)	13G is 4.5 Å away from 71DRA
#1024	mAb, Allo	DR8	74L		25YRF/73ALDT	
#1023	mAb, Allo	DR9, 14; DR53	74E		71RAE	Also 77T
#1014	mAb	DR10; DR53	40Y		40Y+71RRA	Eplets are 4 Å apart
#1408	Allo	DR10; DR53	(38A), 40Y	38A also on DRB3*0202	40Y+71RRA	Same as #1014

Allo, alloserum or eluted alloantibody; mAb, monoclonal antibody; TerEp, Terasaki's epitope.

[a] Amino acids in human leukocyte antigen protein sequence positions are listed with the standard single letter code. Hidden residues not exposed on surface of molecule are in parenthesis. Combinations of residues are shown with + sign.

[b] Two and three unique eplets are separated with slash.

DR3)-specific #1026 is described by 74R, but this residue is also on DRB3*0101. The best description of a DR3 epitope is a pair of eplets: 77GNY and 60Y, which are about 14 Å apart, a distance sufficient for contact by two CDRs of antibody (Figure 1C). TerEps #1040 and #1041 are on the same group of DRB antigens except for DR4 and DR10; they correspond to 140TV (Figure 1B,C) and 149H (Figure 1C), respectively. These eplets are also on DRB3*0301.

The DR15- and DR16 (or DR2)-specific #1603 corresponds to 133L and140AM in the β2 domain; residues (11P)

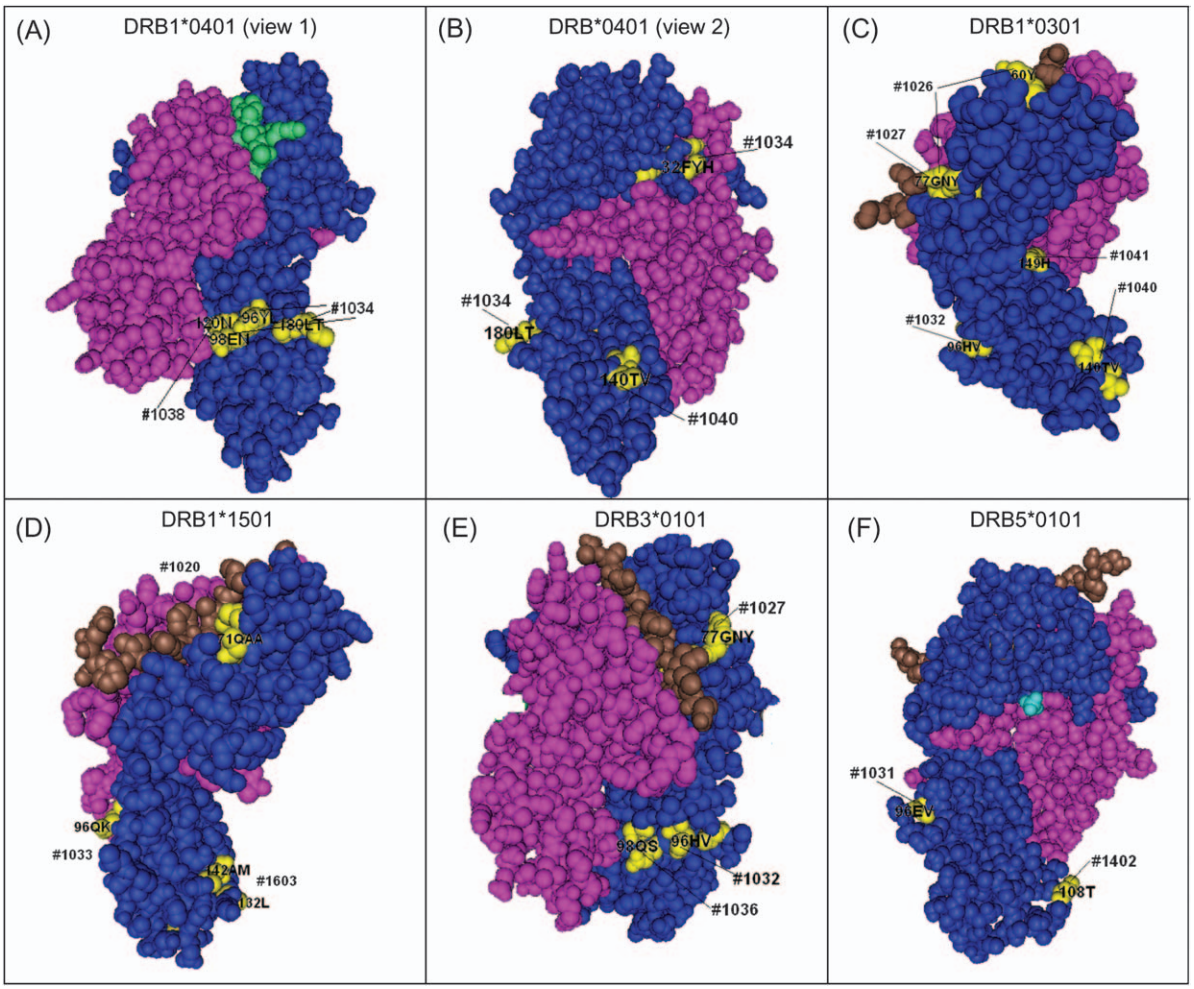

Figure 1 Molecular locations of eplet equivalents of DRB Terasaki's epitopes listed in Table 2 (colour codes: eplet residues are in yellow, α-chain in magenta, β-chain is in blue and peptiae in green).

and (13R) are not antibody accessible (Figure 1D). However, 71QAA on the α-helix is equivalent to the DR15-specific #1020. DR15 and DR16 are in strong linkage equilibrium with DR51, which has the unique #1402 with its 108T equivalent eplet. DR51 and DR1 share #1031 and its equivalent 96EV (Figure 1F). We have recently reported that DR1 or DR51 frequently elicit antibodies that react with 96EV (23).

Whereas no structures of other DRB molecules are available to illustrate their locations, we could readily identify corresponding eplets for the remaining TerEps in Table 2. Several TerEps are antigen specific. For instance, #1017 specific for DR11 is equivalent to 57DE, an eplet also shared with certain HLA-DP antigens (6). The 25Q residue describes the DR7-specific #1008, and we identified two eplets uniquely found on DR7, namely 26QKF and 71DRG. This TerEp seems to be the same as #1602 described by (11G), 14K, 25Q and (30L). We could not find any eplet that corresponds solely to the hidden (11G) and (30L) residues.

Three TerEps are shared between DR7 and one or two other DRB antigens. TerEp #1029 is described by 78V and corresponds to eplets 77TV and 98ES. Although 181M describes #1043 on DR7, DR9 and DR10, this residue is also present on DR53. Nevertheless, this TerEp corresponds to the180VM eplet (DR53 has 180MM). DR7, DR9 and DR12 share #1410 described by (57V) and 60S, but these residues are also present on DRB3*0101 and DRB3*0301. An epitope shared between DR7, DR9 and DR12 can be explained only with 60S paired with 71R about 11 Å away.

Two TerEps relate to serological splits of DR6. DR14 has a unique #1606 that corresponds to 57AA, 60H and 112Y. TerEp #1409 is described by the combination of (57S) and 71K but is only present on DRB1*1303, and this allele has a unique 71DKA eplet. It seems unlikely that (57S) affects

this epitope because this hidden residue is more than 10 Å away from 71K.

The DR8-specific #1024 is defined by 74L and has two equivalent eplets: 73ALDT and 25YRF. DR8 shares #1407 with DR12; this TerEp is described by (13G) and 16Y and corresponds to 14GEY. It is also possible that #1407 is equivalent to 71DRA whose conformation is influenced by 13G below the molecular surface about 4.5 Å away. DR1 and DR12 share #1030 described as 85A, but this residue is absent on DRB1*0101 or DRB1*0103 but present on DRB5*0202. The 85AV eplet corresponds to an epitope shared between DRB1*0102, DR12 and DRB5*0202.

A pair of eplets 71RRA and 40Y separated by about 4 Å can also explain #1014 on DR10 and DR53. This TerEp appears to be same as #1408.

TerEps equivalent to eplets with permissible amino acid combinations

Table 3 lists 10 TerEps with surface residue descriptions that correspond to eplets with permissible residue combinations; 5 of them (#1018, #1021, #1022, #1025 and #1028) are located in the highly variable 67–78 sequence of the α-helix of DRB. Each one has a unique amino acid description, and Figure 2A–F shows the amino acids within a 3 Å radius on informative DR molecules that carry these TerEps. The upper part (yellow) shows the residues of a 3-Å patch. The lower part depicts the patch in detail: polymorphic residues are colored yellow, permissible residue combinations are recorded next to residues colored in magenta and monomorphic residues in yellow are marked only with their sequence position number.

The 77T residue describes #1028, and a visualization of a corresponding 3-Å patch on DRB*0101 (Figure 2A) and DRB5*0101 (Figure 2B) shows that 77T is surrounded by a monomorphic 76D and two residues with permissible combinations. They are 73A (on all #1028-carrying antigens except DR7, which has 73G) and 78Y (on all #1028-carrying antigens except DR7 and DR9, which have 78V). These patch configurations illustrate that #1028 may have a dominant 77T contiguous with the monomorphic 76D and that the A/G and V/Y interchanges in flanking positions 73 and 78 do not significantly affect the specificity of this epitope. We have assigned a corresponding eplet as 77T*, whereby asterisk represents the permissible 73A/G and 78V/Y combinations.

As shown in Figure 2C, on DRB1*0301, the 3-Å patch of the #1022-describing 73G has the monomorphic 69E, 72R and 76D and the permissible 74Q/R and 77N/T combinations. This modeling suggests a dominant role of 73G in this epitope, and the alignment of 69E, 72R and 76D might generate a contiguous configuration as a potential contact site for the specificity-determining CDR loop of antibody. We assigned the corresponding eplet as 73G*, whereby asterisk represents 74Q/R and 77N/T.

TerEp #1025 on DR7 and DR52 (except DRB3*0301) is described by 74Q that forms a 3-Å patch with the monomorphic 75V, 76D and 79C and with the polymorphic 73G and 78V/Y (Figure 2D). The corresponding eplet is 74GQ*, whereby asterisk represents the permissible 78V/Y combination. The 3-Å patch of the #1018 describing 70D has the monomorphic 69E and 67H/I/L, 71E/K/R and 73A/G (Figure 2E). This TerEp corresponds to 70D*, whereby

Table 3 Ten DRB TerEps that correspond to eplets with permissible residue combinations

TerEp	Defined by	Antibody-reactive antigens	Residue description	Comments	Eplets	Comments	Model
#1028	Allo	DR1, 4, 7, 9, 10, 11, 12, 13, 14, 15, 16; DR51; DR53	77T	77T also on DR8	77T*	* = 73A/G and 78V/Y	Figure 2A,B
#1025	mAb, Allo	DR7; DR52	74Q	74Q not on DRB3*0101	74GQ*	* = 78V/Y	Figure 2C
#1018	Allo	DR7, 8, 11, 12, 13, 16, 103; DR51	70D	70D not on DRB5*0202	70D*	* = 67H/I/L, 71E/K/R and 73A/G	Figure 2D
#1022	Allo	DR7, 17, 18; DR52	73G		73G*	* = 74Q/R and 77N/T	Figure 2E
#1021	Allo	DR4, 13, 17, 18; DR52	71K	71K not on DRB1*0404/5 and *1301	71K* (?)	* = 70D/Q	Figure 2F
#1035	Allo	DR4, 7, 9	98E		98E*	* = 120N/S	Figure 2G
#1411	mAb, Allo	DR4, 7, 9	98E, 104A	104A also on DR51 and DR52	98E*	* = 120N/S same as #1035	Figure 2H
#1038	Allo	DR4, 10; DR51; DR53	120N		120N*	* = 98E/K	Figure 2I
#1037	mAb, Allo	DR4, 7, 9; DR51; DR52	104A		104A*	* = 105K/R	Figure 2J
#1039	Allo	DR1, 7, 9, 15, 16; DR51; DR52; DR53	140A	140A not on DRB3*0301	140A*	* = 105K/R	Figure 2K,L

Allo, alloserum or eluted alloantibody; mAb, monoclonal antibody; TerEp, Terasaki's epitope.

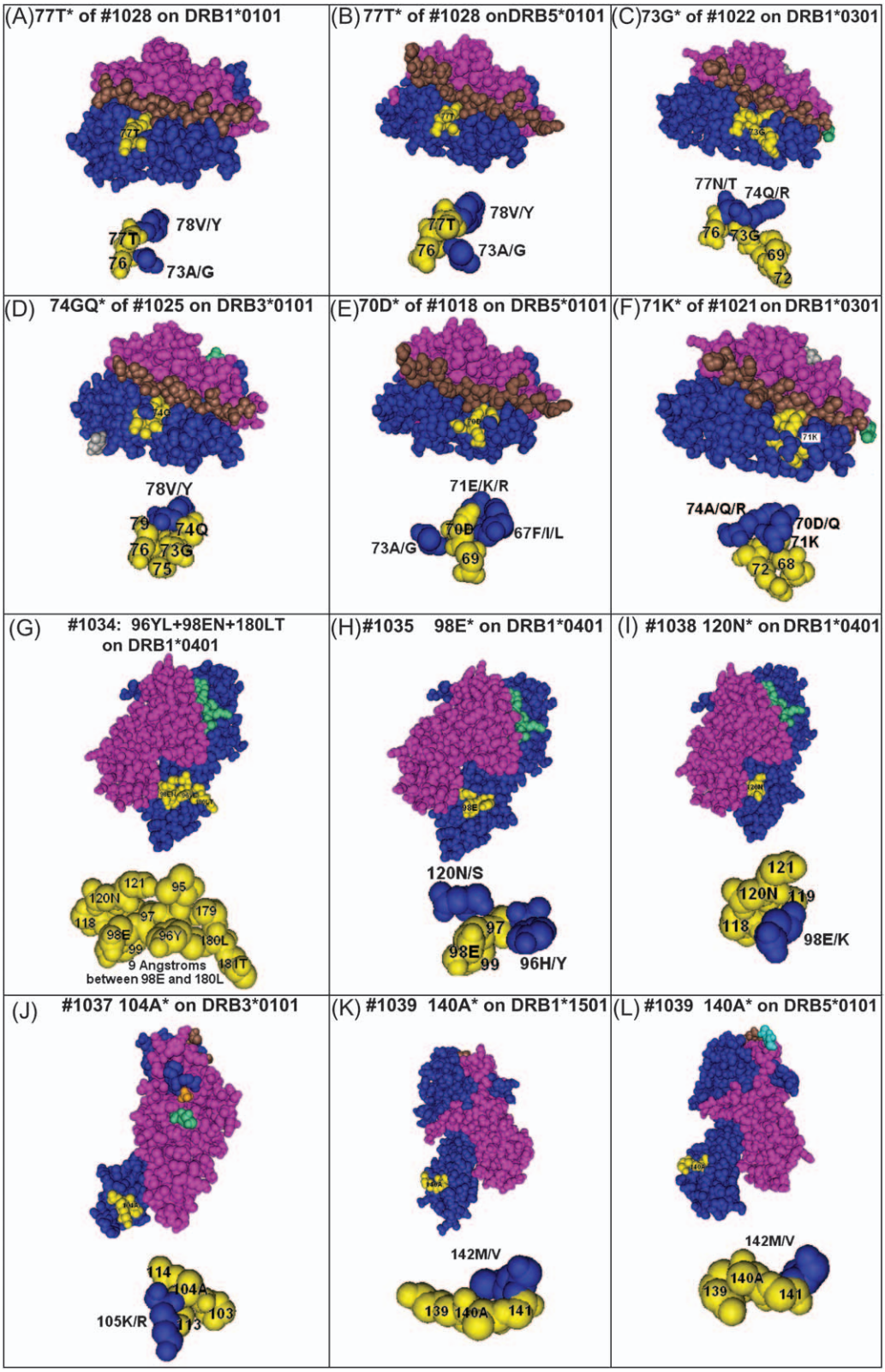

Figure 2 Locations of eplets with permissible residue combinations (colour codes: see Figure 1).

asterisk represents these three permissible polymorphic residue combinations.

The 71K residue that describes #1021 forms a 3-Å patch with the monomorphic 68L and 72R and the polymorphic 70D/Q and 74A/Q/R combinations (Figure 2F). Because 71K is not well expressed on the molecular surface, it is uncertain how this residue can play a dominant role in the specificity of #1021. Moreover, #1021 is on DR4, DR13, DR17, DR18 and DR52, but DRB1*0403, *0405 and *1301 do not have 71K. At this time, the assignment of 71K* must be considered tentative.

As noted above, three DR4-specific eplets 96YL, 98EN and 180LT are in adjoining positions in the β2 domain (Figure 1A). The polymorphic residues of these eplets are 96Y, 98E, 120N, 180L and 181T. Their 3-Å patches put together form a large complex of adjoining residues that may comprise additional DR4-specific eplets, such as 96YE, i.e. 96Y plus 98E (Figure 2G). Moreover, the contiguous alignments of residues within these eplets such as 99V-98E-120N and 97D-98Y-180L-181T seem to meet criteria for contact with the loops of specificity-determining CDRs of antibody. Residues 98E and 180L are about 9 Å apart, and it is also possible that the DR4-specific epitope requires an eplet pair such as 98EN+180LT or 96YL+98EN. Altogether, these findings suggest that DR4-specific antibodies may recognize multiple configurations in that part of the β2 domain.

In this large structural complex, we found two eplets with permissible residue combinations. DR4, DR7 and DR9 share #1035 described by 98E; this residue is in a 3-Å patch that has the monomorphic 97D and 99V and the permissible 96H/Y and 120N/S combinations (Figure 2H). It seems that 98E dominates the specificity of #1035, but the monomorphic 97D and 99V might also be part of this epitope. Although the residue description of #1411 includes 104A also on DR51 and DR52, it appears that #1411 is identical to #1035 because both TerEps are on the same group of DRB antigens. The second example is #1038 on DR4, DR10, DR51 and DR53 and corresponds to 120N*, whereby asterisk is 98E/K (Figure 2I).

TerEp #1037 on DR4, DR7, DR9, DR51 and DR52 has a corresponding 104A*, whereby asterisk represents 105K/R (Figure 2J). All antigens in this group have 105K except DR51, which has 105R. A large group of DRB antigens have #1039 described by 140A (not on DRB3*0301) and that corresponds to 140A*, whereby asterisk is 142M/V (Figure 2K,L).

Eplet assignments to DRB TerEps described by hidden residues

Table 4 lists 20 TerEps described solely by hidden polymorphic residues below the molecular surface and that cannot make direct contact with antibody. These residues are in 14 different sequence locations highlighted in yellow in the DR4 structural model (Figure 3). All these hidden positions are in the peptide-binding groove, and a top view shows their approximate primary locations: below the α-helix of the monomorphic DRA chain (positions 9, 10 and 11), the bound peptide (positions 12, 13, 30, 31, 37, 38 and 57) and the α-helix of the polymorphic DRB chain (positions 14, 28 and 47). A side view suggests a relative prominence of positions 13, 14, 28 and 47 immediately below the molecular surface where epitopes are present.

This analysis addresses the possibility that hidden residues might significantly influence the conformation of an epitope, thereby altering its specificity. Their distance must be close enough for any significant interaction, probably no further away than 6 Å. Several eplets such as 14FEH incorporate hidden residues because they are within 3 Å of the polymorphic surface residues, but we also have searched for eplets that might be influenced by hidden residues up to 6 Å away. We annotated corresponding eplets with hidden residues between parentheses, for example 48YR-(37F) or 32IYN-(9K and 30G). We also searched for hidden-residue-independent eplets (single ones or pairs) expressed by the same DR antigens as those reported for TerEps.

Table 3 lists the DR7-specific #1405 and the DR4-specific #1406 with hidden residue descriptions, but these TerEps appear to be the same as #1008 and #1602 (on DR7) and #1034, #1042 and #1605 (on DR4) shown in Table 2.

Five TerEps are equivalent to eplets that have hidden residues. Residues in hidden position 13 describe three TerEps. For two of them, we readily found corresponding eplets, namely 14FEH for #1005 with (13F) and 14SEH for #1006 with (13S). However, #1007 corresponds to an eplet 48YR, whose conformation seems to be dependent on (13Y) about 5.5 Å away. TerEp #1015 with (47F) corresponds to 48FR. Although #1016 is on DR4, DR8 and DR13, we noted that the descriptive (57S) is only on DRB1*0405, DRB1*0801 and DRB1*1303; this TerEp seems equivalent to 57SA. TerEp #1010 on DR1, DR9, DR51 and DR53 and described by (31I) is equivalent to 32IYN.

The DR12-specific #1012 is described by (37L) and may correspond to 48YR-(37L), but there are other DR12-specific eplets such as 25YRL and 71DRA+85AV, which do not require (37L). DR9 and DR51 share #1404 and they uniquely have (11D) and (28H). Both antigens share 32IYN, and (11D) seems close enough (5.5 Å) to alter the conformation of this eplet; (28H) is more than 9 Å away, too far for any effect. Therefore, #1404 may correspond to 32IYN-(11D).

DR9 has a distinct mAb-defined epitope #1401 and two unique residues (9K) and (30G) hidden in the peptide-binding groove. These residues are about 4 Å from 32IYN sufficiently close for a conformational effect that would render this epitope as DR9 specific. Although #1401 may

Table 4 Eplet equivalents of 20 DRB TerEps described solely by hidden residues

TerEp	Defined by	Antibody-reactive antigens	Residue description	Comments	Eplets	Comments
#1405	Allo	DR7	(11G), (14K)		26QKF/71DRG	Same as #1008 and #1602
#1406	Allo	DR4	(11V), (13H)		32FYH/96YL/98EN/180LT	Same as #1034, #1042 and #1605
#1005	Allo	DR1, 9, 10	(13F)		14FEH	
#1006	Allo	DR11, 13, 14, 17, 18; DR52	(13S)		14SEH	
#1007	Allo	DR7; DR51	(13Y)		48YR-(13Y)	
#1010	Allo	DR1, 9; DR51; DR53	(31I)		32IYN	
#1015	Allo	DR11, 12, 13, 15, 17	(47F)	Not on DRB1*1303	48FR	
#1016	Allo	DR4, 8, 13	(57S)	Only on DRB1*0405, *0801 and *1303	57SA	
#1012	mAb	DR12	(37L)		48FR-(37L)/25YRL/71DRA+85AV	
#1401	mAb	DR9	(9K), (30G)		32IYN-(9K, 30G)/26KYH/74RAE+77TV	
#1404	Allo	DR9; DR51	(11D), (28H)		32IYN-(11D)	(11D) is 5.5 Å away
#1002	Allo	DR1, 7, 15, 16	(9W)		32YN*-(9W10Q)	* = 31I/F, 9W is 3.8 Å away, 10Q is 44 Å away
#1403	Allo	DR1, 7, 15, 16	(9W), (10Q)		32YN*-(9W10Q)	Same as #1002
#1604	Allo	DR11, 13, 14, 17, 18	(11S)+(12T)+(13S)		14SEH-(11S, 12T)	
#1601	mAb, Allo	DR8, 11, 12, 13, 14, 17, 18	(9E)+(10Y)+(11S)+(12T)+(13S)	DR8 and DR12 have 13G, not 13S	96HK	
#1011	Allo	DR7, 14	(37F)	37F also on DRB3*0101 and *0301	48YR-(37F)+77T	48YR and 77T are 12 Å apart
#1004	Allo	DR4, 10	(11V)		140TV+149Q?	Eplets are 19 Å apart
#1003	Allo	DR1, 4, 7, 9, 15, 16; DR51; DR53	(10Q)		32Y*-(10Q)?	
#1009	Allo	DR4, 8, 11, 13, 14, 15, 16, 17	(28D)	28D also on DRB3*0101	?	
#1013	Allo	DR1, 103, 14, 15	(37S)	37S not on DR14 but on DR16	?	

Allo, alloserum or eluted alloantibody; mAb, monoclonal antibody; TerEp, Terasaki's epitope.

correspond to 32IYN-(9K and 30G), there are other DR9-specific eplet configurations including 26KYH and 74RAE+77TV that do not require these hidden residues.

TerEps #1002 and #1403 (both on DR1, DR7, DR15 and DR16) appear to be the same, and the hidden residues 9W and 10Q are closest (3.8 and 4.1 Å, respectively) to 32YN*, whereby asterisk represents the permissible F/I combination in position 31. Accordingly, these TerEps are annotated as 32YN*-(9W and 10Q).

Two TerEps are described by combinations of hidden residues: #1604 has the (11S)+(12T)+(13S) description and corresponds to 14SEH, whose conformation is dependent on (11S) and (12T). The combination of (9E)+(10Y)+(11S)+(12T)+(13S) described #1601. DR8 and DR12 are listed as #1601-carrying antigens, but they do not have (13S), and this residue should probably be excluded from the residue description of #1602. We have not found a corresponding eplet affected by (9E)+(10Y)+(11S)+(12T) with or without (13S). However, the 96HK eplet is equivalent to #1602.

DR7 and DR14 carry #1011 described by (37F); this residue is also on DRB3*0101 and DRB3*0301, and the closest eplet is 48YR about 4.5 Å away. Another residue is necessary to explain the presence of #1011 on only DR7 and DR14: 77T is 12 Å away from 48YR far enough for contact with a second CDR. DRB3*0101 and DRB3*0301 have 77N. Therefore, #1011 may correspond to 48YR-(37F)+77T.

Four TerEps with hidden residue descriptions do not have informative eplet configurations shared between antibody-reactive antigens. A large group of DR antigens have #1003 described by (10Q), and the most nearby eplets

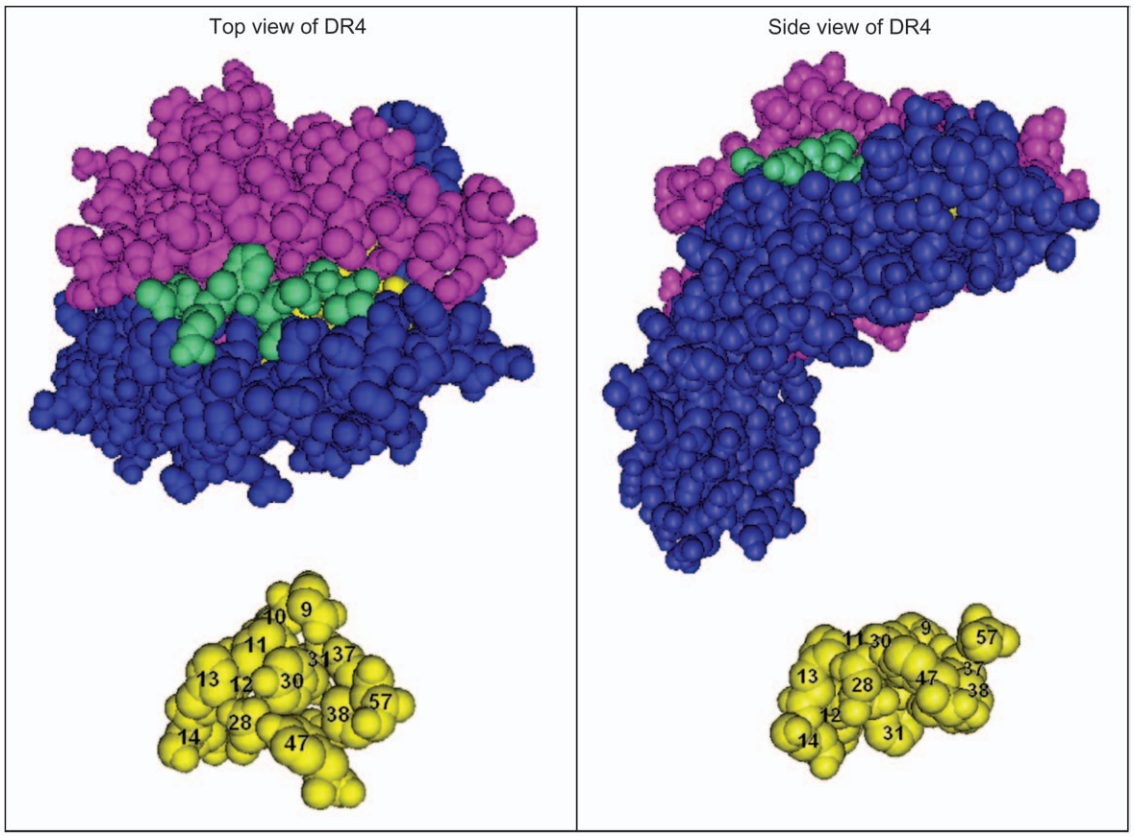

Figure 3 Views of hidden polymorphic residues on the DR4 molecular structure (colour codes: see Figure 1).

are in position 32 about 5 Å away. Three different eplets are on DR antigens with (10Q), namely 32IYN (on DR1, DR9, DR51 and DR53), 32FYH (on DR4) and 32FYN (on DR7), and each one has 32Y. It is possible that #1003 reflects an epitope dominated by 32Y and significantly influenced by 10Q, which is located under the DRA α-helix. However, we question why so many closely located combinations like 31F/I, 33H/N as well as the nearby 9E/K/Q/W combination would not affect the specificity of #1003. We must conclude that the eplet equivalent of this TerEp remains elusive.

There are no eplets even within a 10 Å radius of the hidden (11V), which describes #1004 on DR4 and DR10. This TerEp might correspond to the 140TV+149Q pair, although these eplets are 19 Å apart, probably too far to serve as contact sites for two CDRs of antibody. TerEp #1009 on a large group of antigens is described by (28D), but this residue is also on DRB3*0101. TerEp #1013 on DR1, DR103, DR14 and DR15 and has (37S), but this residue is on DR16 rather than on DR14.

DQ TerEps with corresponding eplets

Table 5 lists 15 DQB TerEps and their residue descriptions, and Figure 4 shows the locations of corresponding eplets on the crystalline structures of DQ2, DQ6 and DQ8 heterodimers. Six TerEps define serological DQ specificities. The DQ1 (i.e. DQ5+DQ6)-specific #2004 corresponds to three eplets: 52PQ and the proximally located 84EV and 89GI (Figure 4D,E). TerEp #2015 is specific for DQ5 and corresponds to 70GA and 116I. A mAb defines #2011 on DQ5 and DQB1*0601, which have (38V), but not on DQB1*0602, which has (38A). This hidden residue is >10 Å away from 46V listed in the description of #2011, and it seems unlikely that it affects the conformation of a 46V-defined epitope. However, the distance between the 38V and the DQ1-defining 52PQ eplet is 5.8 Å, and it is possible that #2011 corresponds to 52PQ-(38V).

Three hidden residues (28S), (30S) and (37I) and two surface residues 52L and 55L describe the DQ2-specific #2001. DQ2 has four more unique residues 46E, 47F, 71K and 74A, and consequently, there are several eplets that correspond to #2001 including 45GE, 52LL and 70RKA (Figure 4A,B).

The DQ3 (i.e. DQ7+DQ8+DQ9)-specific #2006 described by 55P is equivalent to 55PPP (Figure 4G). DQ7 has a unique 45E residue that describes #2005, and 45EV defines this specificity. DQ4 is defined by #2002 described by 56L and has also the distinct 70E and 71D residues; this TerEp corresponds to 56LD and 70ED. DQ3 and DQ4 share #2014 that has five possible residue

Table 5 Eighteen DQ TerEps and their corresponding eplets

TerEp	Defined by	Antigens	Residue description	Eplet	Comments
#2004	mAb, allo	DQ5, 6 (DQ1)	84E/85V/86A/89G/90I/221Q	52PQ/84EV/89GI	Figure 4D,E
#2015	allo	DQ5	70G+71A/116I/125S	70GA/116I	
#2011	mAb	DQ5, DQB1*0601	(38V)+46V	52PQ-(38V)	
#2001	mAb, allo	DQ2	(28S)/(30S)/(37I)/52L/55L	45GE/52LL/71RKA	
#2006	mAb, allo	DQ7, 8, 9 (DQ3)	55P	55PPP	Figure 4A,B
#2005	mAb, allo	DQ7	45E	45EV	
#2002	mAb, allo	DQ4	56L	57LD/70ED	
#2014	allo	DQ4, 7, 8, 9	77T+84Q/77T+85L/77T+86E/77T+87T/182N	52PL/140T/182N	Figure 4G,H
#2013	allo	DQ2, 4, 7, 8, 9	84Q/85L/86E/(87L)/89T/220H/221H	84QL	Figure 4H
#2010	mAb	DQ4, 5, 6, 8, 9	45G+46V	45GV	Figure 4D,E,G
#2012	mAb	DQ8, 9	45G+55P	45GV+55PPP	Figure 4G
#2009	mAb	DQ2, 4, 5, 6, 8, 9	34R (is monomorphic)+45G	45G*	* = 46E/V, Figure 4C
#2007	mAb, allo	DQ4, 5, 6	52P+55R	55PR*	* = 56L/P, Figure 4F
#2003	mAb, allo	DQ4, 5, 6, 7, 8, 9	(28T)/46V/52P	46VY*/52P*	* = 45E/G/* = 53L/Q, Figure 4I
#2008	mAb	DQ2, 5, 7, 8, 9	(9Y)+(11F) (is monomorphic)	Undefined	Xenoepitope?
#2017	allo	DQA1*02	47K/52H/54L	47KL/53HRL	
#2019	allo	DQA1*03	(26S)/47Q/56R/187T	47QL/52FRR/187T	Figure 4H
#2018	allo	DQA1*04, 05, 06	40G/47C	41GR/51VLQ	Figure 4B

Allo, alloserum or eluted alloantibody; mAb, monoclonal antibody; TerEp, Terasaki's epitope.

descriptions; there are three corresponding eplets: 52PL and in the β2 domain, 140T and 182N (Figure 4G).

All DQ antigens except DQ1 have #2013 described by multiple residues; six of them 84Q, 85L, 86E, 87L, 89T and 90T are in a patch of about 5 Å. A single eplet 84QL describes this patch (Figure 4H). The 220H and 221H descriptions of #2013 are not on the external DQ domains and are therefore not considered as epitopes.

The descriptions of three TerEps include 45G. TerEp #2010 on DQ4, DQ5, DQ6, DQ8 and DQ9 is equivalent to 45GV (Figure 4D,E,G). TerEp #2012 with 45G+55P is on DQ8 and DQ9 and corresponds to 45GV+55PPP separated by about 12 Å (Figure 4G). A mAb defines #2009 on all DQ molecules except DQ7; 45G and the monomorphic 34R describe this TerEp. The 3-Å patch of 45G has 46E/V and the monomorphic 41D, 44V and 71R (Figure 4C) and 45G*, whereby the asterisk represents 45E/V corresponds to #2009.

There are two additional DQB TerEps corresponding to eplets with permissible residue combinations. TerEp #2007 on DQ4, DQ5 and DQ6 has a unique 55R, whereas 52P is also on DQ3. The 3-Å patch of 55R has 52P, 56L/P and 58E (monomorphic), and #2007 corresponds to 55PR*, whereby asterisk represents the permissible 56L/P combination. This eplet has a contiguous sequence structure flanked by the permissible residue combination (Figure 4F). All DQ antigens except DQ2 have #2003 described by 46V and 52P that are 15 Å apart; the hidden (28T) is more than 7 Å away, which seems too far for a conformational effect. The 3-Å patch of 46V has 45E/G and 47Y, whereas the 3-Å patch of 52P has the monomorphic 51T and 53L/Q. Figure 4I shows the structural configurations of 46VY* (whereby asterisk is 45E/G) and 52P* (whereby asterisk is 53L/Q).

Two hidden residues (9Y) and the monomorphic (11F) describe the mAb-defined #2008; we could not identify an eplet shared by DQ2, DQ3 and DQ5, and it seems likely that #2008 is a xenoepitope rather than an alloepitope.

Table 4 lists also three DQA TerEps, each of them are defined by alloantibodies eluted from informative DQ heterodimers used to separate DQA from DQB antibodies (8). The DQA1*02-specific #2017 is equivalent to 47KL and 53HRL, which are about 5 Å apart. The DQA1*03-specific #2019 corresponds to 47QL, 52FRR and 187T (Figure 4H). The rather common #2018 on DQA1*04, DQA1*05 and DQA1*06 is analogous to 41GR and 51VLQ (Figure 4B).

DR and DQ eplets without corresponding TerEps

Table 6 represents a list of DRB, DQB and DQA eplets for which no corresponding TerEps have been reported. Our serum screening experience has indicated antibodies with DRB specificities associated with these eplets (data not shown). A recent analysis of sensitized kidney transplant patients has shown rather common antibodies against several DQB eplets such as 57PA and 70GT and DQA eplets such as 169AE and 75SL (6).

Discussion

Similar to our experience with the class I TerEps (16), this analysis has shown that the majority of HLA-DR and -DQ

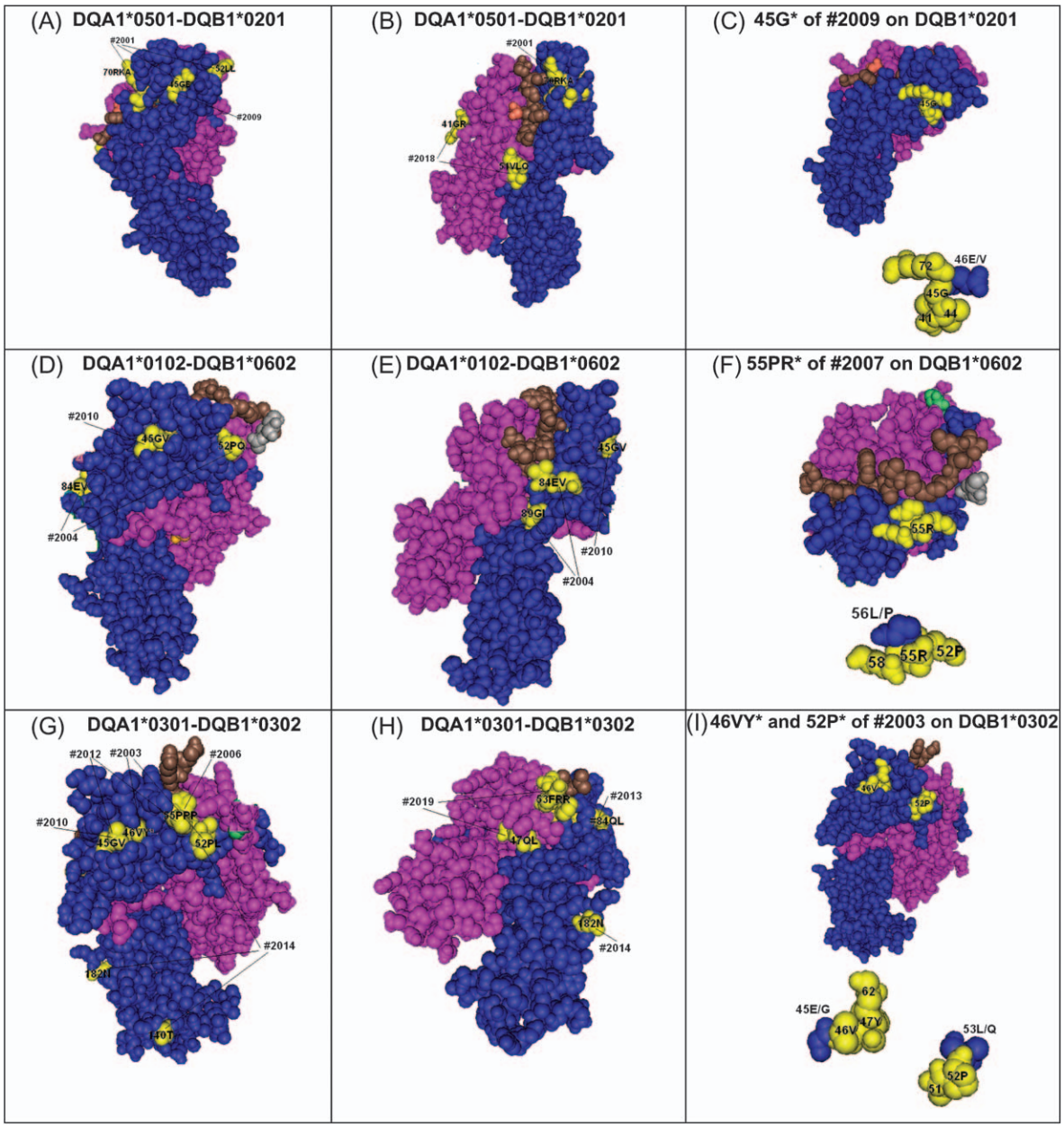

Figure 4 TerEps and their corresponding eplets on human leukocyte antigen-DQ heterodimers (colour codes: see Figure 1).

TerEps correspond to HLAMatchmaker-defined epitopes. This study addressed 60 DRB TerEps, but 7 of them appear to be duplicates because they are on exactly the same group of antigens. We could assign corresponding eplets to 49 of the remaining 53 DRB TerEps and to 17 of 18 DQ TerEps. All four DRB TerEps without corresponding eplets have descriptions with only hidden residues that cannot make direct contact with antibody. About two thirds of TerEp equivalents were single eplets, and the remainder were two or more possible eplets or eplet pairs. There were 10 cases, whereby eplets have permissible residue combinations, and in 5 cases, we found that eplet specificity appeared to be influenced by nearby hidden residues.

Previous studies on human mAbs have demonstrated that certain HLA epitopes require a pair of eplets on the same allele (24). These eplets must be close enough on the molecular surface so they can make contact with the antibody-specificity-mediating CDR loop, and the second CDR loop referred to as a critical contact site necessary for sufficient antibody binding. Antigens that have only the specific eplet will give negative or weak reactions with antibody. This concept may also explain why antibodies

Table 6 HLA-DR and HLA-DQ eplets without corresponding TerEps

DRB eplet	Antigens[a]	DQB eplet	Antigens
12LKF	DR1	14GM	DQ2, 4, 6, 8
25HRL	DR1, 10	52PL	DQ3, 4
26RL	DR1, 10, 12	57PA	DQ2, 8
120S	non-DR4, 51, 53	70GT	DQB1*0602/3
98KS	DR1, 2, 3, 5, 6, 8	74EL	DQ6, 7, 8, 9
104SK	DR1, 2, 3, 5, 6, 8, 53	74SV	DQ4, 5
149Q	non-DR3, 5, 6, 8, 52c	77DR	DQ2, 5
71Qk/rA	DR1, 4	140A	DQ2, 6
98KN	DR10, 51, 53		
81HA	DR12, 51b		
26KFD	DR2, 4	DQA eplet	
25HRF	DR2, 4, 6, 11, 18, 51, 52	48LF	DQA1*02, 03
26RF	DR2, 4, 6, 8, 11, 51, 52bc	50EF	DQA1*01
32FHN	DR3, 6, 12, 52	56RB	DQA1*02, 04, 05, 06
6C	DR51b	60QF	non-DQA1*01
51R	DR52b	69L	DQA1*02, 03, 05
48YQ	DR53	75ILR	DQA1*02, 04, 06
67FR	DR8, 9, 11, 16, 51a	75SL	DQA1*05
189S	DR8, DR52b, 53	80IRS	DQA1*02, 03, 04, 06
14ER	non-DR7	160AE	DQA1*0501/5
4R	non-DR7, 9, 53		
189R	non-DR8, 52b, 53		

[a] DR51a = DRB5*0101; DR51b = DRB5*0202; DR52a = DRB3*0101; DR52b = DRB3*0202; DR52c = DRB3*0301; non, all DRB antigens in the Luminex panel except those listed.

react with certain antigens in binding assays but not in complement-dependent lymphocytotoxicity (25).

This analysis indicates that only about 10% of DRB TerEps are equivalent to eplet pairs, which is much less than the 35% incidence of paired eplet equivalents of TerEps on HLA-A, -B and -C antigens (16). The number of polymorphic or locus-specific residue positions may explain this difference. Virtually, all class I eplet pairs involve the α1 and α2 domains, which have more than 40 such positions. In contrast, there are less than 20 polymorphic positions in β1 domain of DRB1 and none on DRA chains. Although both DQA and DQB chains are polymorphic, we identified only 1/18 DQ TerEps equivalent to an eplet pair. It is possible that the size of the Luminex panel is too small for informative HLA-DQ heterodimers to identify eplet pairs on or shared by DQA and DQB antigens.

DRB antigens appear to have two large regions with overlapping eplets, namely the highly variable 67–78 α-helical sequence of the β1 domain and a discontinuous sequence configuration in the β2 domain dominated by residues in polymorphic positions 96, 98, 120, 180 and 182. Together they comprise almost one half of the eplets that correspond to TerEps. As illustrated in Figure 2, the eplets with permissible residue combinations appear to have contiguous alignments of dominant residues with monomorphic residues, and such structures seem suitable as potential contact sites for CDR loops of antibody. We have found similar structural alignments for HLA class I eplets (16). This concept expands the eplet definition, especially in relation to the antibody analysis version of HLAMatchmaker.

This analysis is not without limitations. The eplet assignments were based on amino acid descriptions of TerEps and/or antigens that reacted with a given mAb or alloserum. Our comparisons of DRB sequences showed that several TerEps had somewhat different residue descriptions as reported by Terasaki's group. This created a minor pitfall because we could choose either the reactive antigens or the reported residue(s) as criteria in the determination of eplets that correspond to a given TerEp. The residue descriptions of TerEps do not consider HLA information about the immunizer and antibody producer, which would have permitted a distinction between nonself and self amino acid residues shared between the immunizing antigen and the antigens in the Luminex panel.

Nevertheless, this study demonstrates that how the HLAMatchmaker interpretation of amino acid residues shared between antibody-reactive antigens can increase our understanding of the structural basis of HLA epitopes. More studies are needed to fully evaluate the HLA epitope repertoire.

Acknowledgments

This study was possible because of the extensive laboratory testing and detailed information about HLA epitopes generated by Paul Terasaki and his group. We gratefully acknowledge their contributions. This study was supported by RO1 grant AI-55933 from the National Institutes of Health.

References

1. Takemoto S, Zeevi A, Feng S et al. National conference to assess antibody-mediated rejection in solid organ transplantation. *Am J Transplant* 2004: **4**: 1033–41.
2. Gebel H, Bray R, Nickerson P. Pre-transplant assessment of donor-reactive, HLA-specific antibodies in renal transplantation: contraindication vs. risk. *Am J Transplant* 2003: **3**: 1488–500.

3. Susal C, Opelz G. Kidney graft failure and presensitization against HLA class I and class II antigens. *Transplantation* 2002: **73**: 1269–73.
4. Pollinger HS, Stegall MD, Gloor JM et al. Kidney transplantation in patients with antibodies against donor HLA class II. *Am J Transplant* 2007: **7**: 857–63.
5. Terasaki PI. Humoral theory of transplantation. *Am J Transplant* 2003: **3**: 665–73.
6. Duquesnoy R, Awadalla Y, Lomago J et al. Retransplant candidates have donor-specific antibodies that react with structurally defined HLA-DR,DQ,DP epitopes. *Transpl Immunol* 2008: **18**: 352–60.
7. Deng CT, Cai J, Tarsitani C, El-Awar N, Lachmann N, Ozawa M. HLA class II DQ epitopes. *Clin Transpl* 2006: 115–22.
8. 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.
9. El-Awar N, Terasaki PI, Cai J et al. Epitopes of the HLA-A, B, C, DR, DQ and MICA antigens. *Clin Transpl* 2007: 175–94.
10. 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. *Am J Transplant* 2006: **6**: 2947–54.
11. Cai J, Kohanof S, Terasaki P. HLA-DR Antibody Epitopes. *Clin Transpl* 2006: 103–14.
12. Cai J, Terasaki P. Post-transplantation antibody monitoring and HLA antibody epitope identification. *Curr Opin Immunol* 2008: **20**: 602–6.
13. Duquesnoy R. Clinical usefulness of HLAMatchmaker in HLA epitope matching for organ transplantation. *Curr Opin Immunol* 2008: **20**: 594–601.
14. Duquesnoy RJ, Askar M. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination V. Eplet matching for HLA-DR, HLA-DQ and HLA-DP. *Hum Immunol* 2007: **68**: 12–25.
15. Duquesnoy RJ. A structurally based approach to determine HLA compatibility at the humoral immune level. *Hum Immunol* 2006: **67**: 847–62.
16. Duquesnoy R, Marrari M. Correlations between Terasaki's HLA class I epitopes and HLAMatchmaker-defined eplets on HLA-A, -B and -C antigens. *Tissue Antigens* 2009: Submitted.
17. Hogue C. Cn3D: a new generation of three-dimensional molecular structure viewer. *Trends Biochem Sci* 1997: **22**: 314–6.
18. Marsh SG, Bodmer JG. HLA-DR and -DQ epitopes and monoclonal antibody specificity. *Immunol Today* 1989: **10**: 305–12.
19. Klohe E, Fu XT, Ballas M, Karr RW. HLA-DR beta chain residues that are predicted to be located in the floor of the peptide-binding groove contribute to antibody-binding epitopes. *Hum Immunol* 1993: **37**: 51–8.
20. Drover S, Marshall WH, Kwok WW, Nepom GT, Karr RW. Amino acids in the peptide-binding groove influence an antibody-defined, disease-associated HLA-DR epitope. *Scand J Immunol* 1994: **39**: 539–50.
21. Fu X-T, Drover S, Marshall WH, Karr RW. HLA-DR residues accessible under the peptide-binding groove contribute to polymorphic antibody epitopes. *Hum Immunol* 1995: **43**: 243–50.
22. Smith KD, Mace BE, Valenzuela A et al. Probing HLA-B7 conformational shifts induced by peptide-binding groove mutations and bound peptide with anti-HLA monoclonal antibodies. *J Immunol* 1996: **157**: 2470–8.
23. Marrari M, Duquesnoy R. Why can sensitization by a HLA-DR2 mismatch lead to antibodies that react also with HLA-DR1? *Hum Immunol* 2009 (in press).
24. Duquesnoy RJ, Mulder A, Askar M, Fernandez-Vina M, Claas FHJ. HLAMatchmaker-based analysis of human monoclonal antibody reactivity demonstrates the importance of an additional contact site for specific recognition of triplet-defined epitopes. *Hum Immunol* 2005: **66**: 749–61.
25. Duquesnoy RJ. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. *ASHI Quarterly* 2002: 60–2.

