INTERNATIONAL JOURNAL OF
IMMUNOGENETICS

Review

doi: 10.1111/j.1744-313X.2011.01042.x

The antibody response to an HLA mismatch: a model for nonself–self discrimination in relation to HLA epitope immunogenicity

R. J. Duquesnoy

Summary

Antibodies to HLA mismatches are specific for epitopes rather than antigens. HLAMatchmaker considers each HLA antigen as a string of eplets that represent key elements of epitopes. Certain antibodies are specific for single eplets, but recent studies have demonstrated that epitopes defined by eplet pairs always involve one nonself-eplet and a self-eplet shared between the immunizing antigen and the antibody producer. This suggests an autoreactive component of the alloantibody response to an HLA mismatch and this report expands this concept. During B-cell development, V_H and V_L gene rearrangements produce a diversity of Ig receptors that can recognize epitopes on autologous proteins. It is hypothesized that B cells carry low-affinity receptors for self-HLA antigens. Their interactions with self-HLA proteins will not lead to B-cell activation or antibody production. In contrast, exposure to HLA mismatches induces often strong alloantibody responses. The activation of self-HLA-specific B cell by a nonself-eplet may require that the remainder of the structural epitope of the immunizing antigen has considerable structural similarity with one of the antibody producer's alleles. This hypothesis has been tested in molecular modelling studies with six epitopes defined by human monoclonal antibodies. In each case, one allele of the antibody producer had no or few differences with the immunizing allele in antibody-accessible positions defined by a 15 Ångstrom radius of the mismatched eplet. The other alleles of the antibody producer showed significantly greater numbers of residue differences with the immunizer (5.8 ± 2.0 versus 1.0 ± 0.6, $P < 0.0001$). These data support the concept that HLA antibodies originate from B cells with self-HLA immunoglobulin receptors that recognize mismatched eplets as nonself entities on immunizing antigens. The nonself–self paradigm provides a new insight of HLA epitope immunogenicity and may explain why sensitized patients have antibodies to a restricted number of mismatched epitopes.

Department of Pathology and The Thomas E. Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Received 5 July 2011; revised NA; accepted 5 September 2011

Correspondence: Rene J. Duquesnoy, University of Pittsburgh Medical Center,Room 5712 - PUH/South Tower, 200 Lothrop Street, Pittsburgh, PA 15213, USA. Tel: 1-412-647-6148; Fax: 1-412-647-1755; E-mail: Duquesnoyr@upmc.edu

Introduction

Antibodies to HLA class I mismatches play an important role in transplant rejection and subsequent failure, and there is now considerable documentation that HLA antibodies are specific for epitopes rather than antigens (Barbetti *et al.*, 1989; Harpprecht *et al.*, 1989; Fuller *et al.*, 1990a,b; Park *et al.*, 1990; Hildebrand *et al.*, 1992a,b; McCutcheon & Lutz, 1992; Lutz *et al.*, 1994; Rodey *et al.*, 1994; El-Awar *et al.*, 2007; Duquesnoy, 2008; Duquesnoy & Marrari, 2009a,b). HLAMatchmaker is a theoretical algorithm developed from HLA molecular modelling and amino acid sequence differences between HLA alleles that permits structural descriptions of HLA epitopes (Duquesnoy, 2006, 2008; Duquesnoy & Marrari, 2009a,b). Each HLA antigen is viewed as a string of amino acid patches in antibody-accessible sequence positions; these so-called eplets represent key elements of epitopes that elicit specific antibodies. From reactivity patterns with HLA alleles, it can readily be established that such antibodies are often specific for a single eplet, but many others recognize epitopes represented by pairs of eplets in different sequence locations (Duquesnoy *et al.*, 2005; Duquesnoy & Marrari, 2009a,b; Marrari *et al.*, 2010). Lutz and co-workers have also observed that certain epitopes are defined by distant residues on reactive antigens (McCutcheon & Lutz, 1992; McCutcheon *et al.*, 1993).

Human monoclonal antibodies (mAb) are especially useful for epitope specificity studies when HLA typing information of the immunizer and antibody producer is available, because this permits a structural description of the immunizing event. Our analysis of mAb reactivity patterns with HLA allele panels has demonstrated that epitopes defined by eplet pairs always involve one nonself-eplet and a self-eplet shared between the immunizing antigen and the antibody producer (Duquesnoy *et al.*, 2005; Marrari *et al.*, 2010). In these pairs, the self-eplet is always expressed on the molecular surface and about 7–16 Ångstroms away from the nonself-eplet. These findings can be inter-

preted in context with current concepts about the structure of the antigen–antibody interface.

The variable domains of immunoglobulin heavy (V_H) and light (V_L) chains determine antibody specificity, and each chain has three complementarity determining regions (CDRs) with hypervariable loops that interact with different parts of a protein epitope (Poljak *et al.*, 1973; Chothia & Lesk, 1987). The third CDR of the heavy chain (CDR-H3) lies generally in the centre of the antigen-binding site and plays a major role in epitope recognition. CDR-H3 shows significant variability in its length (Kabat *et al.*, 1977; Kabat, 1978; Wu *et al.*, 1993; MacCallum *et al.*, 1996; Shirai *et al.*, 1999). CDR-L1, CDR-L2, CDR-L3, CDR-H1 and CDR-H2 have less variability, and they form limited numbers of so-called canonical structures identified from amino acid sequences and stabilize the binding with CDR-H3 (Chothia & Lesk, 1987; Kuroda *et al.*, 2009). Stereochemical analyses of crystallized antigen–antibody complexes have defined a structural epitope as that part of the antigen that makes contact with the six CDRs of antibody (Davies *et al.*, 1990; Padlan, 1994; MacCallum *et al.*, 1996). A structural epitope has about 15–25 surface residues that constitute the interface with the CDRs of antibody. Within it lies the functional epitope consisting of energetic residues in so-called hot spot locations (Getzoff *et al.*, 1988; Novotny, 1991; Laune *et al.*, 1997; Bogan & Thorn, 1998; Van Regenmortel, 2002). An amino acid residue in an epitope is called energetic if a substitution with another residue markedly decreases antibody binding with that epitope (Novotny, 1991). Functional epitopes can be defined by one patch or a pair of patches of energetic residues separated far enough to be contacted by different CDRs of antibody. To be immunogenic, a functional epitope must have at least one nonself residue, i.e. the antibody producer's homologous proteins must have a different residue in the corresponding sequence position. Such residues must be on the molecular surface, so that they can make contact with the specificity-determining CDRs of antibody. The surface of a structural epitope is about 700–900 Å^2 (Davies *et al.*, 1990; Padlan, 1994; MacCallum *et al.*, 1996). In comparison, HLA molecules seen from the top (i.e. including both helices and bound peptide) have surface areas of about 750 Å^2. This means that HLA antibodies make contact with rather substantial parts of the HLA molecular surface.

There is no information about the structure of an HLA antigen complexed with alloantibody, but Ziegler's group has described a crystallized antigen–antibody complex involving class I HLA (Hulsmeyer *et al.*, 2005). They used a Fab fragment of a human monoclonal antibody (Hyb3) specific for a melanoma-associated peptide (MAGE-A1) bound to HLA-A1. Its binding interface (paratope) comprises of contact residues in eleven VH positions (30, 31, 52, 53, 55, 56, 102, 103, 105, 106 and 107) and four VL positions (68, 93, 94 and 95). The structural epitope involves four contact residues of the antigenic peptide (positions 4, 6, 7 and 8), seven contact residues in the α1-helix (positions 65, 69, 72, 73, 76, 80 and 89) and three contact residues in the α2-helix (positions 145, 146 and 155) of HLA-A1. Cn3D modelling of the crystalline structure (PDF 1W72) shows that these contact residues are within a molecular surface of about 900 Å^2 and their locations are depicted in Fig. 1. CDR-H3 contributes the most to antibody binding, and this involves contact with peptide, an arginine residue (R) in position 65 and a glutamine (Q) in position 72 on the α1-helix of HLA-A1 (Hulsmeyer *et al.*, 2005). The latter seems to constitute a functional epitope within the structural epitope. The other CDRs stabilize the antigen–antibody complex.

How can we estimate possible dimensions of structural HLA-specific alloepitopes that would be contacted by the CDRs of antibody? Current definitions of antibody-defined epitopes appear to reflect functional epitopes represented by eplets and eplet pairs (Duquesnoy & Marrari, 2009a,b; Marrari & Duquesnoy, 2009). The eplets in such pairs are about 7–16 Å

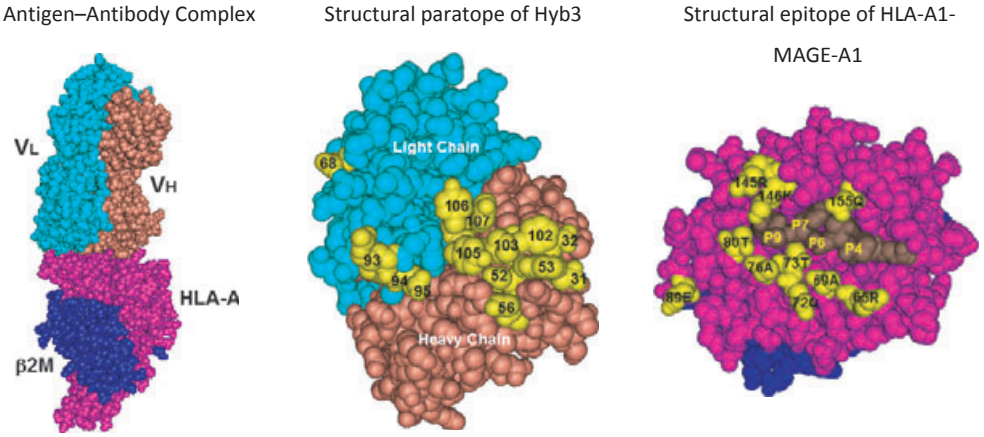

Figure 1. Molecular modeling of the crystalline structure of HLA-A1-MAGE-A1 complexed with the Fab fragment of the Hyb3 monoclonal antibody as reported by Ziegler's group (Hulsmeyer *et al.*, 2005). The contact residues of the paratope and epitope are numbered and highlighted in yellow.

away from each other (Duquesnoy *et al.*, 2005; Marrari *et al.*, 2010). This distance is consistent with the concept that paired eplets make contact with different CDRs of antibody. The mismatched eplet interacts with the CDR (presumably CDR-H3) that plays a primary role in the specificity of antibody, whereas the self-eplet (or self-configuration) serves as a critical contact site with another CDR of antibody. The latter suggests an autoreactive component of the alloantibody response to an HLA mismatch, because the immunizing antigen as well as all mAb-reactive alleles must share this self-configuration with the HLA type of the antibody producer.

Hypothesis

Why and to what extent does an alloantibody response require an autoreactive component of an HLA mismatch? During B-cell development, rearrangements of V_H and V_L genes produce diversity in the antigen-binding sites of immunoglobulins (Kim *et al.*, 1981; Tonegawa, 1983). These processes lead to the expression of immunoglobulin receptors on developing B cells, which go through several stages to become mature B cells. These receptors can recognize epitopes on autologous proteins (Wardemann *et al.*, 2003). Following positive and negative selection (Nossal & Pike, 1980; Hayakawa *et al.*, 1999; Morris *et al.*, 2000; Heltemes & Manser, 2002; Pillai *et al.*, 2004) and receptor editing (Gay *et al.*, 1993; Tiegs *et al.*, 1993; Pelanda & Torre, 2006), the remaining B cells carry only low-avidity receptors, and their interactions with autologous proteins will not lead to B-cell activation or antibody production. Accordingly, each person can be expected to have B cells with low-avidity immunoglobulin receptors for their own HLA molecules. In other words, the immune repertoire has B cells specific for self-HLA-A, self-HLA-B and self-HLA-C antigens. These receptors can interact, although their CDRs are with different portions of the HLA molecular surface, but the binding strength is so weak that B-cell activation and antibody production cannot occur.

In contrast, exposure to HLA mismatches induces often strong alloantibody responses. It seems apparent that this must involve the activation of self-HLA-specific B cells by distinct nonself-amino acid configurations on immunizing antigens; such alloepitopes would be equivalent to eplets. It is well known that among the six CDRs immunoglobulins, the centrally located CDR-H3 plays a pivotal role in determining antibody specificity. CDR-H3 has a considerably longer amino acid sequence than the other CDRs and displays significant sequence variability and structure (Wu *et al.*, 1993; Shirai *et al.*, 1999). It seems likely that CDR-H3 can form multiple loops one of which has a very low binding ability with a self-epitope, whereas other loops with different configurations might permit strong interactions with an alloepitope. In other words, CDR-H3 could refold itself to specifically recognize a mismatched eplet and, in combination with the binding of the other CDRs to self-configurations on the HLA molecule, this would be sufficient to trigger a B-cell response. This concept raises the requirement that the immunizing HLA antigen must have one distinct nonself-amino acid configuration (the mismatched eplet), whereas the other amino residues contacted by antibody should be the same or very similar as those on a self-HLA antigen of the antibody producer. The experimental evidence described later supports this contention.

Methods

The hypothesized nonself–self model of HLA class I epitope immunogenicity has been tested in six cases, whereby a human monoclonal antibody is specific for an epitope defined by a pair of a nonself and a self-eplet (Table 1). A recent publication describes the reactivity of these epitopes (Marrari *et al.*, 2010). Two mismatched eplets 65RNA and 65QIA are located in the α1-domain, and four mismatched eplets 142MI, 144TKR, 163EW and 163LW are on the α2-domain. Three immunizing epitopes reside on HLA-A, whereas the other three are on HLA-B. Although these epitope-specific antibodies originated during pregnancy, it has become apparent that similar antibodies can result from sensitization induced by a transplant or a blood transfusion.

This analysis consisted of comparisons between antibody-accessible residues on the immunizing antigen and all the antigens of the antibody producer. Considering the 700–900 $Å^2$ surface of a structural epitope, one can calculate the locations of surface residues within a 15 Å radius of a mismatched eplet including those that can make contact with the CDRs of a given antibody. These residues can be identified with the 'select by distance' command of the Cn3D structure software program (Hogue, 1997) using informative HLA models downloaded from Entrez Molecular Modeling Database on the National Center for Biotechnology Information website: http://www.ncbi.nlm.nih.gov/Structure. Excluding the residues comprising a mismatched eplet, this analysis was designed to determine surface residue differences between the immunizing allele and the alleles of the antibody producer. According to the aforementioned hypothesis, one of the antibody producer's alleles should have no or very few residue differences with the immunizing allele. The space fill command of Cn3D was used to illustrate the locations of eplets and self-residues on informative molecular structures.

Results

Table 2 shows details of surface amino acid differences between immunizing allele and the alleles of the antibody producer within a 15 Å radius of a mis-

Table 1. Antibody-defined HLA class I epitopes analyzed in this study as described in Marrari *et al.*, 2010.

Case	Monoclonal	Isotype	HLA type – antibody producer	Immunizer	Eplet specificity	Distance	Reactive antigens	Self eplet on
1	HDG4B1	IgG	A2, 24; B7, 60; Cw7, w10	A32	65RNA+s82LR	11 Å	A25/A32/B17/B63	A24
2	WK1D12	IgG	A1,–; B8,–; Cw7,–	B27	163EW+s73TE	16 Å	B7/B13/B27/B40/B47/B48/B81	B8
3	HDG11G12	IgG	A2, 24; B7, 60; Cw7, w10	B35	163LW+s62RQI	8 Å	B15/B35/B49/B50/B51/B52/B53/B56/B78	B7, B60
4	MUL4C8	IgG	A2, 25; B18, 51; Cw12, 15	A11	144TKR+s151H	7 Å	A1/A3/A11/A24/A36	A2, A25
5	MUL9F4	IgM	A2, 25; B18, 51; Cw12, 15	B55	65QIA+s76ES	7 Å	B7/B*2708/B42/B54/B55/B56/B67/B81/B82	A25, B18
6	OK4F10	IgM	A2, 28; B7, 27; Cw2, w7	A3	142MI+s79GT	13 A	A1/A3/A11/A26/A29/A30/A31/A33/A34/A36/A43/A66/A74/A80	A2, A28

matched eplet. These residue differences pertain to antibody-accessible polymorphic positions on putative structural epitopes. In five cases, one of the antibody producer's alleles has no or few differences except of course for the mismatched eplet specifically recognized. The numbers of residue differences are as follows: two between A*24:02 with the 65RNA+s82LR carrying A*32:01 (case 1), 2 between B*08:01 and 163EW+s73TE carrying B*27:05 (case 2), 0 between B*40:01 and the 163LW+s65RQI carrying B*35:01 (case 3), 1 between A*02:01 and the 144TKR+s151H carrying A*11:01 (case 4), and 0 between B*18:01 and the 65QIA+s76ES carrying B*55:01 (case 5). Case 6 had two self-alleles, namely A*02:01 and A*68:01, that differed by one residue with the 142MI+s79GT carrying A*03:01. In contrast, the other alleles of the antibody producer showed significantly greater numbers of residue differences with the immunizing alleles (5.8 ± 2.0 versus 1.0 ± 0.6, $P < 0.0001$).

These data must be viewed in context with the fact that each of these selected epitopes consists of a mismatched eplet paired with a self-configuration that serves as a second critical contact site with antibody. The latter could have biased the identification of the antibody producer's allele with the fewest number of residue differences. In four cases, two of the antibody producer's alleles have the same self-configuration as a critical contact site. Regarding the 163LW+s65RQI epitope, B*07:02 and B*40:01 have both s65RQI, but B*40:01 has no residue differences with the immunizing B*35:01, whereas B*07:02 has 3 such differences (case 3). The self-configuration of 144TKR+s151H is present in the antibody producer's A*02:01 and A*25:01, but these alleles have considerably different numbers of residue difference with the immunizer: 1 versus 6 (case 4). For the 65QIA+s76ES epitope, B*18:01 has 0 residue difference versus 6 differences for A*25:01 (case 5). Altogether, this analysis shows that one allele of the antibody producer has no or very few residue differences with the immunizing antigen, although in case 6, it was not possible to distinguish between two alleles namely A*02:01 and A*68:01; each one has one difference. These findings are consistent with the hypothesis that each of these monoclonal antibodies was derived from a B cell with a self-HLA antigen-specific immunoglobulin receptor that interacted with the immunizing antigen.

Figure 2 illustrates the locations of eplets and surrounding residues on molecular models of informative HLA antigens. The yellow area represents the molecular surface within 15 Å of the eplet, the locations and letter codes of its residues are depicted in white font (e.g. 65G, 66K and 69A, case 1). Single numbers refer to sequence locations of monomorphic residues (e.g. 57 and 170, case 1), and underlined numbers with letters describe polymorphic residues shared between the immunizing allele and the self-allele with the fewest residue differences, but not with the other alleles of

Table 2. Polymorphic surface residue differences between alleles of immunizers and producers of eplet -specific antibodies

	Eplet			Number of differences	Polymorphic surface residues											
Case 1																
65RNA+s82LR	65	66	69		43	62	73	76	79	80	82	83	152	163	166	
A*32:01 Immunizer	R	N	A		Q	Q	T	E	R	I	L	R	V	T	E	
Antibody producer																
A*02:01	R	K	A	6	–	G	–	V	G	T	R	G	–	–	–	
A*24:02	**G**	**K**	**A**	**2**	**–**	**E**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**D**	
B*07:02	Q	I	A	7	P	R	–	–	–	N	R	G	E	E	–	
B*40:01	Q	I	T	6	P	R	–	–	–	N	R	G	–	E	–	
C*07:02	Q	K	R	7	P	R	A	V	–	N	R	G	–	–	–	
C*03:04	Q	K	R	8	P	R	T	V	–	N	R	G	E	L	–	
Case 2																
163EW+s73TE	163	167			62	65	66	69	71	73	76	109	131	151	158	166
B*27:05 Immunizer	E	W			R	Q	I	A	T	T	E	L	S	R	A	E
Antibody producer																
A*01:01	R	G		10	Q	R	N	–	S	–	A	F	R	H	V	D
B*08:01	**T**	**W**		**2**	**–**	**–**	**–**	**T**	**–**	**–**	**–**	**–**	**R**	**–**	**–**	**–**
C*07:01	T	W		6	–	–	N	R	A	A	V	–	R	–	–	–
Case 3																
163LW+s65RQI	163	167			62	65	66	69	71	73	76	107	109	151	152	166
B*35:01 Immunizer	L	W			R	Q	I	T	T	T	E	G	L	R	V	E
Antibody producer																
A*02:01	T	W		9	G	R	K	A	S	–	V	W	F	H	–	–
A*24:02	T	G		8	E	G	K	A	S	–	–	–	F	H	–	D
B*07:02	E	W		3	–	–	–	A	A	–	–	–	–	–	E	–
B*40:01	**E**	**W**		**0**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**
C*07:02	T	W		6	–	–	K	R	A	A	V	–	–	–	A	–
Case 4																
144KR+s151H	142	144	145		73	76	79	80	82	83	127	131	138	149	151	
A*11:01 Immunizer	I	K	R		T	V	G	T	R	G	N	R	M	A	H	
Antibody producer																
A*02:01	**T**	**K**	**H**	**1**	**–**	**–**	**–**	**–**	**–**	**–**	**K**	**–**	**–**	**–**	**–**	
A*25:01	I	Q	R	6	–	E	R	I	L	R	–	–	–	T	–	
B*18:01	I	Q	R	6	–	E	R	N	–	–	–	S	T	–	R	
B*51:01	I	Q	R	8	–	E	R	I	L	R	–	S	T	–	R	
C*12:03	I	Q	R	5	A	–	R	N	–	–	–	–	T	–	R	
C*15:02	I	Q	R	4	–	–	R	K	–	–	–	–	T	–	R	
Case 5																
65QIA+s76ES	65	66	69		43	62	73	76	79	80	82	83	151	163		
B*55:01 Immunizer	Q	I	A		P	R	T	E	R	N	R	G	R	T		
Antibody producer																
A*02:01	R	K	A	6	Q	G	–	V	G	T	–	–	H	–		
A*25:01	R	N	A	6	Q	–	–	–	–	I	L	R	H	R		
B*18:01	**Q**	**I**	**T**	**0**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**		
B*51:01	Q	I	T	4	–	–	–	–	–	I	L	R	–	L		
C*12:03	Q	K	R	2	–	–	A	V	–	–	–	–	–	–		
C*15:02	Q	N	R	2	–	–	–	V	–	K	–	–	–	–		
Case 6																
142MI+s79GT	138	142	145		73	76	79	80	82	83	127	131	144	151		
A*03:01 Immunizer	M	I	R		T	V	G	T	R	G	N	R	K	H		
Antibody producer																
A*02:01	**M**	**T**	**H**	**1**	**–**	**–**	**–**	**–**	**–**	**–**	**K**	**–**	**–**	**–**		
A*68:01	**M**	**T**	**H**	**1**	**–**	**–**	**–**	**–**	**–**	**–**	**K**	**–**	**–**	**–**		
B*07:02	T	I	R	5	–	E	R	N	–	–	–	–	Q	R		
B*27:05	T	I	R	7	–	E	R	–	L	R	–	S	Q	R		
C*02:02	T	I	R	4	–	–	R	K	–	–	–	–	Q	R		
C*07:02	T	I	R	5	A	–	R	N	–	–	–	–	Q	R		

the antibody producer. Positions with residue differences between self-allele and the immunizing allele have an underlined number and two letters separated by a slash.

It should be noted that all residues in close vicinity to the eplet are identical between the immunizing allele and the antibody producer's allele with the fewest residue differences. Case 1 is the exception: the

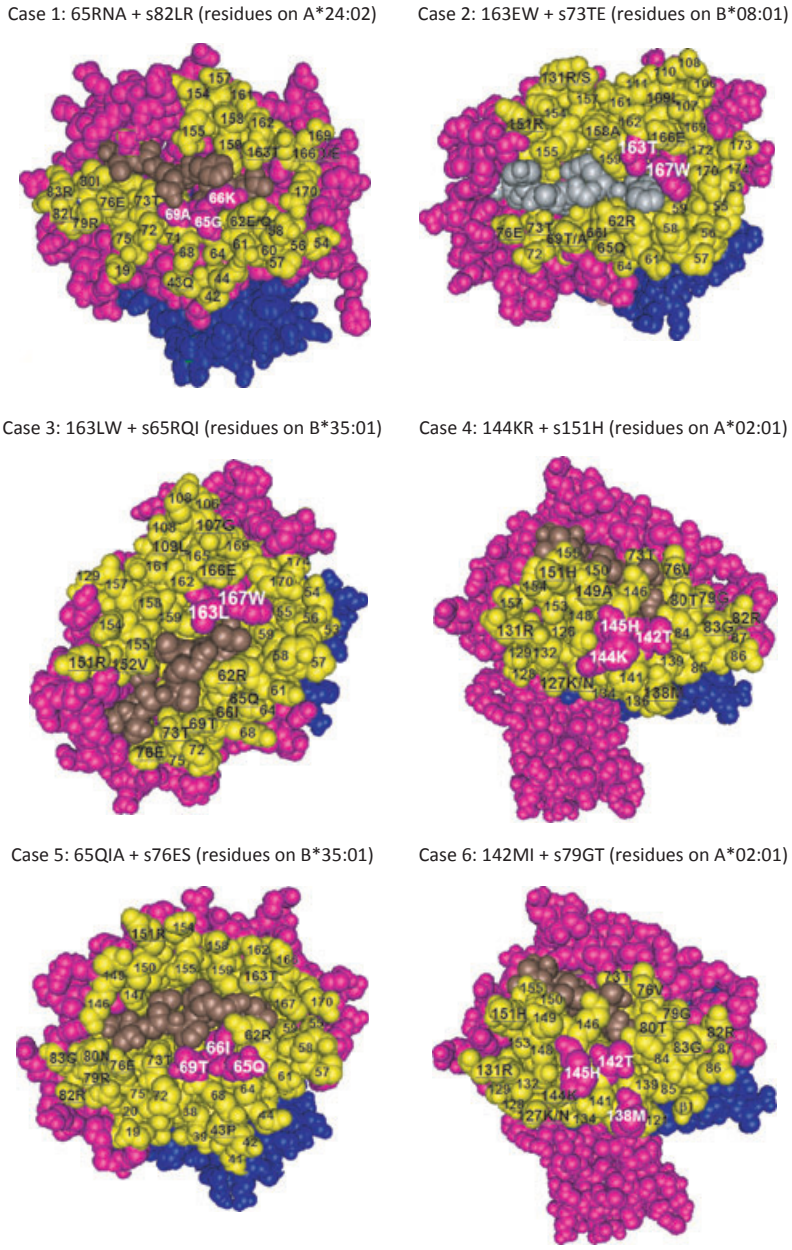

Figure 2. Molecular models of crystalline HLA structures showing surface-exposed residues surrounding eplets (in white fonts) within a radius of about 15 Å (in yellow). Single numbers refer to sequence locations of monomorphic residues and underlined numbers with letters describe polymorphic residues shared between the immunizing allele and the self allele with the fewest residue differences. Positions with residue differences between self and the immunizing allele have an underlined number and two letters separated by a slash. Cases 1, 2, 4 and 6 are self alleles, case 3 is the immunizing allele and case 5 is an antibody-reactive allele because no crystalline models for the self B*18:01 and the immunizing B*55:01 are available).

immunizer's 62Q is nonself for all alleles of the antibody producer, and this residue might have been part of the immunizing epitope. Table 2 lists a few residue differences between the immunizing allele and the antibody producer's allele that presumably has the self-HLA-reactive immunoglobulin receptor. Interestingly, most of them are in positions at the periphery of the 15 Å radius (case 1: 166D/G, case 2: 131R/S, case 4: 127K/N, case 6:127K/N), and they may be outside the actual structural epitope.

The molecular models show for these six cases an average of 36 residues (range 30–41) in 15-Å^2 areas, but the actual numbers of residues making contact with antibody range from 15 to 25 (Getzoff *et al.*, 1988; Laune *et al.*, 1997; Bogan & Thorn, 1998; Van Regenmortel, 2002). Therefore, one would expect that

several residues cannot be part of the structural epitopes recognized by HLA antibodies. Nevertheless, analyses of antibody reactivity patterns with single allele panels have shown that certain self-residues play a critical role in the binding with antibody, and they can be considered important elements of structural HLA epitopes. More detailed information about structural epitopes seems possible if the testing includes alleles with informative point mutations (McCutcheon & Lutz, 1992; McCutcheon *et al.*, 1993).

Figure 2 shows also other parts of the molecular HLA structure including β2-microglobulin (in blue) and peptide (in brown). It should be noted that in each case, some peptide residues reside within the 15-Å radius. Although antibodies specific for peptide-dependent HLA epitopes have been reported (Mulder *et al.*, 2005), it seems that none of the antibodies in this analysis are influenced by peptide.

A mismatched eplet can be considered the driving force of the alloantibody response that seems to begin with the activation of a B cell with a self-HLA class I immunoglobulin receptor. Interestingly, there are also structural similarities between the mismatched eplet of the immunizing allele and the self-eplet on the antibody producer's allele with the fewest residue differences. The eplets are defined by two or three polymorphic residues, and in each case, they share at least one of them (Table 3). The hypothesis described earlier states that CDR-H3 can recognize a mismatched eplet by refolding itself to specifically recognize a different amino acid configuration. It seems that some of the interactions with self-residues in maintained. This could mean that the specific reaction of CDR-H3 with a mismatched eplet would include an interaction with a self-residue.

Discussion

The nonself–self paradigm of HLA epitope immunogenicity represents a significant step in the development of the HLAMatchmaker algorithm, since the introduction of the eplet concept in 2006. It is based on the postulate that HLA antibodies originate from B cells with self-HLA immunoglobulin receptors that recognize a mismatched eplet within the structural epitope of the immunizing antigen. The antibody response requires that the remainder of that structural epitope must consist of residues shared with one of the alleles of the antibody producer. Indeed, for all six cases of monoclonal antibody-defined epitopes it was possible to identify an HLA allele of the antibody producer with an identical or very similar amino acid composition as the immunizing allele. Unpublished data with other class I HLA epitope-specific antibodies provide further support of this concept.

Table 3. Amino acid similarities between eplets on alleles of the immunizer and antibody producer. Shared residues are in bold font

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Immunizing allele	65R	163E	163L	142I	**65Q**	**138M**
	66N	**167W**	**167W**	**144K**	**66I**	142I
	69A			145R	69A	145R
Allele of antibody producer	65G	163T	163E	142T	**65Q**	**138M**
	66K	**167W**	**167W**	**144K**	**66I**	142T
	69A			145H	69T	145H

Among the CDRs of antibody, the centrally located CDR-H3 has the greatest sequence variability and plays a dominant role in determining antibody specificity (Wu *et al.*, 1993; Wedemayer *et al.*, 1997; Shirai *et al.*, 1999; Babor & Kortemme, 2009; Schroeder *et al.*, 2010). The rather long length of CDR-H3 permits it to make different loops, some of which will weakly bind to a self-configuration at a given sequence location, whereas other 'specificity-determining' loops can bind strongly to different amino acid compositions in the same sequence position. It seems likely that CDR-H3 loops of HLA alloantibodies may react with mismatched eplets.

The humoral alloresponse to an HLA mismatch is not well understood. It is well known that sensitized patients develop specific antibodies to a restricted number of mismatched epitopes (Duquesnoy *et al.*, 1990; Rodey *et al.*, 1994). The nonself–self paradigm of HLA epitope immunogenicity may explain this phenomenon. It is possible that certain mismatched eplet-carrying antigens have significant structural epitope differences with all patient alleles, and this would prevent B-cell activation through its self-HLA immunoglobulin receptor. Accordingly, one might predict that the 65RNA+s82LR epitope on A*32:01 induces antibodies in patients who type for A*24:02, which has only 2 residue differences with A*32:01, but less likely in patients who are homozygous for HLA-A*02:01, which has six residue differences (Table 2, Case 1). A similar lack of a specific antibody response to 144TKR+s151H on A*03:01 might occur in patients who are homozygous for A*25:01, which has six residue differences (Table 2, Case 4). Another explanation is that the patient's self-HLA-reactive B-cell repertoire is restricted to some molecular configurations, because other potentially reactive B cells have been deleted through negative selection, anergy and receptor editing. The nonself–self concept of HLA immunogenicity may become clinically useful regarding predicting antibody responses to HLA mismatches, but this needs, of course, experimental verification.

These findings support the hypothesis that HLA antibody-producing cells originally had B-cell receptors specific for self-HLA constituents. This concept is consistent with previously reported findings that antibody specificity is often directed against HLA epitopes defined by a pair of nonself and self-eplets (Duquesnoy *et al.*, 2005; Marrari *et al.*, 2010). Antibody reactivity to eplet pairs raises questions about determining mismatch acceptability for sensitized patients. If the patient has antibodies against a mismatched eplet

paired with self-configuration, can we consider an eplet-carrying allele as an acceptable mismatch if it lacks this self-configuration?

The nonself–self paradigm of HLA immunogenicity provides a model to address the question why and when an immune response occurs. Since the 1950s, there has been a considerable debate of Burnet's original theory that the immune response is based on the discrimination between self and nonself (Burnet & Fenner, 1949; Burnet, 1959, 1969). Every element that distinctively belongs to the organism does not induce an immune response, whereas every foreign element triggers an immune response. Nevertheless, this criterion has been criticized both conceptually and experimentally (Jerne, 1955, 1974; Lederberg, 1959; Cohen, 1992; Janeway, 1992; Matzinger, 1994; Tauber, 1994; Silverstein & Rose, 1997; Cohn *et al.*, 2007). More recently, Pradeu and Carosella have proposed the 'criterion of continuity' to explain self and nonself discrimination (Pradeu & Carosella, 2006). Accordingly, an antibody response is triggered not by every foreign entity, but by every strong discontinuity of the antigenic patterns (whether endogenous or exogenous) reacting with immune receptors. In other words, the immune system does not respond to self molecules, but rather to certain modifications within self molecules. Mismatched eplets may represent such modifications, whereas the remainder of a structural epitope consists of self-residues shared with an allele of the antibody producer. Given the extensive polymorphism of HLA and the considerable diversity of HLA antibodies, it seems that the humoral response to an HLA mismatch offers an attractive approach to study the self–nonself discrimination hypothesis.

References

Babor, M. & Kortemme, T. (2009) Multi-constraint computational design suggests that native sequences of germline antibody H3 loops are nearly optimal for conformational flexibility. *Proteins*, **75**, 846.

Barbetti, A.A., Park, M.S., Terasaki, P.I., Clark, B. & Fan, J.L. (1989) HLA serologic epitopes. *Clinical Transplants*, 477.

Bogan, A.A. & Thorn, K.S. (1998) Anatomy of hot spots in protein interfaces. *Journal of Molecular Biology*, **280**, 1.

Burnet, F.M. (1959) *The Clonal Selection Theory of Acquired Immunity*. Cambridge Univ. Press, Cambridge,UK.

Burnet, F. (1969) *Self and Not-Self*. Melbourne Univ Press and Cambridge Univ Press, Cambridge, UK.

Burnet, F. & Fenner, F. (1949) *The Production of Antibodies*. Macmillan, Melbourne.

Chothia, C. & Lesk, A. (1987) Canonical structures for the hypervariable regions of immunoglobulins. *Journal of Molecular Biology*, **196**, 901.

Cohen, I. (1992) The cognitive priciple challenges clonal selection. *Immunology Today*, **13**, 441.

Cohn, M., Mitchison, N.A., Paul, W., Silverstein, A.M., Talmage, D. & Weigert, M. (2007) Reflections on the clonal-selection theory. *Nature Reviews Immunology*, **7**, 823.

Davies, D., Padlan, E. & Sheriff, S. (1990) Antibody-antigen complexes. *Annual Review of Biochemistry*, **59**, 439.

Duquesnoy, R.J. (2006) A structurally based approach to determine HLA compatibility at the humoral immune level. *Human Immunology*, **67**, 847.

Duquesnoy, R. (2008) Clinical usefulness of HLAMatchmaker in HLA epitope matching for organ transplantation. *Current Opinion in Immunology*, **20**, 594.

Duquesnoy, R. & Marrari, M. (2009a) Correlations between Terasaki's HLA class I epitopes and HLAMatchmaker-defined eplets on HLA-A, -B and -C antigens. *Tissue Antigens*, **74**, 117.

Duquesnoy, R.J. & Marrari, M. (2009b) HLAMatchmaker-based definition of structural HLA epitopes detected by alloantibodies. *Current Opinion in Organ Transplantation*, **14**, 403.

Duquesnoy, R.J., White, L.T., Fierst, J.W., Vanek, M., Banner, B.F., Iwaki, Y. *et al.* (1990) Multiscreen serum analysis of highly sensitized renal dialysis patients for antibodies toward public and private class I HLA determinants. Implications for computer-predicted acceptable and unacceptable donor mismatches in kidney transplantation. *Transplantation*, **50**, 427.

Duquesnoy, R.J., Mulder, A., Askar, M., Fernandez-Vina, M. & Claas, F.H.J. (2005) 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.

El-Awar, N.R., Akaza, T., Terasaki, P.I. & Nguyen, A. (2007) Human leukocyte antigen class I epitopes: update to 103 total epitopes, including the C locus. *Transplantation*, **84**, 532.

Fuller, A.A., Rodey, G.E., Parham, P. & Fuller, T.C. (1990a) Epitope map of the HLA-B7 CREG using affinity-purified human alloantibody probes. *Human Immunology*, **28**, 306.

Fuller, A.A., Trevithick, J.E., Rodey, G.E., Parham, P. & Fuller, T.C. (1990b) Topographic map of the HLA-A2 CREG epitopes using human alloantibody probes. *Human Immunology*, **28**, 284.

Gay, D., Saunders, T., Camper, S. & Weigert, M. (1993) Receptor editing: an approach by autoreactive B cells to escape tolerance. *Journal of Experimental Medicine*, **177**, 999.

Getzoff, E.D., Tainer, J.A., Lerner, R.A. & Geysen, H.M. (1988) The chemistry and mechanisnm of antibody binding to protein antigens. *Advances in Immunology*, **43**, 1.

Harpprecht, J., Olde, K., Westphal, E. & Muller-Ruchholtz, W. (1989) Serological mapping of HLA-epitopes with monoclonal antibodies and its interpretation by sequenced HLA-molecules. *Tissue Antigens*, **34**, 170.

Hayakawa, K., Asano, M., Shinton, S., Gui, M., Allman, D., Stewart, C. *et al.* (1999) Positive selection of natural autoreactive B cells. *Science*, **285**, 113.

Heltemes, L. & Manser, T. (2002) Level of B cell antigen receptor surface expression influences both positive and negative selection of B cells during primary development. *Journal of Immunology*, **169**, 1283.

Hildebrand, W.H., Madrigal, J.A., Belich, M.P., Zemmour, J., Ward, F.E., Williams, R.C. *et al.* (1992a) Serologic cross-reactivities poorly reflect allelic relationships in the HLA-B12 and HLA-B21 groups. Dominant epitopes of the alpha 2 helix. *Journal of Immunology*, **149**, 3563.

Hildebrand, W.H., Madrigal, J.A., Little, A.M. & Parham, P. (1992b) HLA-Bw22: a family of molecules with identity to HLA-B7 in the alpha 1-helix. *Journal of Immunology*, **148**, 1155.

Hogue, C. (1997) Cn3D: a new generation of three-dimensional molecular structure viewer. *Trends in Biochemical Sciences*, **22**, 314.

Hulsmeyer, M., Chames, P., Hillig, R., Stanfield, R., Held, G., Coulie, P. *et al.* (2005) A major histocompatibility complex-peptide-restricted antibody and T cell receptor molecules

recognize their Target by distinct binding modes. Crystal structure of human leukocyte antigen (HLA)-A1 -MAGE-A1 complex with Fab-Hyb3. *Journal of Biological Chemistry*, **280**, 2972.

Janeway, C. (1992) The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunology Today*, **13**, 11.

Jerne, N.K. (1955) The natural selection theory of antibody formation. *Proceedings of the National Academy of Sciences of the United States of America*, **41**, 849.

Jerne, N. (1974) Towards a network theory of the immune system. *Annals de l''Institute Pasteur Immunol (Paris)*, **125**, 373.

Kabat, E.A. (1978) The structural basis of antibody complementarity. *Advances in Protein Chemistry*, **32**, 1.

Kabat, E.A., Wu, T. & Bilofsky, H. (1977) Unusual distribution of amino acids in complementarity-determining (hypervarible) segments of heavy and light chains of immunoglobulins and their possible roles in specificity of antibody-combining sites. *Journal of Biological Chemistry*, **252**, 6609.

Kim, S., Davis, M., Sinn, E., Patten, P. & Hood, L. (1981) Antibody diversity: somatic hypermutation of rearranged VH genes. *Cell*, **27**, 573.

Kuroda, D., Shirai, H., Kobori, M. & Nakamura, H. (2009) Systematic classification of CDR-L3 in antibodies: implications of the light chain subtypes and the VL-VH interface. *Proteins*, **75**, 139.

Laune, D., Molina, K., Ferrieres, G., Mani, J.-C., Cohen, P., Simon, D. *et al.* (1997) Systematic exploration of the antigen binding activity of synthetic peptides isolated from the variable regions of immunoglobulins. *Journal of Biological Chemistry*, **272**, 30937.

Lederberg, J. (1959) Genes and antibodies: do antigens bear instructions for antibody specificity or do they select cell lines that arise by mutation? *Science*, **129**, 1649.

Lutz, C.T., Smith, K.D., Greazel, N.S., Mace, B.E., Jensen, D.A., McCutcheon, J.A. *et al.* (1994) Bw4-reactive and Bw6-reactive antibodies recognize multiple distinct HLA structures that partially overlap in the alpha-1 helix. *Journal of Immunology*, **153**, 4099.

MacCallum, R.M., Martin, A.C.R. & Thornton, J.M. (1996) Antibody-antigen interactions: contact analysis and binding site topography. *Journal of Molecular Biology*, **262**, 732.

Marrari, M. & Duquesnoy, R. (2009) Correlations between Terasaki's HLA class II epitopes and HLAMatchmaker-defined eplets on HLA-DR, -DQ and -DP antigens. *Tissue Antigens*, **74**, 134.

Marrari, M., Mostecki, J., Mulder, A., Balazs, I., Claas, F. & Duquesnoy, R. (2010) Human monoclonal antibody reactivity with HLA class I epitopes defined by pairs of mismatched eplets and self eplets. *Transplantation*, **90**, 1468.

Matzinger, P. (1994) Tolerance, danger, and the extended family. *Annual Review of Immunology*, **12**, 991.

McCutcheon, J.A. & Lutz, C.T. (1992) Mutagenesis around residue 176 on HLA-B*0702 characterizes multiple distinct epitopes for anti-HLA antibodies. *Human Immunology*, **35**, 125.

McCutcheon, J.A., Smith, K.D., Valenzuela, A., Aalbers, K. & Lutz, C.T. (1993) HLA-B*0702 antibody epitopes are affected indirectly by distant antigen residues. *Human Immunology*, **36**, 69.

Morris, S., Moroldo, M., Giannini, E., Orekhova, T. & Finkelman, F. (2000) In vivo survival of autoreactive B cells: characterization of long-lived B cells. *Journal of Immunology*, **164**, 3035.

Mulder, M., Eijsink, C., Kester, M.G.D., Franke, M.E.I., Kardol, M.J., Heemskerk, M.H.M. *et al.* (2005) Impact of peptides on the recognition of HLA class I molecules by human HLA antibodies. *Journal of Immunology*, **175**, 5950.

Nossal, G. & Pike, B. (1980) Clonal anergy: persistence in tolerant mice of antigen-binding B lymphocytes incapable of responding to antigen or mitogen. *Proceedings of the National Academy of Sciences of the United States of America*, **77**, 1602.

Novotny, J. (1991) Protein antigenicity: a thermodynamic approach. *Molecular Immunology*, **28**, 201.

Padlan, E.A. (1994) Anatomy of the antibody molecule. *Molecular Immunology*, **31**, 169.

Park, M.S., Barbetti, A.A., Geer, L.I., Clark, B.D., Terasaki, P.I., Aoki, J. *et al.* (1990) HLA epitopes detected by serology. *Clinical Transplants*, 515.

Pelanda, R. & Torre, R. (2006) Receptor editing for better or for worse. *Current Opinion in Immunology*, **18**, 184.

Pillai, S., Cariappa, A. & Moran, S. (2004) Positive selection and lineage commitment during peripheral B-lymphocyte development. *Immunological Reviews*, **197**, 206.

Poljak, R., Amzel, L., Avey, H., Chen, B., Phizackerley, R. & Saul, F. (1973) Three-dimensional structure of the Fab' fragment of a human immunoglobulin at 2,8-A resolution. *Proceedings of the National Academy of Sciences of the United States of America*, **70**, 3305.

Pradeu, T. & Carosella, E. (2006) On the definition of a criterion of immunogenicity. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 17858.

Rodey, G.E., Neylan, J.F., Whelchel, J.D., Revels, K.W. & Bray, R.A. (1994) Epitope specificity of HLA class I alloantibodies. I. Frequency analysis of antibodies to private versus public specificities in potential transplant recipients. *Human Immunology*, **39**, 272.

Schroeder Jr, H., Zemlin, M., Khass, M., Nguyen, H. & Schelonka, R. (2010) Genetic control of DH reading frame and its effect on B-cell development and antigen-specifc antibody production. *Critical Reviews in Immunology*, **30**, 327.

Shirai, H., Kidera, A. & Nakamura, H. (1999) H3-rules: identification of CDR-H3 structures in antibodies. *FEBS Letters*, **455**, 188.

Silverstein, A. & Rose, N. (1997) On the mystique of the immunological self. *Immunological Reviews*, **159**, 197.

Tauber, A. (1994) *The Immune Self: Theory or Metaphor?* Cambridge Univ Press, Cambridge, UK.

Tiegs, S., Russell, D. & Nemazee, D. (1993) Receptor editing in self-reactive bone marrow B cells. *Journal of Experimental Medicine*, **177**, 1009.

Tonegawa, S. (1983) Somatic generation of antibody diversity. *Nature*, **302**, 575.

Van Regenmortel, M. (2002) Reductionism and the search for structure-function relationships in antibody molecules. *Journal of Molecular Recognition*, **15**, 240.

Wardemann, H., Yurasov, S., Schaefer, A., Young, J., Meffre, E. & Nussenzweig, M. (2003) Predominant autoantibody production by early human B cell precursors. *Science*, **301**, 1374.

Wedemayer, G., Patten, P., Wang, L., Schultz, P. & Stevens, R. (1997) Structural insights into the evolution of an antibody combining site. *Science*, **276**, 1665.

Wu, T., Johnson, G. & Kabat, E. (1993) Length distribution of CDRH3 in antibodies. *Proteins*, **16**, 1.