

# Human Leukocyte Antigen Class II DQ Alpha and Beta Epitopes Identified From Sera of Kidney Allograft Recipients

Chun-Tsan Deng,<sup>1,3</sup> Nadim El-Awar,<sup>1</sup> Miyuki Ozawa,<sup>1</sup> Junchao Cai,<sup>2</sup> Nils Lachmann,<sup>2</sup> and Paul I. Terasaki<sup>2</sup>

**Background.** Epitopes are the sites to which antibodies bind. Both alpha and beta peptide chains of the human leukocyte antigen-DQ heterodimers (DQA1 and DQB1, respectively) contain polymorphic regions. We can identify DQA1 and DQB1 epitopes by DQ single antigen beads assay of the antibodies, correlating the beads' reaction patterns with either DQA1 or DQB1 alleles.

**Methods.** Sera from 74 transplant patients and 35 mouse DQB1 monoclonal antibodies were tested with DQ single antigen beads for their DQ allelic and serological specificities. Epitopes were defined by amino acids shared by the positive antigens of the antibodies. Unique amino acids were identified as potential epitope sites by comparing the peptide sequences of all human leukocyte antigen class II alleles. For the absorption or elution, patient's serum sample was absorbed by a homozygous B-lymphoblast cell line of specific DQ typing, the eluted antibody then tested with single antigen beads to demonstrate that the antibody reacted to a single epitope shared by multiple DQ antigens.

**Results.** Three DQA1 and 15 DQB1 epitopes were identified. We found that 21 patients produced antibodies against one of the DQA1 epitopes; 27 patients produced antibodies against one of the DQB1 epitopes.

**Conclusion.** The DQA1 and DQB1 epitopes identified here seem to be immunogenic and to elicit DQ antibodies. For the DQB1 epitopes, multiple DQ serological specificities that were detected in the serum of a transplant patient could be explained as a single donor-specific DQ antibody reacting to a mismatched DQ epitope of the donor. Ten examples are shown here.

**Keywords:** HLA-DQ epitopes, DQ antibodies, HLA class II antigens.

(*Transplantation* 2008;86: 452–459)

Previous studies have discussed human leukocyte antigen (HLA) class I epitopes (1–4) and class II DR epitopes (5, 6). The recent development of class II HLA-DQ single antigen beads has now made it possible to distinguish HLA-DQ antibodies from class II HLA-DR and HLA-DP antibodies. The polymorphic nature of the DQ molecule's DQA1 and DQB1 alleles make it a challenge to show whether DQ antibodies are reacting to DQA1 or DQB1 antigens. We identified DQA1 antibodies—by the correlation of the DQ single antigen beads' reaction patterns with DQA1 alleles—and defined three DQA1 epitopes in the sera of posttransplant patients. We also defined 15 DQB1 epitopes, using mouse DQB1 monoclonal antibodies (mAbs) and sera from transplant patients.

<sup>1</sup> One Lambda Inc., Canoga Park, CA.

<sup>2</sup> Terasaki Foundation Laboratory, Los Angeles, CA.

<sup>3</sup> Address correspondence to: Chun-Tsan Deng, Ph.D., 11570 W. Olympic Boulevard, Los Angeles, CA 90064.

E-mail: cdeng@terasakilab.org

Received 19 December 2007. Revision requested 4 January 2008.

Accepted 26 March 2008.

Copyright © 2008 by Lippincott Williams & Wilkins

ISSN 0041-1337/08/8603-452

DOI: 10.1097/TP.0b013e3181804cd2

## MATERIALS AND METHODS

### Monoclonal Antibodies and Sera

Sera and mAbs were tested to show their DQ single antigen beads' reaction patterns. We used 35 mouse IgM mAbs (One Lambda Inc., Canoga Park, CA) with proven DQB1 serological specificities (7). Either human cell lines or peripheral blood lymphocytes were used to immunize the mice. The human cell lines were not recombinant cells with recombinant DQ alpha and beta chains. Therefore, the mAbs were unlikely to be raised against the denatured or distorted versions of solubilized HLA molecules. A dilution of ascites (1:10,000) or hybridoma supernatants of the mAbs were used for the single antigen beads assay. All sera used for this study's single antigen beads assay were diluted 1:3. They included 72 from recipients of kidney allograft at the Charité-Universitätsmedizin, Berlin (Germany) and two from the Brody School of Medicine at East Carolina University, Greenville, North Carolina.

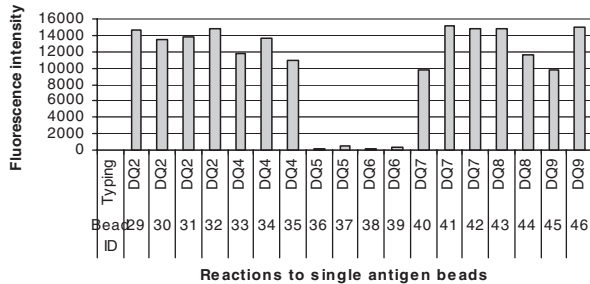
### Single Antigen Beads Assay

Patient sera and mAbs were tested with a panel of 59 single antigen beads (LABScreen HLA class II single antigen

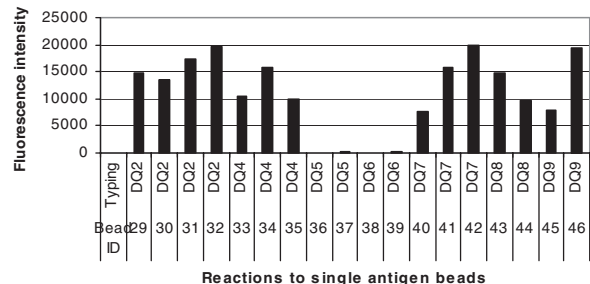
**A**

			DQB1 pattern no.	#2001	#2002	#2003	#2004	#2005	#2006	#2007	#2008	#2009	#2010	#2011	#2012	#2013	#2014	#2015
			Serological spec.	DQ4,5,6, 7,8,9					DQ4,5, DQ2,5,7,8,9					DQ2,4, DQ4,7, 7,8,9				
Bead ID	Molecular DQA1*	Typing DQB1*	Serological Typing	(6 mAbs) (1 Ab)	(6 mAbs) (0 Ab)	(2 mAbs) (1 Ab)	(5 mAbs) (3 Abs)	(5 mAbs) (0 Ab)	(5 mAbs) (4 Abs)	(1 mAb) (1 Ab)	(1 mAb) (0 Ab)	(1 mAb) (0 Ab)	(1 mAb) (3 Abs)	(1 mAb) (2 Abs)	(1 mAb) (0 Ab)	(No equivalent mAb) (2 Abs) (1 Ab) (2 Abs)		
29	201	201	DQ2	+	-	-	-	-	-	-	+	+	-	-	-	+	-	-
30	301	201	DQ2	+	-	-	-	-	-	-	+	+	-	-	-	+	-	-
31	501	201	DQ2	+	-	-	-	-	-	-	+	+	-	-	-	+	-	-
32	201	202	DQ2	+	-	-	-	-	-	-	+	+	-	-	-	+	-	-
33	201	401	DQ4	-	+	+	-	-	-	+	-	+	+	-	-	+	+	-
34	201	402	DQ4	-	+	+	-	-	-	+	-	+	+	-	-	+	+	-
35	401	402	DQ4	-	+	+	-	-	-	+	-	+	+	-	-	+	+	-
36	101	501	DQ5	-	-	+	+	-	-	+	+	+	+	+	-	-	-	+
37	102	502	DQ5	-	-	+	+	-	-	+	+	+	+	+	-	-	-	+
38	103	601	DQ6	-	-	+	+	-	-	+	-	+	+	+	-	-	-	-
39	102	602	DQ6	-	-	+	+	-	-	+	-	+	+	+	-	-	-	-
40	301	301	DQ7	-	-	+	-	+	+	-	+	-	-	-	-	+	+	-
41	303/505	301	DQ7	-	-	+	-	+	+	-	+	-	-	-	-	+	+	-
42	601	301	DQ7	-	-	+	-	+	+	-	+	-	-	-	-	+	+	-
43	101	302	DQ8	-	-	+	-	-	+	-	+	+	+	-	+	+	+	-
44	301	302	DQ8	-	-	+	-	-	+	-	+	+	+	-	+	+	+	-
45	301	303	DQ9	-	-	+	-	-	-	-	+	+	+	+	-	+	+	-
46	201	303	DQ9	-	-	+	-	-	+	-	+	+	+	-	+	+	+	-

**B** Ab pattern #2013 DQ2,4,7,8,9 in serum 16975 before absorption



**C** Eluted Ab pattern #2013 of serum 16975 after absorbed by DQ7 cell line



**FIGURE 1.** Fifteen DQB1 patterns #2001–2015 are defined by reactions of 35 mAbs and 20 sera (Ab) with 18 HLA-DQ single antigen beads. The number of antibody samples tested with the distinct reaction pattern is shown in parenthesis.

beads: LS2A01 lot #4, One Lambda Inc., Canoga Park, CA). The DQ single antigen beads can clearly distinguish DQ antibodies independent of the DR or DP antibodies. The DR and DP specificities that are tested by DR and DP single antigen beads (included in the panel) were not present in some of the 74 patients. Some other patients had DQ and other class II antibodies. However, the DQ reactions of the DQ single antigen beads were clearly for DQ specificities, and not for the other class II specificities. Testing was performed in accordance with the manufacturer’s protocol. Briefly, 20  $\mu$ L of serum or mAb was added to 2.5  $\mu$ L of beads, which were incubated for 30 min in the dark at room temperature, and then washed with wash buffer. For the sera, 100  $\mu$ L of PE-conjugated goat anti-human IgG was added to the beads, which were again incubated for 30 min in the dark at room temperature. For the mAbs, goat anti-mouse IgM was used. The beads were washed again with wash buffer and suspended in phosphate buffered saline (PBS). Reactions were analyzed with the Luminex reader (LABScan 100, One Lambda Inc.), and then analyzed and graphed for the fluorescence values of the single antigen beads using HLA Visual software (One Lambda Inc.). The human leukocyte antigen-DQ alleles on the single antigen beads are listed in Figure 1.

**Antibody Absorption by Cell Lines and Elution of Absorbed Antibody**

Absorption or elution was performed to separate DQB1 antibody from DQA1 antibody and to show a single antibody reactive to an epitope shared by multiple antigens. Homozygous B-lymphoblast cell lines of defined DQ typing were harvested and washed two to four times with PBS, and then spun at 3000g for 1 min. For absorption, the cell pellet (consisting of about 8 million cells) was mixed with 60  $\mu$ L of serum diluted 1:5 or 1:10, and then incubated for 30 min on a shaker. After absorption, the cells were spun at 3000g for 2 min and the supernatant was transferred to a fresh tube for the single-antigen beads assay.

The cell pellet with the absorbed antibody was washed two to four times with PBS. To elute the absorbed antibody from the cells, 60  $\mu$ L of ImmunoPure IgG Elution Buffer (Pierce #21004) was added and the pellet was then incubated for 10 min at room temperature on a shaker. Next, cells were spun at 3000g for 2 min. Sixty microliters of the resulting supernatant—which contained the eluted antibody—was transferred to a fresh tube that had in it 3  $\mu$ L of neutralization buffer (1 M Tris-HCL at pH 9.5), the supernatant and buffer immediately mixed and saved for

the single antigen beads assay that would determine the eluted antibody's specificity.

### Human Leukocyte Antigen Peptide Sequences and Epitopes

Epitopes were defined by amino acids shared by the positive antigens of the antibodies. Human leukocyte antigen class II peptide sequences were obtained from the HLA informatics section of the Anthony Nolan website (8). For ease of identifying DQB1 and DQA1 epitope sites, we extracted from the website the positions of polymorphic amino acids of the DQB1 and DQA1 alleles that are present on the single antigen beads, using those positions to construct DQB1 and DQA1 amino acid tables. By checking the reaction pattern of each antibody that had been tested with DQ single antigen beads against the DQ polymorphic amino acid sequences on the tables generated from the Nolan website, we could identify the unique amino acids that might be epitopes. Furthermore, by comparing the DQ unique amino acids with class II DR and DP amino acids, we can rule out as epitope sites the amino acids that are shared among DQ, DR, and DP sequences. So the final DQ epitope sites in Table 1 are unique both to the reaction patterns of DQ beads and to DQB1 or DQA1 peptide sequences.

The NCBI website (9) provides three-dimensional (3-D) models of the DQ2 and DQ0602 molecules that can be used to see whether a particular amino acid is on the surface of a DQ molecule—and therefore accessible for antibody binding—or is buried under the surface. Table 1 lists the exposed amino acids of epitope sites in bold face.

### DQ Antibodies and Epitopes Postulated from Published Reports of Sera from Kidney Transplant Patients

Recent studies (10–18) reported that DQ antibodies were detected in the posttransplant sera of 93 patients, all reports combined. As stated in all these studies, DQ antibodies were among the HLA antibodies that were produced. Almost all DQ specificities that were detected by the recombinant DQ antigens were similar to the antigens in the single antigen panel we used to test the sera from the 74 patients in our study. Considering the DQ specificities of these sera, we postulate potential DQ epitopes for these antibodies. These studies also specified whether the kidneys functioning had experienced rejection episodes or had failed. Table 1 summarizes the postulated DQ epitopes for these 93 patients as well as for 48 patients of the 74 whose sera were analyzed with single antigen beads for our study.

## RESULTS

Figure 1(A) shows 15 distinct reactions produced by 35 DQ-specific mouse DQB1 mAbs and 20 sera tested by class II single antigen beads. These DQB1 patterns, with proposed numbering, are shown in the first row. The second row shows their serological DQ specificities, the third row indicates the number of mAbs and sera that reacted with the patterns shown below. The left-hand columns show the bead number and its associated DQA1 and DQB1 alleles. As noted earlier, each DQ antigen bead necessarily has a combination of DQA1 and DQB1 alleles. The body of the table shows the

reaction patterns of each group of antibodies. For pattern #2001, one serum and six DQB1 mAbs are positive to serological DQ2 and negative to the non-DQ2 single antigen beads. These antibodies may react with the DQB1\*02 allele on single antigen beads. Only posttransplant sera define patterns #2013 to 2015 because no equivalent mAbs are available. Patterns #2002, 2005, 2008, 2009, and 2012 are defined by DQB1 mAbs, but no equivalent sera from the 74 patients show similar patterns.

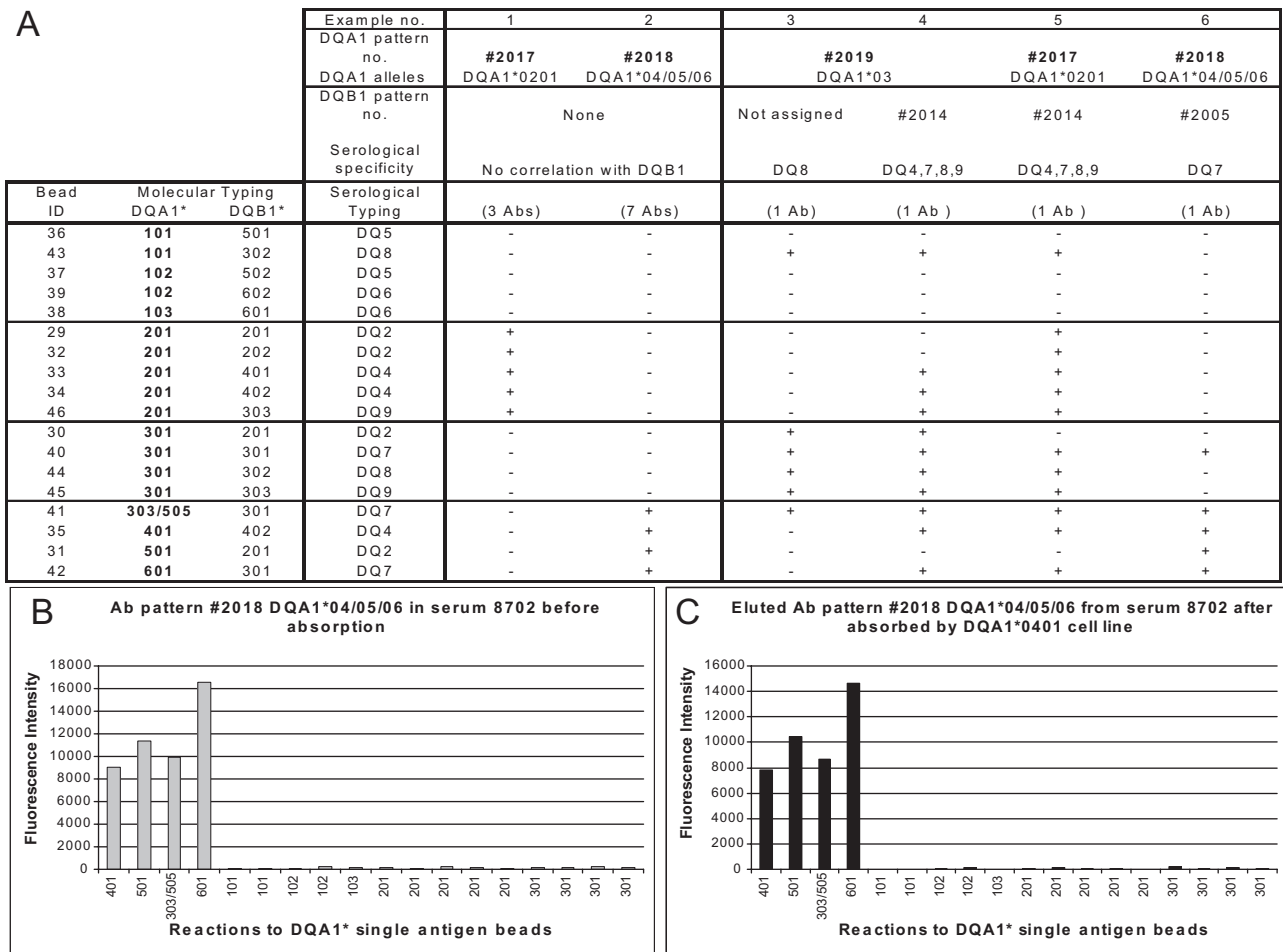
Figure 1(B) of reaction #2013 shows the reaction intensity of serum 16975, which has specificities of DQ2, 4, 7, 8, and 9. A homozygous B-lymphoblast cell line—of a typing of DQ7 as antigen for the absorption of DQ7 antibody—was used to absorb the DQ antibody from the serum, and then the eluted antibody was tested by single antigen beads. As shown in Figure 1(C), the eluted antibody reacts with DQ7 as expected—and also with DQ2, 4, 8, 9 antigen beads. The data show that serum 16975 contains a single DQ antibody reacting with an epitope that is shared by DQ2, 4, 7, 8 and 9 specificities.

Figure 2(A) shows three DQA1 reaction patterns, #2017 to 2019, identified by 14 posttransplant sera. The left-hand columns show the 18 HLA-DQ single antigen beads in ascending order of their DQA1 alleles. Example 1 shows the DQA1\*0201 pattern #2017 of three sera and their positive reaction patterns correlated with the DQA1\*0201 allele on all five single antigen beads, displaying neither false positive nor false negative reactions. Example 2 shows the DQA1\*04/05/06 pattern #2018 for seven sera, of their positive reactions to DQA1\*0505, DQA1\*0401, DQA1\*0501, and DQA1\*0601 alleles (beads 41, 35, 31, and 42).

Figure 2(B) of reaction #2018 shows the reaction intensity of serum 8702 to the antigen beads of DQA1\*04/05/06 specificities. The same absorption or elution process was used, except that the homozygous B-lymphoblast cell line used to absorb the DQ antibody had a DQA1\*0401 typing. In Figure 2(C), the eluted antibody shows a reaction to DQA1\*04—again as expected—and also to DQA1\*05 and DQA1\*06 antigen beads. These data show that serum 8702 contains a single DQA1 antibody reactive to an epitope shared by DQA1\*04, 05, and 06 specificities.

Example 3 in Figure 2(A) shows DQA1\*03 pattern #2019, which is defined by positive beads of DQA1\*0301 and DQA1\*0303 alleles. Bead 43 (with DQA1\*0101 and DQB1\*0302) is also positive. This patient's serum may contain two antibodies, one reacting to the DQA1\*03 allele, the other to the DQB1\*0302 (serological DQ8) allele. Positive reaction to the other DQB1\*0302 bead (44 is DQA1\*0301) is masked by the DQA1\*0301 allele. Example 4 shows another patient's serum that may contain two antibodies, one reactive to the DQA1\*03 allele, the other with the same reactivity as that of DQB1 pattern #2014 (shared by DQ 4, 7, 8, 9 specificities). Examples 5 and 6 also include patients' sera that may contain two antibodies, one reactive to DQA1 alleles and the other to DQB1 alleles.

As shown in Figure 3, DQB1 antibody can be separated from the DQA1 antibody in a patient's serum by absorption of the serum with a cell line of specific DQB1 antigen. Patient serum 338 (Fig. 2A, example 5) may contain DQB1 antibody of pattern #2014 DQ4, 7, 8, 9 specificities and DQA1 antibody of pattern #2017 DQA1\*0201 specificity. After being absorbed by a cell line with typing of DQA1\*0401 and



**FIGURE 2.** Three DQA1 patterns #2017–2019 are defined by reactions of 14 sera (Ab) with 18 HLA-DQ single antigen beads. The number of antibody samples tested with the distinct reaction pattern is shown in parenthesis.

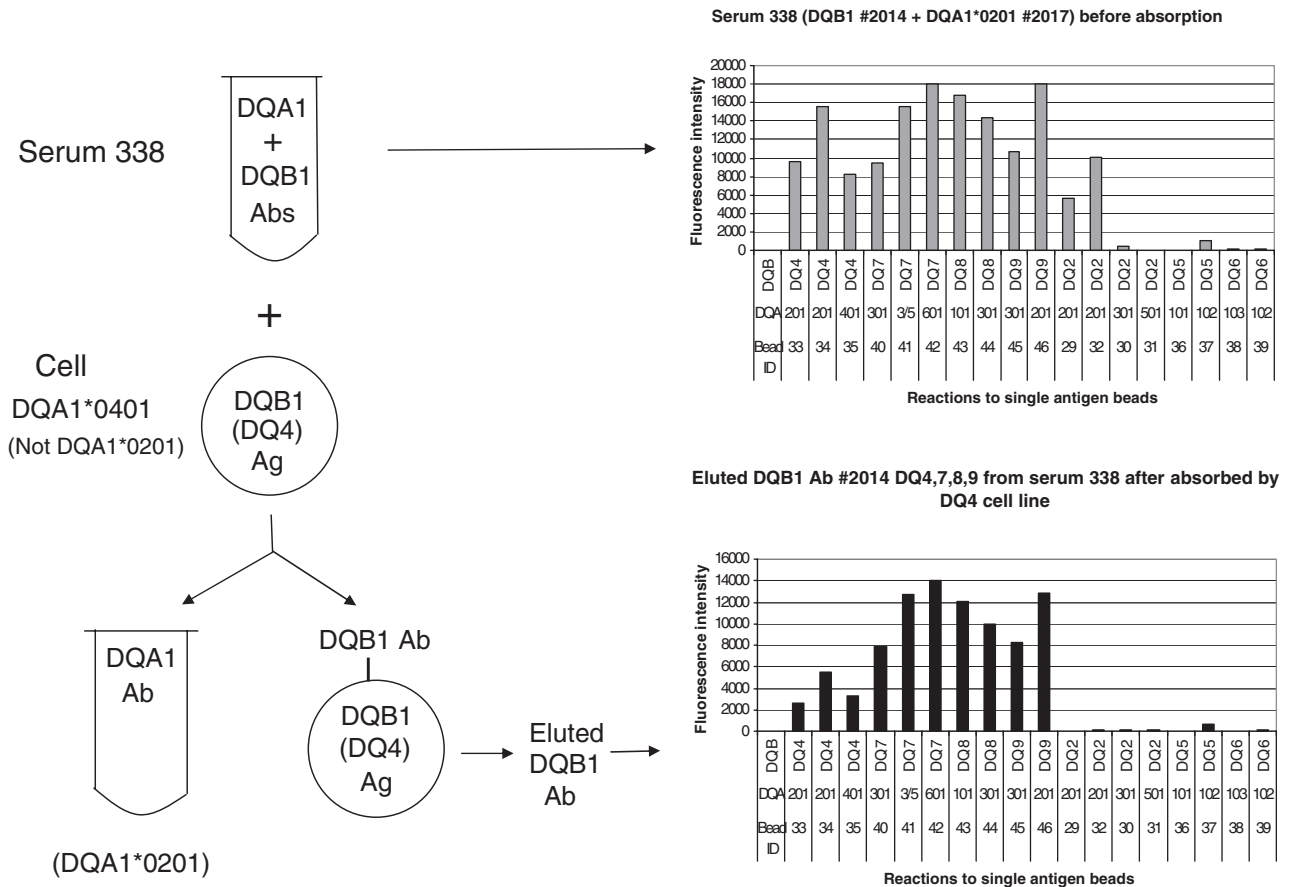
DQB1\*0402 (DQ4), the eluted antibody reacted to the DQB1 epitope #2014, shared by DQ4, 7, 8, 9, and was negative to two DQA1\*0201/DQ2 beads (29 and 32)—whereas before absorption, these two beads were positive because of the DQA1\*0201 antibody in serum 338. Since the cell line’s typing was not DQA1\*0201, it did not absorb the DQA1\*0201 antibody.

Table 1 lists the possible unique amino acid sites of 15 DQB1 patterns that we can now call epitopes: #2001 to 2015. It also lists three DQA1 patterns—now identified as epitopes—#2017 to 2019. For example, pattern #2001, which defined a DQ2 epitope, may be mapped by any one or combination of several unique amino acid positions: 28S, 30S, 37I, 52L, or 55L (shown in Table 1 separated by “/”). Amino acids 52L and 55L (in bold face) are on the surface of the DQ 3-D molecule (9), so are more likely to define this epitope. Epitope #2007—shared by specificities DQ4, 5, 6—requires a combination of amino acids 52P and 55R to form a unique epitope site (shown in Table 1 by “+”). The 3-D model shows that 52P and 55R are close to each other and therefore may define the potential conformational epitope.

Table 1 also summarizes the epitopes that correlate with the DQ antibodies in the sera of the renal transplant patients.

These patients include 48 of the 74 for this study and 93 from the other published studies (10–18). As shown in Figure 1(A), #2002, 2005, 2008, 2009, and 2012 are defined by mouse DQB1 mAbs. No equivalent sera were found in the 74 patients, but from the published studies of the 93 patients, we deduced examples of specificities equivalent to the DQ antibodies that react with these epitopes—except for epitope #2008. This is the only epitope whose possible epitope-site amino acids (9Y and 11F) are not on the surface of the DQ molecule. Except for the antibody to this epitope, examples of DQ antibodies reactive to the epitopes listed in Table 1 can be found in the posttransplant sera whether the patients’ kidneys were functioning, had experienced rejection episodes, or had failed.

Figure 4(A) includes 10 cases in which each graft recipient produced a single donor-specific DQ antibody reacted to a DQ epitope that was shared among DQ specificities in recipient’s serum, as detected by single antigen beads. For example, in case 1—with DQ6 the mismatched typing—the patient produced a donor-specific DQ antibody reacted to epitope #2003, shared by DQ4, 5, 6, 7, 8, 9 specificities. In case 2, DQ6 was also the mismatched DQ, but the patient produced a donor-specific DQ antibody that reacted to epitope #2004, shared by DQ5 and DQ6 antigens. Again, for absorp-



**FIGURE 3.** The DQB1 antibody was separated from the DQA1 antibody in serum 338 by absorption with a cell line of DQA1\*0401 and DQB1\*0402 (DQ4) typing, and then the eluted DQB1 antibody—reacting to DQB1 pattern #2014 DQ4, 7, 8, 9 antigens—was shown at the lower chart. Upper chart showed that serum 338 may contain both DQB1 and DQA1\*0201 antibodies before the absorption.

tion/elution and single antigen beads assay, the case 1 serum was absorbed by a homozygous B-lymphoblast cell line of DQ7 typing. As shown in Figure 4(B), the eluted antibody reacts to DQ7, as expected, and also to DQ4, 5, 6, 8, 9 antigen beads. And again, the data show that the case 1 serum contains a single DQ antibody that reacted to an epitope shared by DQ4, 5, 6, 7, 8, 9 specificities.

Figure 5(A) gives an example of sequential monitoring of the antibodies detected in the serum of patient R33 and gives the graft outcome (10). All the potential HLA antibodies were tested by single antigen beads for patient R33 and only the DQ specificities were detected. No other antibody of either HLA class I or class II (DR) was detected even though there were mismatched antigens that could have elicited an immune response to these antigens. Strong DQ serological specificities were present at the time when rejection was diagnosed 50 months after transplantation. As shown in Figure 5(A), both donor and patient shared the same DQB1 serological DQ2 and DQ8 typing, with DQA1 typing unknown. Although it seemed that the patient had made antibodies against multiple HLA class II DQ specificities—detected by two of four DQ2 antigen beads, two of three DQ4 and one of two DQ9 beads—the patient actually had produced a single class II antibody that reacted to a single epitope #2017 of

DQA1\*0201, with the unique amino acid positions: 47K/52H/54L on the surface of the alpha chain. Because the donor had not been typed for DQA antigen, we were not certain that this DQA1\*0201 antibody had been critical in the rejection of this graft. Figure 5(B) shows that one serum sample from the same patient, tested by single antigen beads, displays strong positive fluorescence intensity to five of five DQA1\*0201 single antigen beads (46, 34, 32, 33, 29) and negative reactions to the beads that are not DQA1\*0201 (35, 30, 31, 45, etc.). Similarly, reaction #2017 is detected in two sera from different patients (Fig. 2A, example 1). The unique epitope sites of amino acid positions: 47K/52H/54L of the DQA1\*0201 allele are also shown in the Figure 5(C) sequence table. The consensus amino acid shared by the DQA1\*010101 amino acid sequence (on the top line) is represented by “-”.

**DISCUSSION**

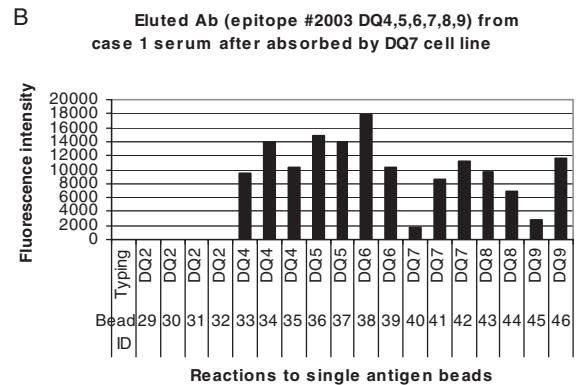
When donor-specific antibodies are found after transplantation, they can be more confidently identified as de novo, and may be harmful to the transplant (13, 14, 19–23). Worthington et al. (17) noted the high frequency of DQ antibodies in patients who had rejected a transplant. Piazza et al. (13) noted that DQ antibodies often proliferate before transplant rejection.

**TABLE 1.** Unique amino acids for 15 DQB1 and 3 DQA1 epitope sites and related DQ antibodies found in patients with various outcome of kidney grafts

Pattern or epitope no.	DQB1 (#2001–2015) or DQA1 (#2017–2019) epitopes	Position & unique amino acids for possible epitopes sites	Total # with DQ epitopes	# with functioning kidneys	# with rejections	# with failed kidneys
#2001	DQ2	28S/30S/37I/52L/55L	14	7	2	5
#2002	DQ4	<b>56L</b>	5	2	1	2
#2003	DQ4, 5, 6, 7, 8, 9	28T/ <b>46V</b> /52P	5	2	0	3
#2004	DQ5, 6	<b>84E</b> / <b>85V</b> /86A/89G/90I/221Q	22	3	2	17
#2005	DQ7	<b>45E</b>	4	4	0	0
#2006	DQ7, 8, 9	<b>55P</b>	27	15	4	8
#2007	DQ4, 5, 6	<b>52P</b> + <b>55R</b>	3	1	0	2
#2008	DQ2, 5, 7, 8, 9	9Y+11F	0 <sup>a</sup>	0	0	0
#2009	DQ2, 4, 5, 6, 8, 9	<b>34R</b> + <b>45G</b>	1	1	0	0
#2010	DQ4, 5, 6, 8, 9	<b>45G</b> + <b>46V</b>	4	2	0	2
#2011	DQ5, 0601	38V+ <b>46V</b>	2	2	0	0
#2012	DQ8, 9	<b>45G</b> + <b>55P</b>	6	3	0	3
#2013	DQ2, 4, 7, 8, 9	<b>84Q</b> / <b>85L</b> /86E/ <b>87L</b> /89T/220H/221H	11	6	2	3
#2014	DQ4, 7, 8, 9	<b>77T</b> + <b>84Q</b> / <b>77T</b> + <b>85L</b> / <b>77T</b> + 86E/ <b>77T</b> + <b>87L</b> / <b>182N</b>	10	8	1	1
#2015	DQ5	<b>70G</b> + <b>71A</b> / <b>116I</b> / <b>125S</b>	5	3	0	2
#2017	DQA1*0201	<b>47K</b> / <b>52H</b> / <b>54L</b>	5	2	1	2
#2018	DQA1*04/05/06	<b>40G</b> / <b>47C</b>	13	10	0	3
#2019	DQA1*03	26S/ <b>47Q</b> /56R/187T	4	3	0	1
		Total	141	74	13	54

Bold text indicates amino acids that are on surface of DQ molecule.  
 “/” indicates that each single amino acid could be a unique site of multiple potential sites.  
 “+” indicates 2 or 3 amino acids would be required to form the unique site.  
<sup>a</sup> Epitopes #2008 is defined by mAb.

A	DQ specificities detected in recipients by mismatched DQ type				
Case no.	Recipient DQ type	Donor DQ type	Mismatched DQ type	single antigen beads	DQB1 Epitope
1	DQ2	DQ2, DQ6	DQ6	DQ4, 5, 6, 7, 8, 9	#2003
2	DQ7, DQ8	DQ6, DQ7	DQ6	DQ5, 6	#2004
3	DQ2, DQ4	DQ2, DQ5	DQ5	DQ5, 6	#2004
4	DQ3**	DQ1*, DQ3**	DQ1*	DQ5, 6	#2004
5	DQ6	DQ6, DQ9	DQ9	DQ7, 8, 9	#2006
6	DQ7, DQ9	DQ1*	DQ1*	DQ4, 5, 6	#2007
7	DQ2, DQ7	DQ4, DQ7	DQ4	DQ4, 5, 6, 8, 9	#2010
8	DQ2, DQ7	DQ1*	DQ1*	DQ4, 5, 6, 8, 9	#2010
9	DQ5, DQ6	DQ4, DQ5	DQ4	DQ2, 4, 7, 8, 9	#2013
10	DQ2	DQ2, DQ3**	DQ3**	DQ4, 7, 8, 9	#2014



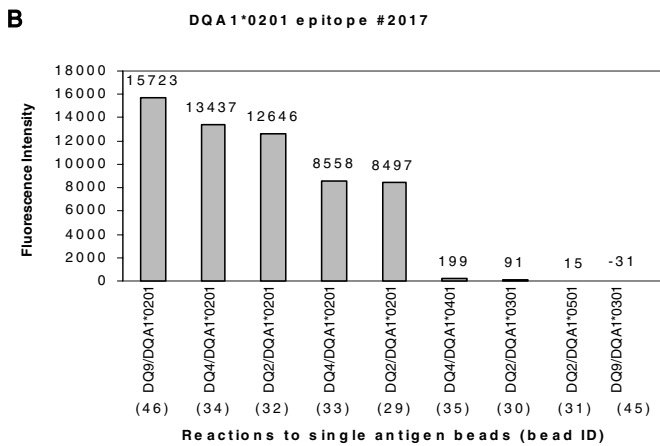
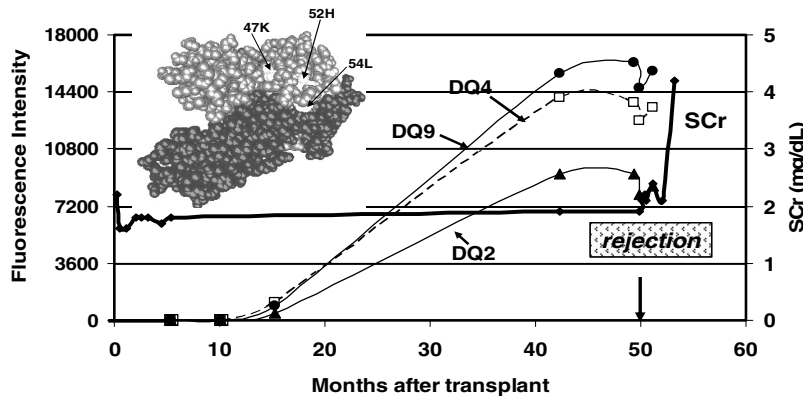
**FIGURE 4.** Donor-specific DQ antibodies against the mismatched DQ type that shared epitope among DQ specificities are detected in patients’ sera. DQ1 splits to DQ5 and DQ6. DQ3 splits to DQ7, DQ8 and DQ9.

Whether DQ antibodies are the sufficient cause of rejection or are just part of the total HLA antibodies attack on kidneys is still to be determined. Regarding the HLA class II antibodies detected in the 74 patients in this study, some produced only DQ antibodies detected by the DQ single antigen beads, whereas other patients produced DQ plus DR or DP antibodies detected by the DR single antigen or DP single antigen beads.

Development of HLA single antigen beads permitted us to identify potential epitopes that could be responsible for

HLA antibodies (1–6). Because the genes encoding the alpha and beta chains of DQ antigens are both polymorphic—unlike HLA-DR antigens, which are polymorphic in the beta chain only—it is significantly more complicated to identify DQ epitopes. In this study, 35 mouse DQB1 mAbs specific for the DQ beta chain are very useful for identifying DQB1 epitopes. The lesser number of identified DQA1 epitopes may be due to several factors, such as lack of DQA1 mouse mAbs, scarcity of donor and recipient DQA1 typing, and fewer single

**A** R33 Patient: A2,28, B18,27, DR4,17, DQ2,8 DQ Ab: DQ2, 4, 9 (all DQA1\*0201)  
 Donor: A24,32, B14,60, DR4,7, DQ2,8 **DQA1\*0201 epitope #2017**  
 Serum creatinine (SCr) mg/dL



**C**

Amino acid pos.	45	47	50	52	54
DQA1*010101	A	W	R	W	P
DQA1*010101	-	-	-	-	-
DQA1*010201	-	-	-	-	-
DQA1*010202	-	-	-	-	-
DQA1*010203	-	-	-	-	-
DQA1*010204	-	-	-	-	-
DQA1*0103	-	-	-	-	-
<b>DQA1*0201</b>	V	-	<b>K</b>	L	-
DQA1*030101	V	-	Q	L	-
DQA1*0303	V	-	Q	L	-
DQA1*040101	V	-	C	L	-
DQA1*040102	V	-	C	L	-
DQA1*0404	V	-	C	L	-
DQA1*050101	V	-	C	L	-
DQA1*050102	V	-	C	L	-
DQA1*0505	V	-	C	L	-
DQA1*060101	V	-	C	L	-
DQA1*060102	V	-	C	L	-

**FIGURE 5.** Patient R33 shared DQ beta DQ2, DQ8 typing with the donor. DQ alpha typing was unknown. DQ antibody against DQA1\*0201 epitope #2017 was present at the time that rejection was diagnosed 50 months after transplantation. In one serum sample of the sequential sera collected, reactions were detected by all five DQA1\*0201 antigen beads (46, 34, 32, 33, 29). The DQA1\*0201 unique amino acid positions 47K/52H/54L are shown to be epitope sites and are on the surface of the alpha chain of the 3-D DQ model.

antigen beads that target DQA1 alleles. Currently, we cannot identify potential interchain epitopes that are defined by the combined unique amino acids of both DQA1 and DQB1 alleles.

The concept that HLA antigens share epitopes was first proposed in the early 1980s when investigators found that a significant number of individual mAbs would react to multiple different serological HLA antigens (24–26). Furthermore, Viken et al. (27) identified one DQB1 epitope using three HLA class II mAbs that were tested by a panel of transfected cell lines. All three mAbs showed the same reaction pattern: positive to DQ4, 5, 6, 8, 9 but negative to DQ2 and DQ7 antigens. The epitope was identified as being on a DQ beta chain, not on a DQ alpha chain. In this study, we identify the same DQB1 epitope (#2010) with one mAb and three sera (Fig. 1A). We found that DQB1 mouse mAb was useful in identifying a single epitope shared by all DQB1 antigens positive with the antibody. However, not all DQB1 epitopes that are defined by mAbs may be clinically relevant in human patients. Interestingly, some of the DQ epitopes defined by mAbs could also be defined by antibodies in sera of the 74

patients of this study and the 93 patients of the referred studies (10–18). One exception was the mAb defined DQB1 epitope #2008 (Fig. 1A). This epitope seems to be a nonclinically relevant epitope because no equivalent antibody was detected in the patients’ sera. Furthermore, the amino acids defining this epitope were buried inside the DQ molecule and therefore are unlikely to elicit the specific antibody in patients.

Since no DQA1 mAbs were available, we relied on absorptions and elutions to show that certain serum contained a single DQA1 antibody reactive to an epitope shared by multiple antigens. For example, serum 8702 contained the antibody reactive to DQA1\*04/05/06 antigens. This antibody could be absorbed by antigenic relevant cell line 9021 (DQA1\*04) or 9064 (DQA1\*0503, DQB1\*0301), and then the eluted antibody was shown to react to DQA1\*04, 05, 06 antigen beads. DQA1 antibody in serum 8702 could not be absorbed by the antigenic irrelevant cell line 9066 (DQA1\*0103, DQB1\*0601) that does not have either of the DQA1\*04, 05, 06 antigens. However, cell line 9066 could absorb the DQB1 antibody (serum 1119 in Fig. 4A case 2) reac-

tive to the epitope shared by DQ5 and DQ6 antigens. On the other hand, this DQB1 antibody could not be absorbed by cell line 9064 of DQB1\*0301 (Data not shown). Thus, the DQ antibodies recovered after the absorption then elution were truly specific to the DQ antigens on the cell lines.

Cumulative knowledge of immunogenic HLA DQ epitopes should be useful in analyzing sera from patients with high panel reactive antibodies. Such analysis may permit more accurate prospective crossmatching of DQ epitopes for transplantation. Virtual crossmatching may be of importance particularly with kidneys for highly sensitized patients. It is of critical importance for heart and lung transplants, with whom there is often insufficient time to perform the crossmatch and for whom the cost of a failed transplant is so high.

### ACKNOWLEDGMENTS

The authors thank Mamie Lias and Mike Chen for providing them the mAbs and cell lines; Fay Jahr, Tho Pham and Adam Idica for their excellent assistance.

### REFERENCES

1. El-Awar N, Lee JH, Tarsitani C, et al. HLA class I epitopes: Recognition of binding sites by mAbs or eluted alloantibody confirmed with single recombinant antigens. *Hum Immunol* 2007; 68: 170.
2. El-Awar N, Akaza T, Terasaki PI, et al. Human Leukocyte Antigen class I epitopes: Update to 103 total epitopes, including the C locus. *Transplantation* 2007; 84: 532.
3. El-Awar N, Cook D, Terasaki PI. HLA class I epitopes: A and B Loci. In: Terasaki PI, ed. *Clinical transplantation 2006*. Los Angeles, CA, Terasaki Foundation Laboratory 2006, pp 79.
4. Akaza T, El-Awar N, Hguyen A, et al. HLA class I epitopes: C-Locus. In: Terasaki PI, ed. *Clinical transplantation 2006*. Los Angeles, CA, Terasaki Foundation Laboratory 2006, pp 95.
5. 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.
6. Cai J, Kohanof S, Terasaki PI. HLA-DR antibody epitopes. In: Terasaki PI, ed. *Clinical transplantation 2006*. Los Angeles, CA, Terasaki Foundation Laboratory 2006, pp 103.
7. Lee J-H, Lias M, Deng C-T, et al. A one-step monoclonal antibody typing procedure that simplifies HLA class I and class II typing. *Tissue Antigens* 1994; 44: 34.
8. Anthony Nolan Research Institute: IMGT/HLA Sequence Database. Available at: <http://www.anthonynolan.org.uk/HIG/data.html>.
9. NCBI Entrez structure website. Available at: <http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml>.
10. Ozawa M, Rebellato LM, Terasaki PI, et al. Longitudinal testing of 266 renal allograft patients for HLA and MICA antibodies: Greenville experience. In: Terasaki PI, ed. *Clinical transplantation 2006*. Los Angeles, CA, Terasaki Foundation Laboratory 2006, pp 265.
11. Kinukawa T, Kato M, Terasaki PI, et al. Retrospective antibody analysis of thirty patients with kidney graft failure. In: Terasaki PI, ed. *Clinical transplantation 2006*. Los Angeles, CA, Terasaki Foundation Laboratory 2006, pp 291.
12. Van den Berg-Loonen EM, Terasaki PI, Kohanof S, et al. Longitudinal testing of seventy-six renal allograft patients for HLA antibodies: Maastricht experience. In: Terasaki PI, ed. *Clinical transplantation 2006*. Los Angeles, CA, Terasaki Foundation Laboratory 2006, pp 305.
13. Piazza A, Poggi E, Ozzella G, et al. Post-transplant donor-specific antibody production and graft outcome in kidney transplantation: Results of sixteen-year monitoring by flow cytometry. In: Terasaki PI, ed. *Clinical transplantation 2006*. Los Angeles, CA, Terasaki Foundation Laboratory 2006, pp 323.
14. Lachmann N, Terasaki PI, Schönemann C. Donor-specific HLA antibodies in chronic renal allograft rejection: A prospective trial with a four-year follow-up. In: Terasaki PI, ed. *Clinical transplantation 2006*. Los Angeles, CA, Terasaki Foundation Laboratory 2006, pp 171.
15. Rebellato LM, Ozawa M, Verbanac KM, et al. Clinical and anti-HLA antibody profile of nine renal transplant recipients with failed grafts: Donor-specific and nondonor-specific antibody development. In: Terasaki PI, ed. *Clinical transplantation 2006*. Los Angeles, CA, Terasaki Foundation Laboratory 2006, pp 241.
16. Mizutani K, Shibata L, Ozawa M, et al. Detection of HLA and MICA antibodies before kidney graft failure. In: Terasaki PI, ed. *Clinical transplantation 2006*. Los Angeles, CA, Terasaki Foundation Laboratory 2006, pp 255.
17. Worthington JE, Martin S, Barker AJ, et al. The role of HLA-specific antibodies in kidney transplant rejection: Published studies and local data. In: Terasaki PI, ed. *Clinical transplantation 2006*. Los Angeles, CA, Terasaki Foundation Laboratory 2006, pp 349.
18. Wade E, Goral S, Kearns J, et al. Experience with antibody-mediated rejection in kidney allograft recipients. In: Terasaki PI, ed. *Clinical transplantation 2006*. Los Angeles, CA, Terasaki Foundation Laboratory 2006, pp 439.
19. Hourmant M, Cesbron-Gautier A, Terasaki PI, et al. Frequency and clinical implications of development of donor-specific and nondonor-specific HLA antibodies after kidney transplantation. *J Am Soc Nephrol* 2005; 16: 2804.
20. Mizutani K, Terasaki PI, Rosen A, et al. Serial ten-year follow-up of HLA and MICA antibody production prior to kidney graft failure. *Am J Transplant* 2005; 5: 2265.
21. Terasaki PI, Ozawa M. Predicting kidney graft failure by HLA antibodies: A prospective trial. *Am J Transplant* 2004; 4: 438.
22. Worthington JE, Martin S, Al-Husseini DM, et al. Posttransplantation production of donor HLA-specific antibodies as a predictor of renal transplant outcome. *Transplantation* 2003; 75: 1034.
23. Piazza A, Poggi E, Ozzella G, et al. Public epitope specificity of HLA class I antibodies induced by a failed kidney transplant: Alloantibody characterization by flow cytometric techniques. *Transplantation* 2006; 81: 1298.
24. Igarashi M, Gebhard DH, Chen YX, et al. Identification with the monoclonal antibody 26.163 of a determinant shared by the beta chains of the gene products of the HLA-DR, DQ, and DP loci. *Hum Immunol* 1986; 16: 347.
25. McMichael AJ, Parham P, Rust N, et al. A monoclonal antibody that recognizes an antigenic determinant shared by HLA A2 and B17. *Hum Immunol* 1980; 1: 121.
26. Parham P, McLean J. Characterization, evolution and molecular basis of a polymorphic antigenic determinant shared by HLA-A and B products. *Hum Immunol* 1980; 1: 131.
27. Viken HD, Thorsby E, Gaudernack G. Characterization and epitope mapping of four HLA class II reactive mouse mAbs using transfected L cells and human cells transfected with mutants of DQB1\*0302. *Tissue Antigens* 1995; 45: 250.