Transplant Immunology 38 (2016) 15–18

Contents lists available at ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

Brief communication

Epitope analysis of DQ6-reactive antibodies in sera from a DQ6-positive transplant candidate sensitized during pregnancy

Liesbeth Daniëls [a,*], Marie-Paule Emonds [a], Jean-Louis Bosmans [b], Marilyn Marrari [c], Rene J. Duquesnoy [c]

[a] Histocompatibility and Immunogenetics Laboratory (HILA), Red Cross-Flanders, Mechelen, Belgium
[b] Department of Nephrology, Antwerp University Hospital, Edegem, Belgium
[c] Division of Transplantation Pathology, Thomas E. Starzl Transplantation Institute, University of Pittsburgh Medical Center, PA, USA

ARTICLE INFO

Article history:
Received 6 June 2016
Received in revised form 22 July 2016
Accepted 25 July 2016
Available online 26 July 2016

Keywords:
HLA
HLAMatchmaker
HLA antibodies
Epitope
Nonself-self

ABSTRACT

This case report describes DQ6-reactive serum antibody reactivity in a patient who types as DQ6. DNA typing showed DQB1*06:09 on the antibody producer and serum reactivity with DQB1*06:01, *06:02 and *06:03 but not with *06:04 and *06:09. HLAMatchmaker serum analysis showed antibody reactivity with a new antibody-verified 85VA eplet on DQB but additional reactivity with DQB1*02:01 could not be readily interpreted. After applying the nonself-self algorithm of HLA immunogenicity we have identified a new DQB epitope structurally described as $140A_2 + 130R + 135D$ and shared by DQB1*02:01 and DQB1*05:01 and DQB1*06:02 of the immunizer.

1. Introduction

One of the major tasks of histocompatibility testing laboratories is the determination of acceptable HLA mismatches from serum antibody reactivity patterns with HLA panels. Certain sera have unexpected reactivity patterns with two or more alleles corresponding to the same antigen. This can create a dilemma in the interpretation of mismatch acceptability at the antigen level especially if the reactivity difference involves an allele of the recipient. Therefore, mismatch acceptability should be determined at the allele rather than the antigen level [1].

HLA antibodies are specific for epitopes rather than alleles or antigens. HLA structural modeling has identified small patches of polymorphic amino residues referred to as eplets as essential components of structural epitopes that make contact with the six Complementarity Determining Regions (CDRs) of antibody [2]. HLAMatchmaker has become a useful tool to analyze epitope specificities of antisera tested with HLA panels and determinations of mismatch acceptability. This algorithm is based on the concept that antibodies recognize mismatched eplets that are not on any HLA allele of the antibody producer. Although HLAMatchmaker offers an effective analysis of most sera, certain reactivity patterns cannot be readily interpreted because the antibody recognizes an eplet which is also present in the HLA type of the antibody producer. The so-called nonself-self paradigm of HLA epitope immunogenicity offers a new approach to this problem [3].

This paradigm emerged from observations that antibody-reactive eplets are surrounded by self-residues shared with an allele of the antibody producer. During B-cell development, V_H and V_L gene rearrangements produce a diversity of immunoglobulin receptors that can recognize epitopes on autologous proteins. One may hypothesize that certain B cells carry low-affinity receptors for self-HLA epitopes. Their interactions with self-HLA proteins will not lead to B-cell activation or antibody production. In contrast, HLA mismatches can elicit strong alloantibody responses but the activation of self-HLA-specific B-cells by a nonself-eplet would probably require that the remainder of the structural epitope consists largely of self-residues on one of the antibody producer's alleles [3].

Recent studies have demonstrated the successful application of the nonself-self paradigm of HLA epitope immunogenicity in the antibody verification of HLA-ABC and DRB epitopes [4–7]. This case describes DQ6-reactive serum antibody reactivity in a patient who types as DQ6 and for the first time, a nonself-self paradigm-based interpretation of a DQB-reactive antibody pattern.

2. Case description and epitope analysis

A 54-year-old Caucasoid female patient on the kidney transplant waiting list with a vPRA of 97% had been sensitized during pregnancy. Her serological HLA-DQ type was DQ4,6 and high-resolution typing showed DQB1*04:02, *06:09; DQA1*01:02, *04:01. Two children were mismatched for DQB1*06:02 only and one child was mismatched for

* Corresponding author at: Motstraat 42, 2800 Mechelen, Belgium.
E-mail address: liesbeth.daniels@rodekruis.be (L. Daniëls).

http://dx.doi.org/10.1016/j.trim.2016.07.004
0966-3274/

Table 1
HLA-DQ typing and mismatches.

Patient		Child 1		Child 2		Child 3	
DQB1*06:09	DQA1*01:02	DQB1*06:09	DQA1*01:02	DQB1*06:02	DQA1*01:02	DQB1*05:01	DQA1*01:01
DQB1*04:02	DQA1*04:01	DQB1*06:02	DQA1*01:02	DQB1*04:02	DQA1*04:01	DQB1*04:02	DQA1*04:01

DQB1*05:01 and DQA1*01:01. Table 1 shows the HLA-DQ typing and the mismatches.

Several sera had a very similar reactivity pattern with two DQ heterodimer panels (Life Codes, Immucor; One Lambda, ThermoFischer) tested in IgG-binding assays on a Luminex platform. Table 1 shows a typical result demonstrating antibody reactivity of DQ5 and most DQ6 alleles even though the patient typed as DQ6. High-resolution type information permitted an HLAMatchmaker analysis which then revealed that the reactive DQB1*05:01, *05:02, *05:03, *06:01, *06:02 and *06:03 shared a distinct 85VA eplet with children's DQB alleles. The non-reactive DQB1*06:09 (self) and DQB1*06:04 have 85VG instead of 85VA.

While there were no indications of DQA-reactive antibodies, this serum reacted also with all DQB1*02:01 heterodimers albeit with somewhat lower MFI values than those with the 85VA-carrying alleles (Table 2). This suggested the presence of antibodies against a second epitope which had to be shared between DQB1*02:01 and the immunizing DQB mismatch, i.e. DQB1*05:01 or DQB1*06:02 or both during the three pregnancies.

It should be noted that DQB1*02:02 was non-reactive; this allele has only one residue difference with the reactive DQB1*02:01, namely 135G versus 135D which is present on all other DQB alleles in the panel including those of the antibody producer. This means that 135D, as a self-residue, plays a critical role in the epitope responsible for the reactivity of DQB1*02:01. But which other residues contribute to this epitope? According to the structural epitope concept [2], 135D must be located within a 15 Ångstrom radius of the specificity-determining eplet or some other polymorphic residue configuration on the molecular surface.

Cn3D modeling [8] of a HLA-DQ6 molecular structure (DQB1*06:02, DQA1*01:02, PDB:1UVQ) identified four polymorphic residues within a 15 Ångstrom radius of 135D, namely 130R, 140A, 182S and 185T that are distinctly shared between DQB1*02:01 and the children's DQB1*05:01 and DQB1*06:02. Which of them contribute to the epitope which includes 135D as a critical contact site and are they nonself or self?

The antibody producer's DQB1 alleles have the following residues in these four sequence positions: 130R, 140T, 182N and 185I are on DQB1*04:02, and 130Q, 140A, 182S, and 185T are on DQB1*06:09. It should be noted that the immunizer's 130R is non-self for DQB1*06:09 but self for DQB1*04:02, whereas the immunizer's 140A, 182S, and 185T are non-self for DQB1*04:02 but self for DQB1*06:02. In other words, each residue is a mismatch for one DQB1 allele but a match for the other DQB1 allele of the antibody producer. Is it possible that such polymorphic residue cannot solely describe an eplet and that another nearby polymorphic residue is necessary to define a mismatch?

Eplets are key elements of HLA epitopes and they can be determined in structural models by identifying amino acid residues within a 3.5 Ångstrom radius of a polymorphic residue. With the DQB1*06:02, DQA1*01:02 model (PDB:1UVQ) we could not find any additional

Table 2
Serum reactivity patterns with HLA-DQ panels reflect antibodies specific for a new antibody-verified 85VA epitope and a second epitope on DQB1*02:01.

				Immucor	ThermoFischer**			Immucor	ThermoFischer**
	DQB		DQA	MFI	MFI	DQB	DQA	MFI	MFI
	Positive Control			18622	14658	Negative Control		153	13
Immunizer	DQB1*05:01	(85VA)	DQA1*01:01	5443	2879	DQB1*02:02	DQA1*02:01	149	50
Immunizer	DQB1*05:01	(85VA)	DQA1*01:02	7310		DQB1*02:02	DQA1*03:02	152	
Immunizer	DQB1*06:02	(85VA)	DQA1*01:01			DQB1*02:02	DQA1*05:01	184	
Immunizer	DQB1*06:02	(85VA)	DQA1*01:02	9898	1779	DQB1*03:01	DQA1*02:01		46
Immunizer	DQB1*06:02	(85VA)	DQA1*01:01		2170	DQB1*03:01	DQA1*03:01		64
	DQB1*05:02	(85VA)	DQA1*01:02		1360	DQB1*03:01	DQA1*03:02	125	
	DQB1*05:03	(85VA)	DQA1*01:04	5813		DQB1*03:01	DQA1*05:01	101	
	DQB1*06:01	(85VA)	DQA1*01:03	4968	1527	DQB1*03:01	DQA1*05:03		104
	DQB1*06:01	(85VA)	DQA1*01:04	3476		DQB1*03:01	DQA1*05:05		45
	DQB1*06:01	(85VA)	DQA1*02:01	5396		DQB1*03:01	DQA1*06:01	234	21
	DQB1*06:03	(85VA)	DQA1*01:03	8161	3689	DQB1*03:02	DQA1*02:01	117	52
	Mean±Standard Deviation			6308±2034	2225±818*	DQB1*03:02	DQA1*03:01	113	54
	DQB1*02:01		DQA1*02:01		589	DQB1*03:02	DQA1*03:02	113	257
	DQB1*02:01		DQA1*03:01		968	DQB1*03:03	DQA1*02:01		47
	DQB1*02:01		DQA1*04:01		817	DQB1*03:03	DQA1*03:01		143
	DQB1*02:01		DQA1*05:01	3984	1325	DQB1*03:03	DQA1*03:02	132	151
	Mean±Standard Deviation			3984	1037±261*	DQB1*03:03	DQA1*04:01	202	
						DQB1*03:03	DQA1*06:01	87	
	DQB1*04:02		DQA1*02:01		42	DQB1*04:01	DQA1*02:01	90	48
	DQB1*04:02		DQA1*03:01	90		DQB1*04:01	DQA1*03:03		46
Self	DQB1*04:02		DQA1*04:01	98	34	DQB1*04:01	DQA1*04:01	137	
	DQB1*04:02		DQA1*06:01	87		DQB1*04:01	DQA1*05:01	94	
Self	DQB1*06:09		DQA1*01:02		103	DQB1*06:04	DQA1*01:02	172	91
	Mean±Standard Deviation			122±40	68±34	Mean±Standard Deviation		135±43	81±64

*p=0.004 **1/10 diluted serum

Table 3
Reactivity patterns for four possible eplets and self-residues in corresponding structural epitopes.

Possibility 1	Allele	Eplet	135	140	182	185
Self	DQB1*04:02	130R	-	T	N	I
Self B-cell Receptor	DQB1*06:09	s130Q	sD	sA	sS	sT
Child	DQB1*05:01	130R	-	-	-	-
Child	DQB1*06:02	130R	-	-	-	-
Reactive	DQB1*02:01	130R	-	-	-	-
Non-reactive	DQB1*02:02	130R	G	-	-	-
Non-reactive	DQB1*03:01	130R	-	T	N	-
Non-reactive	DQB1*03:02	130R	-	T	N	I
Non-reactive	DQB1*03:03	130R	-	T	N	I
Non-reactive	DQB1*04:01	130R	-	T	N	I
Non-reactive	DQB1*06:04	s130Q	-	-	-	-

Possibility 2	Allele	Eplet	130	135
Self	DQB1*06:09	140A	Q	-
Self B-cell Receptor	DQB1*04:02	s140T	sR	sD
Child	DQB1*05:01	140A	-	-
Child	DQB1*06:02	140A	-	-
Reactive	DQB1*02:01	140A	-	-
Non-reactive	DQB1*02:02	140A	-	G
Non-reactive	DQB1*06:04	140A	Q	-
Non-reactive	DQB1*03:01	s140T	-	-
Non-reactive	DQB1*03:02	s140T	-	-
Non-reactive	DQB1*03:03	s140T	-	-
Non-reactive	DQB1*04:01	s140T	-	-

Possibility 3	Allele	Eplet	130	135	185
Self	DQB1*06:09	182S	Q	-	T
Self B-cell Receptor	DQB1*04:02	s182N	sR	sD	sI
Child	DQB1*05:01	182S	-	-	T
Child	DQB1*06:02	182S	-	-	T
Reactive	DQB1*02:01	182S	-	-	T
Non-reactive	DQB1*02:02	182S	-	G	T
Non-reactive	DQB1*06:04	182S	Q	-	T
Non-reactive	DQB1*03:01	s182N	-	-	T
Non-reactive	DQB1*03:02	s182N	-	-	-
Non-reactive	DQB1*03:03	s182N	-	-	-
Non-reactive	DQB1*04:01	s182N	-	-	-

Possibility 4	Allele	Eplet	130	135	182
Self	DQB1*06:09	185T	Q	-	S
Self B-cell Receptor	DQB1*04:02	s185I	sR	sD	sN
Child	DQB1*05:01	185T	-	-	S
Child	DQB1*06:02	185T	-	-	S
Reactive	DQB1*02:01	185T	-	-	S
Non-reactive	DQB1*02:02	185T	-	G	S
Non-reactive	DQB1*06:04	185T	Q	-	S
Non-reactive	DQB1*03:01	185T	-	-	-
Non-reactive	DQB1*03:02	s185I	-	-	-
Non-reactive	DQB1*03:03	s185I	-	-	-
Non-reactive	DQB1*04:01	s185I	-	-	-

polymorphic residues nearby 130R, 140A, 182S and 185T even after increasing the radius to 6 Å. Therefore, single residues rather than residue combinations define these eplets and all of them are located in antibody-accessible positions on the β2 chain of DQB1.

Eplets are centrally located within corresponding structural epitopes so we determined with the DQB1*06:02, DQA1*01:02 model, which polymorphic positions on the molecular surface are within 15 Å of each of these four eplets (Table 3). There are four such positions for 130R, two for 140A, three for 182S and three for 185T. For each DQB1 allele in the panel we determined the residues in these positions. In this search for a new epitope we excluded the reactive DQB1*05:02, *05:03, *06:01 and *06:03 beads because these alleles carry the 85VA epitope already recognized by antibodies in these sera; such alleles cannot be informative for the new epitope.

All four eplets are on the children's DQB1*05:01 and DQB1*06:09 but as described above, each eplet is a mismatch for one DQB1 allele but a match for the other DQB1 allele of the antibody producer (Table 3). According to the nonself-self paradigm of eplet immunogenicity [3], we postulated that antibodies specific for this yet undefined epitope originated from B-cells with immunoglobulin receptors for self-structural epitope on one of the DQB1 alleles of the antibody producer. Table 3 has for that DQB1 allele the notation "self B-cell receptor" and polymorphic residue descriptions within the context of structural epitopes. Self-residues have the prefix "s" and dashes in the columns represent the same self-residue.

We have hypothesized that in order to have an effective HLA antibody response induced by nonself-eplet it is necessary that the remainder of the corresponding structural epitope of the immunizing allele must largely consist of self-residues [3]. This requires that a non-self eplet on the immunizer and reactive alleles must be surrounded by self-residues and that unreactive, non-self eplet-carrying alleles lack certain critical self-residues. These criteria were investigated for each of these four possible eplet mismatches (Table 3).

As the first possibility, 130R is a mismatch for the 130Q-carrying DQB1*06:09. This would mean that 130R-reactive antibodies originated from B-cells with self-receptors for a structural epitope corresponding to 130Q on DQB1*06:09 of the antibody producer and includes the polymorphic 135D, 140A, 182S and 185T self-residues; all of them have the "s" prefix. The child's DQB1*06:02 and DQB1*05:01 have the mismatched 130R surrounded by the same self residues and either one would qualify as the immunizing allele.

The reactive DQB1*02:01 has 130R together with the same self-residues as the antibody producer's DQB1*06:09. The remaining 130R-carrying alleles are non-reactive and they have residue differences such as 135G versus 135D for DQB1*02:02 and 140T versus 140A and 182N versus 182S for DQB1*03:01, *03:02, *03:03, *04:01 and DQB1*04:02 of the antibody producer. The non-reactive DQB1*06:04 has 130Q instead of 130R. These findings indicate that a 130R-defined epitope would require the presence of 135D + 140A + 182S. The non-reactive DQB1*03:01 shares 185T with the reactive alleles; this self-residue seems less important for the epitope.

As a second possibility, 140A is a mismatch for antibody producer's DQB1*04:02 which has 140T instead and is surrounded by self-residues 130R and 135D (Table 3). The children's DQB alleles and the reactive DQB1*02:01 have the same self residues. The non-reactive 140A-carrying DQB1*06:04 and DQB1*02:02 have 130Q and 135G, respectively. Since the remaining non-reactive alleles have 140T, these can be interpreted that a 140A-defined epitope would require the presence of the 130R + 135D combination.

Thirdly, 182S is a mismatch for antibody producer's DQB1*04:02 which has 182N instead; the latter is surrounded by self-residues 130R, 135D and 185I (Table 3). Both children's DQB alleles and the reactive DQB1*02:01 share 130R and 135D with DQB1*04:02 and substitutions such as 130Q on DQB1*06:04 and 135G on DQB1*02:02 correlate with non-reactivity. On the other hand, all 182S-carrying alleles have 185T which is non-self for DQB1*04:02. As noted above, 182S and 185T cannot be on the same eplet because these residues are 6.6 Å apart.

The fourth possibility involves the same residues but in this case 185T is a mismatch for the antibody producer's DQB1*04:02 which has 185I; self-residues 130R and 135D are informative in explaining the reactivity differences between DQB1*02:01 versus DQB1*02:02 and DQB1*06:04 which have 135G and 130Q, respectively. However, the 185T-carrying DQB1*03:01 has also 130R + 135D but is non-reactive. This rules out 185T + 130R + 135D combination as the epitope.

Altogether, there are three remaining possible combinations to describe the epitope shared between DQB1*02:01 and the children's DQB1*05:01 and DQB1*06:02, and either one could be the immunizing allele. They are 130R with 135D + 140A + 182S, 140A with 130R + 135D and 182S with 130R + 135D. They involve just four residues which are depicted in a structural model (Fig. 1). 140A and 182S are 23 Å apart which is too far to be included in the same structural epitope. As recorded in the HLA Epitope Registry, the antibody-verified $140A_2$ describes two separate eplets 140A and 182S shared between the same group of DQB1*02, *05 and *06 alleles. The Luminex kits do not have alleles that distinguish 140A from 182S so we use the $140A_2$ designation.

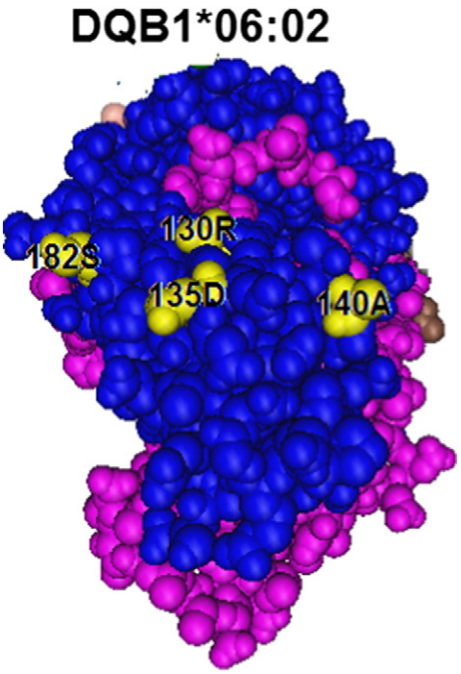

Fig. 1. Locations of four polymorphic residues defining a DQB epitope structurally described as $140A_2 + 130R + 135D$ which could be 140A + 130R + 135D or 182S + 130R + 135D.

In conclusion, this patient has antibodies that react with $140A_2$-carrying alleles which also must have 130R and 135D. Three non-reactive $140A_2$-carrying alleles and DQB1*02:02, DQB1*06:04 and DQB1*06:09 have 135G or 130Q. Therefore, the DQB epitope recognized by antibodies in this patient can be described structurally as $140A_2 + 130R + 135D$.

3. Discussion

This report illustrates the importance of high-resolution HLA typing information and the usefulness of epitope-based antibody specificity analysis to understand the degree of humoral HLA sensitization and the determination of acceptable mismatches for sensitized transplant candidates. HLA antigen-based acceptable mismatching for this highly immunized patient is problematic because from a conventional perspective her serum has anti-DQ6 antibodies but she types as DQ6. DNA typing showed DQB1*06:09 on the antibody producer and serum reactivity with DQB1*06:01, *06:02 and *06:03 but not with *06:04 and *06:09. Conventional analysis of antibody reactivity patterns may not be sufficient to identify all clinically relevant epitopes that may induce antibodies. Developing new methods to identify antibody specificities, such as the nonself-self paradigm, will allow us to better determine acceptable mismatches for transplant candidates.

The initial HLAMatchmaker serum analysis showed antibody reactivity with a new antibody-verified 85VA eplet on DQB1*05 and some DQB1*06 alleles. But additional reactivity with DQB1*02:01 could only be interpreted by applying the nonself-self algorithm of HLA immunogenicity.

A major clue was the non-reactivity of DQB1*02:02 which has only one residue difference with DQB1*02:01, namely 135G versus 135D. This means that position 135 is important for the epitope. DQB1*02:01 shares 135D with the children's DQB1*05:01 and DQB1*06:02. However, this residue is also on both DQB alleles of the mother and therefore must be a self-residue as an important part of the structural epitope recognized by this antibody. A Cn3D search with a molecular DQB model yielded four polymorphic residues within 15 Å of 135D. Each of them defines an eplet which turned out in each case a mismatch for one DQB allele but a match for the other DQB allele of the antibody producer. Since HLAMatchmaker could not be used for this analysis we proceeded to apply the nonself-self algorithm of eplet immunogenicity. Eplets are centrally located within their corresponding structural epitopes and for each of these four residues we determined which polymorphic residues are within a 15 Ångstrom radius.

After comparing these molecular models, we concluded the presence of a new epitope which can be best described as $140A_2 + 130R + 135D$ and which is shared by all positively reacting DQB alleles. Although each residue is self on one of the DQB1 alleles of the antibody producer, the combination can be considered nonself for the entire DQ phenotype. Therefore, the epitope can be defined as $140A_2 + 130R + 135D$ and it should be noted that each component is far enough from the others to be contacted by separate CDRs of antibody. This new epitope will be considered for inclusion in the HLA Epitope Registry as antibody-verified.

Disclosure

The authors of this manuscript have no conflict of interest to disclose as described by Transplant Immunology.

Acknowledgments

The authors would like to thank Marian Witvliet, Dave Roelen and Frans Claas for testing the patient serum with the One Lambda, ThermoFisher IgG-binding assay in the HLA laboratory, Leiden University Medical Center, The Netherlands.

The authors would also like to thank Gerda Van Beeumen for sample coordination.

References

[1] R.J. Duquesnoy, M. Kamoun, L.A. Baxter-Lowe, E.S. Woodle, R.A. Bray, F.H.J. Claas, D.D. Eckels, J.J. Friedewald, S.V. Fuggle, H.M. Gebel, J.A. Gerlach, J.J. Fung, D. Middleton, P. Nickerson, R. Shapiro, A.R. Tambur, C.J. Taylor, K. Tinckam, A. Zeevi, Personal viewpoint: should HLA mismatch acceptability for sensitized transplant candidates be determined at the high-resolution rather than the antigen level? Am. J. Transplant. 20 (2015) 1–6.
[2] R.J. Duquesnoy, Epitope-based human leukocyte antigen matching for transplantation, Curr. Opin. Organ Transplant. 19 (2014) 418–419.
[3] R.J. Duquesnoy, The antibody response to an HLA mismatch: a model for nonself-self discrimination in relation to HLA epitope immunogenicity, Int. J. Immunogenet. 39 (2011) 1–9.
[4] M. Marrari, R. Conca, L. Praticò-Barbato, A. Amoroso, R. Duquesnoy, Brief report: why did two patients who type for HLA-B13 have antibodies that react with all Bw4 antigens except HLA-B13? Transplant. Immunol. 25 (2011) 217–220.
[5] R.J. Duquesnoy, M. Marrari, A. Mulder, Usefulness of the nonself-self algorithm of HLA epitope immunogenicity in the specificity analysis of monospecific antibodies induced during pregnancy, Front. Immunol. 6 (2015) (Article 160:Article 160).
[6] A. Hahn, V. Bravo-Egana, J. Jackstadt, D. Conti, R.J. Duquesnoy, HLA-A2 reactive antibodies in a patient who types as HLA-A2: the importance of high 3 resolution typing and epitope-based antibody analysis, Transpl. Immunol. 32 (2015) 141–143.
[7] M. Resse, R. Paolillo, B. Minucci, G. Moccia, C. Napoli, Antibody-reactive class I epitopes defined by pairs of mismatched eplets and self-eplets, Tissue Antigens 86 (2015) 368–372.
[8] C. Hogue, Cn3D: a new generation of three-dimensional molecular structure viewer, Trends Biochem. Sci. 22 (1997) 314–316.