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How did a patient who types for HLA-B*4403 develop antibodies that react with HLA-B*4402?

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ABSTRACT

This case report shows that the sensitization of a HLA-B*4403 patient by a kidney transplant with a HLA-Cw*0704 mismatch led to antibodies reacting with the 156DA eplet shared with B*4402 and other HLA-B antigens including B*0801, B*3701, B*4101, B*4201, B*4501, and B*8201. It demonstrates that antibodies induced by an HLA-C mismatch can render certain HLA-B antigens unacceptable mismatches although the patient has never been exposed to them. This finding illustrates the importance of analyzing antibody specificities against HLA epitopes in the determination of mismatch acceptability for sensitized patients.

1. Introduction

Recently developed solid-phase antibody detection assays with single HLA alleles have improved the analysis of antibody specificity patterns of sera for HLA sensitized patients and the determination of mismatch acceptability of potential transplant donors. Often enough however, these assays yield surprising findings that appear difficult to interpret. Here we report a case whereby a patient whose type had B44 and who, after kidney transplantation, developed antibodies that reacted strongly with B*4402 but not with B*4403, and how an HLAMatchmaker analysis of patient's serum reactivity provided an explanation.

2. Case description

This 61-year-old male patient typed as HLA-A1,66; B44,58; Cw4,6 and in October 2001, received a kidney transplant from his cousin who typed as HLA-A1,3; B57,62; Cw6,7. There was no evidence of pretransplantation sensitization and the direct and anti-globulin augmented lymphocytotoxicity cross-matches were negative. Three weeks post-transplantation, a biopsy showed moderate acute cellular rejection and the patient was successfully treated with increased immunosuppression. The transplant failed due to chronic rejection and the patient was put on hemodialysis in September 2005. Serum screenings with ELISA showed no HLA class I antibody reactivity until March 2007. Subsequent sera tested with single class I allele Luminex panels (LabScreen; OneLambda, Canoga Park, CA) showed similar antibody reactivity patterns that included Cw7 (strong), B62 (moderate), A3 (low) and there was no reactivity with the donor's B57 mismatch (Fig. 1).

This patient typed as expressing B44 but his serum reacted strongly with B*4402 but not with B*4403. These alleles have only one eplet difference, namely 156DA versus 156LA. Patient's serum reacted also strong with all 156DA-carrying B*0801, B*3701, B*4101, B*4201, B*4501 and B*8201 alleles in the Luminex panel but none of them including B*4402 were in the donor's phenotype. After ruling out that the 156DA-specific antibody was the result of a third-party sensitization, we considered that the immunizing Cw7 must have 156DA. The common Cw7 alleles Cw*0701 and Cw*0702 do not have this eplet so we postulated that this donor's type must have the uncommon 156DA-carrying Cw*0704 allele. Indeed, high-resolution typing revealed the donor as A*0101, *0301; B*1501, *5,701; Cw*0602, *0704 and the patient as A*0101, *6601; B*4403, *5,801; Cw*0401, *0602.

We conducted an HLAMatchmaker analysis of serum reactivity with single alleles to determine which epitopes, referred to as eplets, were recognized by patient's antibodies. The eplet version HLAMatchmaker algorithm has been described elsewhere [1,2] and a dedicated website http://www.HLAMatchmaker.net has more details about the antibody analysis programs. Briefly, these programs identify for each allele which eplets are mismatched for the patient. In the Luminex panel, any allele that gives a negative reaction with patient's serum is recorded in HLAMatchmaker and their corresponding eplets are interpreted as acceptable mismatches. The remaining eplets on reactive alleles are then compared with those of the immunizing donor to assess the antibody specificities of the serum.

Table 1 summarizes the results of this analysis of serum antibody reactivity tested with Luminex kits from two manufacturers (LabScreen, OneLambda, Canoga Park, CA and Lifecodes, Tepnel Corporation, Stamford, CT). The transplant donor had four mismatched alleles: Cw*0704 with 12 mismatched eplets had the highest epitope load followed by B*1501 (six eplets), A*0301 (three

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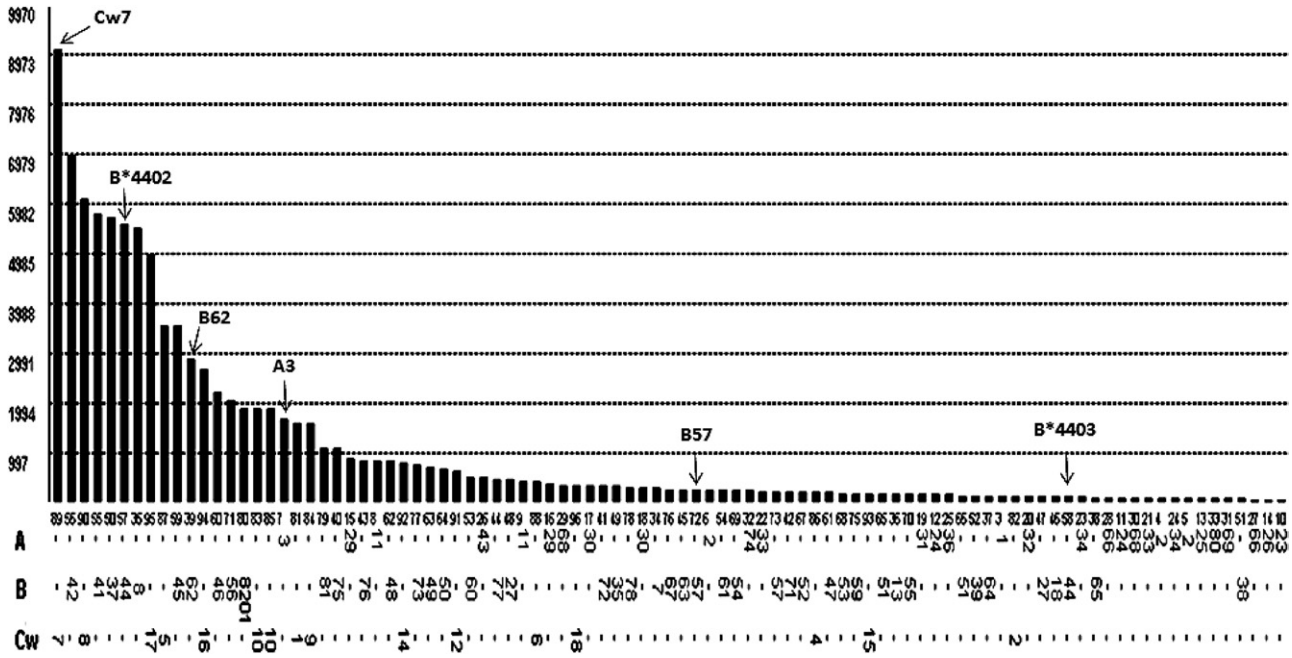

Fig. 1. Serum reactivity (in MFI values) with a Luminex panel (Labscreen,OneLambda), The arrows point to the reactive B*4402 and the non-reactive B*4403 as well as the mismatched antigens of the transplant donor.

eplets) and B*5701 which was a structural match for this patient. The Luminex reactivities are shown as median MFI values; their average with self alleles were 258 ± 146 and 1174 ± 518, respectively. Sixty-three alleles were considered negative their mean MFI values were 387 ± 308 and 1160 ± 527. The remainder of Table 1 shows the MFI values of the reactive alleles in both kits along with the mismatched eplets of the immunizer. Only the Lifecodes kit had Cw*0704 (MFI = 9427), and eight eplets are shared with the reactive alleles.

This study addressed the interpretation of the B*4402 reactivity in this B*4403 patient. Its MFI with B*4402 were 5612 and 3202 and this allele shares the 156DA eplet with the immunizing Cw*0704. Moreover, seven reactive alleles B*0801, B*3701, B*4101, B*4201, B*4501, B*8201 and B*8202 shared only 156DA with Cw*0704 (the negative reaction with B*3701 in the LifeCodes kit reflects a technical problem with this allele). These findings explain that the serum reactivity with B*4402 reflects the presence of a 156DA-specific antibody induced by the Cw*0704 mismatch.

Cw*0704 had additional eplets that induced specific antibodies. For instance, two reactive alleles Cw*0501 and Cw*0801 shared 177KT with Cw*0704. Two HLA-C alleles Cw*0702 and Cw*1701 shared 267QE with Cw*0704 and reacted strongly but the 267QE-carrying B*7301 allele was less reactive. B*4601 and a large group of HLA-C alleles shared 77VSN and 79VRN with Cw*0704 and their reactivity with patient's serum was intermediate or weak.

This transplant had three additional HLA class I allele mismatches. A*0301 induced antibodies with weak reactivity with 161D, an eplet found uniquely on A*0301. Although B*1501 had six mismatched eplets, there was weak antibody reactivity with77TTS paired with 44RM and, possibly with 80SRN paired with 156WA. This finding is analogous to previously reported observations that the epitope specificity of certain antibodies can be defined by pairs of eplets [3,4]. Not surprisingly, the donor's B*5701 which had no mismatched eplets for this patient did not elicit antibodies.

3. Discussion

This study demonstrates how HLAMatchmaker can facilitate the interpretation of serum antibody reactivity patterns with HLA panels. This algorithm is based on the premise that HLA-specific antibodies recognize epitopes rather than antigens and it uses eplets, that is, small patches with polymorphic amino acids on the molecular surface, to describe HLA epitopes [1,2]. Information about the immunogenetic relationship between immunizer and antibody producer greatly enhances the antibody analysis and this can be readily obtained by determining the spectrum of mismatched eplets on the HLA alleles of the immunizer. This case illustrates the need for high-resolution typing (at the four-digit level) to obtain accurate information about donor–recipient compatibility at the epitope level and to optimize the interpretation of serum antibody reactivity for sensitized patients. Under these conditions, we could resolve the question why a patient who types for B*4403 had antibodies reacting with B*4402 although this patient had not been exposed to B*4402.

High-resolution typing often show eplet differences between alleles belonging to the same antigen. For instance, the common B44 subtypes B*4402 and B*4403 have one eplet difference 156DA versus 156LA whereby D (aspartic acid) and L (leucine) are in position 156 (not well exposed on the molecular surface) and A (alanine) is in position 158. This structural difference has been shown to adversely affect the outcome of B*4402/03 mismatched hematopoietic stem cell transplants through greater graft-versus-host reactivity [5]. Moreover, Bray *et al.* have reported the presence of B*4402-reactive antibodies in the serum of an African-American patient who typed as B*4403 but no HLA information was provided about the immunizer [6].

In this case, sensitization to 156DA was induced by a Cw*0704 mismatch which led to antibodies that reacted with all 156DA-carrying HLA-B alleles in the Luminex panel, including B*4402. It demonstrates that antibodies induced by an HLA-C mismatch can render certain HLA-B antigens unacceptable mismatches although the patient has never been exposed to them. Because of the persistently strong anti-156DA reactivity of this serum one might postulate that all 156DA-carrying alleles, including those not in these Luminex panels (such as B*0704, B*4102, B*4405, B*5108 and B*8301) could be considered unacceptable mismatches.

Table 1
HLAMatchmaker analysis of antibody reactivity with single HLA alleles in two Luminex kits

Patient: A*0101,A*6601; B*4403,B*5801; Cw*0401,Cw*0602				
Mismatched donor alleles			Nr	Mismatched eplets for patient
A*0301			3	105S, 161D, 150AAH
B*1501			6	73TTS, 76TES, 77ESN, 80SRN, 82RNR
B*5701			0	—
Cw*0704			12	73AS, 77VSN, 79VRN, 80SRN, 82RNR, 152RA, 156DA, 177KT, 193PL, 211T, 267QE
		OneLambda	Tepnel	
Negative cont		38	252	—
Positive cont		9273	2807	—
Average self-reactivity		258±146	1174±518	
				Immunizer eplets on reactive alleles
Negative Alleles	N = 63	387±308	1160±527	None
Cw*0704	Imm	nt	9427	73AS, 77VSN, 79VRN, 152RA, 156DA, 177KT, 193PL, 267QE
B*4402		5622	3202	156DA
B*0801		5770	8818	156DA
B*3701		5775	747	156DA
B*4101		5872	3237	156DA
B*4201		6967	3882	156DA
B*4501		3620	4468	156DA
B*8201		1997	Nt	156DA
B*8202		nt	5062	156DA
Cw*0501		3730	6063	177KT
Cw*0801		6319	8155	77VSN, 79VRN, 177KT
Cw*0802		nt	6471	76TVS, 77VSN, 79VRN, 138K, 177KT
Cw*1701		5159	9149	267QE
B*7301		859	3645	79VRN, 267QE
Cw*0701		nt	8307	73AS, 77VSN, 79VRN, 152RA, 193PL, 267QE
Cw*0702		9182	7150	73AS, 77VSN, 79VRN, 152RA, 193PL, 267QE
Cw*1601		2735	3131	77VSN, 79VRN, 152RA
Cw*1202		nt	7611	73AS, 77VSN, 79VRN
Cw*1203		737	Nt	73AS, 77VSN, 79VRN
Cw*0102		1679	3939	77VSN, 79VRN
Cw*0302		1938	Nt	77VSN, 79VRN
Cw*0303		1669	3190	77VSN, 79VRN
Cw*0304		1926	3060	77VSN, 79VRN
Cw*1402		918	2429	77VSN, 79VRN
B*4601		2305	2061	77VSN, 79VRN
A*0301	Imm	1766	2312	161D
B*1501	Imm	2877	1946	44RM73TTS, 80SRN156WA
B*1512		939	1255	44RM73TTS, 80SRN156WA
B*1502		1155	1214	44RM73TTS
B*5701	Imm	426	746	None

Nr = Number; Nt/nt = not tested; Imm = Immunizer; Cont = Control.

The demonstration of epitope-specific antibodies has important implications for organ transplant allocation. Gebel *et al.* pointed out recently that, in the USA, patients with antibodies reacting with B*4402 but not B*4403 are not distinguished from patients who have antibodies reacting with both alleles [7]. In the United Network for Organ Sharing (UNOS), the reporting of unacceptable mismatches is limited to antigens, not alleles. This creates a dilemma because a patient with only B*4402 reactive antibodies might be listed as having B44 as an unacceptable mismatch whereas B*4403 could be acceptable. Moreover, as this case illustrates, current UNOS criteria consider B44 as a match for a patient who types as B*4403 although he has B*4402-reactive antibodies. Because these alleles have similar frequencies, one might expect about one-half of cross-matches with B44 will be positive and this is not a desirable approach to allocate organ transplants. Gebel *et al.* have suggested a solution of this problem by allowing the entry of allele-specific antibodies in the UNOS matching system especially for the identification of acceptable mismatches for sensitized patients [7]. We agree with this approach although we would prefer the incorporation of epitope-reactive antibodies for determining allelic mismatch acceptability.

In summary, these findings illustrate the usefulness of determining antibody specificities against epitopes from serum reactivity patterns with single alleles, especially if the patient and the immunizing donor have been typed with high-resolution molecular methods.

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