Utility of HLAMatchmaker and Single-Antigen HLA-Antibody Detection Beads for Identification of Acceptable Mismatches in Highly Sensitized Patients Awaiting Kidney Transplantation

Reyna S. Goodman,[1] Craig J. Taylor,[1,4] Cheryl M. O'Rourke,[1] Andrew Lynch,[2] J. Andrew Bradley,[3] and Tim Key[1]

Background. In highly sensitized patients (HSP) awaiting renal transplantation, accurate delineation of acceptable human leukocyte antigen (HLA) mismatches (AMM) aids identification of suitable crossmatch negative donors. Comparison of differences in polymorphic triplet amino acid sequences in antibody accessible regions of HLA may predict immunogenicity. We have examined the ability of the HLAMatchmaker computer algorithm to predict AMM determined by antibody screening using the full repertoire of single-antigen HLA-A and -B specificities.

Methods. The HLA types of 24 HSP awaiting kidney transplantation were analyzed using HLAMatchmaker to determine the number of triplet amino acid (TAA) mismatches for each of 64 mismatched HLA-A and -B specificities. Patient sera with the highest immunoglobulin (Ig)G HLA-specific antibody reactivity were tested against the 64 individual HLA-A and -B specificities using single-antigen HLA antibody detection beads. Logistic regression analysis was performed to determine the association between AMM and the number of TAA mismatches.

Results. There was a strong positive association between the number of TAA mismatches and the presence of HLA-specific antibody. HLA specificities with zero TAA mismatches were antibody positive in only 4 of 47 (9%) cases. A single TAA mismatches was sufficient to invoke an antibody response in 40 (41%) of 98 cases, increasing to 97 (87%) of 112 cases with 9 or more TAA mismatches. However, there was considerable heterogeneity between individual patients, and only 16 (67%) of the 24 HSP studied fitted the logistic regression model for TAA mismatches and HLA-specific antibody.

Conclusions. Identification of TAA mismatches using HLAMatchmaker is a helpful tool for predicting potential donors with an acceptable HLA mismatch in HSP.

(*Transplantation* 2006;81: 1331–1336)

Up to one third of patients on the waiting list for a primary cadaveric donor renal transplant are sensitized to human leukocyte antigens (HLA) through pregnancy and/or transfusion of blood products. The sensitization rate increases to approximately 80% in patients listed for a second or subsequent renal transplant (*1*). In all sensitized patients, it is essential to identify a crossmatch negative donor to avoid the risk of hyperacute or accelerated renal allograft rejection (*2*, *3*), and finding a suitable donor is most difficult for those patients with high levels of HLA-specific sensitization. Between 5% and 10% of all patients registered for renal transplantation are classified as highly sensitized (defined by Eurotransplant as having immunoglobulin [Ig]G HLA-specific antibodies that react with ≥85% of potential donors), and in this patient subgroup, it is particularly difficult to find a suitable crossmatch negative donor (*1*).

The likelihood of a highly sensitized patient (HSP) receiving a transplant can be greatly increased by the identification of acceptable HLA mismatches (AMM) to which the recipient has no detectable circulating alloantibody (*4*, *5*). Whereas it is possible to identify AMM using conventional antibody screening techniques that use lymphocyte or purified antigen panels, there are major logistic and practical problems with this approach because the assay targets express multiple HLA specificities. There is, therefore, a need for better ways to identify AMM, and two recent advances, namely single-antigen HLA class I antibody detection systems and HLAMatchmaker, may fulfill this role.

Commercially available single-antigen HLA class I antibody detection systems comprise the full repertoire of serologically defined HLA-A and -B specificities bound to a solid phase that enables accurate detection and characterization of HLA-specific antibodies in patient sera without the ambiguities encountered using conventional techniques, where several HLA specificities are present (*6*). HLAMatchmaker is a computer algorithm that determines HLA compatibility at a structural level by comparing differences between polymorphic triplet amino acid sequences in the antibody accessible regions of the HLA molecule (*7*). The algorithm performs intra- and inter-locus comparisons between the patient's HLA type and each mismatched HLA specificity and calculates the number of triplet amino acid (TAA) sequence mismatches. This information can then be used to identify HLA

[1] Tissue Typing Laboratory, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom.
[2] Centre for Applied Medical Statistics, Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge, Cambridge, United Kingdom.
[3] Department of Surgery, School of Clinical Medicine, University of Cambridge, Cambridge, United Kingdom.
[4] Address correspondence to: Craig J. Taylor, Ph.D., FRCPath, Tissue Typing Laboratory (Box 209), Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom.
E-mail: craig.taylor@addenbrookes.nhs.uk
Received 17 October 2005.
Accepted 6 January 2006.

ISSN 0041-1337/06/8109-1331
DOI: 10.1097/01.tp.0000205202.56915.f5

mismatches that are likely to be acceptable for a given recipient (*8*). HLAMatchmaker has been applied retrospectively to renal transplant registry data with varying results. Analysis of the United Network for Organ Sharing and Eurotransplant registry data showed a good correlation between the numbers of TAA mismatches and graft survival (*9*), whereas analysis of the Collaborative Transplant Study registry data showed no such correlation (*10*).

The ability of HLAMatchmaker to predict AMM (as defined by antibody screening using the full repertoire of single-antigen HLA class I specificities) has not been previously assessed. We report an analysis undertaken in HSP awaiting renal transplantation in which we compare the number of TAA mismatches identified by HLAMatchmaker for each HLA specificity with acceptable (antibody negative) and unacceptable (antibody positive) mismatches identified using single-antigen HLA class I antibody detection beads.

METHODS

Patient Samples Selected for Study

All patients awaiting renal transplantation at Addenbrooke's Hospital, Cambridge, United Kingdom, had routine blood samples taken at two monthly intervals to screen for the presence of HLA-specific antibodies. IgM and IgG HLA class I- and class II-specific antibodies were detected by a complement-dependent cytotoxicity assay using an HLA-typed lymphocyte panel. In addition, IgG HLA class I- and class II-specific antibodies were identified using GTI Quickscreen and GTI B-screen, and antibody specificity was determined by GTI Quick ID enzyme-linked immunosorbent assay (GTI 20925; Crossroads Circle, Waukesha, WI). Of 406 patients on the transplant waiting list in August 2004, 24 were identified as being highly sensitized, defined as having IgG antibodies against ≥85% of the HLA class I antigen panel. Of the 24 HSP, 14 were female and 10 were male, with a median age of 43 years (range, 27–60 years), and the highest level of panel reactive antibodies (PRA) for individual patients ranged from 89% to 100% (Table 1).

Antibody Screening Using Single-Antigen Human Leukocyte Antigen Class I Antibody Detection Beads

From each of the 24 HSP, the serum sample with the highest PRA was selected and screened for IgG HLA-specific antibodies using single-antigen HLA class I antibody detection beads (LABScreen, One Lambda, Inc., Canoga Park, CA). Data acquisition was carried out using a LABScan 100 Luminex platform. Antibody binding was assessed using the median channel fluorescent signal for each HLA-coated bead after correction for nonspecific binding to negative control beads. All data were normalized to the results obtained for the negative control serum using Luminex software. The normalized background ratio was used to assign the strength of HLA-specific antibody binding. The cut-off between negative, equivocal, weak positive, and strong positive results were defined as 0% to 15%, 16% to 30%, 31% to 70%, and >70% between the minimum and maximum bead ratios. In all cases, antibodies binding to single-antigen beads coated with "self" antigens present on the patient's own HLA phenotype were negative (≤15%), and therefore, for the purposes of this study, equivocal results (16%–30% above the negative control serum) were considered positive. One sample (patient 1)

TABLE 1. Patient demographics for study cohort of 24 highly sensitized patients awaiting renal transplantation

Patient No.	Gender	Age	Number of pregnancies	Number of blood transfusions	Number of previous renal transplants	Highest IgG HLA class I %PRA
1	F	39	2	6	0	92
2	M	53	N/A	5	0	90
3	F	42	0	>10	1	92
4	M	31	N/A	>10	2	93
5	F	33	1	49	1	90
6	F	33	0	>10	2	100
7	M	40	N/A	>15	1	100
8	M	44	N/A	>20	1	100
9	F	35	3	5	0	93
10	M	49	N/A	>20	0	95
11	F	39	2	>20	1	98
12	F	48	2	5	0	100
13	F	48	0	>10	2	95
14	M	27	N/A	8	3	92
15	F	55	5	>40	0	100
16	F	44	4	2	0	93
17	F	32	0	>20	1	100
18	M	54	N/A	9	1	89
19	M	49	N/A	0	1	91
20	F	56	3	>10	1	98
21	M	34	N/A	6	1	95
22	M	60	N/A	>10	1	98
23	F	43	2	>10	2	97
24	F	46	0	>70	1	100

Ig, immunoglobulin; HLA, human leukocyte antigen; PRA, panel reactive anitbodies; N/A, not applicable.

was noted to have high background antibody binding against the negative control bead population and was treated with Adsorb Out beads (non–HLA-coated micro-beads; One Lambda, Inc) to remove nonspecific antibody binding before further analysis.

Determination of Triplet Amino Acid Mismatches Using HLAMatchmaker

For each of the 24 HSP, the HLA-A, -B, and -C types, defined at the serological equivalent split specificity level, were entered into the HLAMatchmaker program. The HLAMatchmaker algorithm defines the TAA sequences of 79 HLA class I specificities, of which 64 (21 HLA-A and 43 HLA-B) were also represented in the LABScreen antibody screening assay (Table 2). The HLAMatchmaker program was used to determine the number of TAA differences for each of the 64 HLA-A and -B specificities for each HSP.

Data Analysis

The absence or presence of antibody to each mismatched HLA-A and -B specificity was determined using LABScreen, and the number of TAA mismatches was determined by HLAMatchmaker. Logistic regression analysis, weighted for the number of observations in each TAA group, was then used to determine the correlation between the number of TAA mismatches and HLA-A and -B specific antibody for each mismatched antigen using the R 1.9 statistical package (*11*). Logistic regression plots were generated for the combined study cohort and for each individual patient. *P* values <0.05 were considered significant.

RESULTS

Antibody Screening Using Single-Antigen Human Leukocyte Antigen Class I Antibody Detection Beads

Sera from the 24 HSP were screened for IgG antibodies against the 65 individual HLA-A (n=21) and -B (n=44) specificities represented in the LABScreen single-antigen HLA class I antibody detection beads. HLA-B4005 is represented in the LABScreen kit but not in the HLAMatchmaker algorithm and for the purposes of this study was excluded from the data analysis. Of the 64 HLA specificities analyzed, the number of mismatched HLA-A and -B specificities identified for each patient ranged from 60 to 62 depending on whether patients were homozygous at HLA-A and/or HLA-B.

There were a total of 1,451 mismatched combinations, of which antibody was detected in 972 (67%) (Table 3). Patient sera were antibody negative against a mean of 19 (range, 1–41) of the 60 to 62 possible mismatched HLA specificities, which, in many cases, included antigens that are common in the potential United Kingdom donor population. Although all HSP had, by definition, high (≥85%) IgG PRA, there were considerable differences in the number of HLA specificities to which they had IgG antibodies. For example, patient 16 had an IgG PRA of 93% (Table 1) but was only positive with 20 of the 61 (33%) mismatched HLA specificities, whereas patient 15 (with 100% IgG PRA) was positive with 61 of the 62 (98%) mismatched HLA specificities. Ten or more AMM were identified in 19 patients, and the remaining 5 patients (patients 6, 7, 15, 17, and 24 all having 100% PRA) had between 1 and 9 AMM. As may be expected, AMM were frequently found to include HLA specificities that are serologically cross-reactive with the patient's own HLA type (data not shown). In addition, there was considerable overlap between the antibody specificities identified using single-antigen HLA antibody detection beads and those previously defined using conventional antibody screening techniques (cytotoxicity against a lymphocyte panel and GTI Quick ID enzyme-linked immunosorbent assay), but the lack of ambiguity and extended repertoire of single-antigen screening provided a more complete assessment of antibody specificities.

TABLE 2. List of HLA-A and -B antibody specificities detected by LABScreen single-antigen HLA class I antibody detection beads and represented in the HLAMatchmaker algorithm

HLA-A	1, 2, 3, 11, 23, 24, 25, 26, 29, 30, 31, 32, 33, 34, 36, 43, 66, 68, 69, 74, 80.
HLA-B	7, 8, 13, 18, 27, 35, 37, 38, 39, 41, 42, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 67, 71, 72, 73, 75, 76, 77, 78, 81, 82.

HLA, human leukocyte antigens.

TABLE 3. Relationship between antibody binding to single HLA specificities and the number of triplet amino acid mismatches. Sera from 24 highly sensitized patients were screened to detect antibody binding to single HLA-A and –B specificities (n=64, LABScreen) and triplet amino acid mismatches were determined by the HLAMatchmaker program

Number of triplet amino acid mismatches	Number of mismatched HLA specificities	Number antibody positive (%)
0	46	4 (9)
1	98	40 (41)
2	145	61 (42)
3	205	121 (59)
4	214	136 (64)
5	197	155 (79)
6	178	141 (79)
7	141	116 (82)
8	115	101 (88)
9	58	53 (91)
10	30	27 (90)
11	15	12 (80)
12	7	5 (71)
13	2	0 (0)
Total	1,451	972 (67)

Determination of Triplet Amino Acid Mismatches Using HLAMatchmaker

For each of the 24 HSP, HLAMatchmaker was used to compare the HLA-A, -B, and -C type with each of the 64 mismatched HLA-A and -B specificities represented in the single-antigen antibody detection system, and the number of TAA mismatches was determined. The number of TAA mismatches identified for each mismatched HLA specificity ranged from 0 to 13 (mode, four TAA mismatches). There

was a strong positive association between the presence of antibody to mismatched HLA specificities and the number of TAA mismatches (Table 3). Of the 46 HLA specificities with zero TAA mismatches, only four (9%) were antibody positive, and in all four cases, the antibody binding was equivocal (ratio, 16%–30%). In contrast, there were 112 HLA specificities with 9 or more TAA mismatches, of which 97 (87%) were antibody positive ($P<0.001$).

The relationship between the presence of antibody to each mismatched HLA specificity and the number of TAA mismatches was analyzed using a logistic regression model for grouped data. The HLA-specific antibody screening data obtained using single-antigen beads was combined for all patients into marginal totals and compared with the number of TAA mismatches and weighted by the number of observations in each group (Fig. 1).

There was a positive association between the presence of HLA-specific antibody and the number of TAA mismatches, but the overall model fit was poor, indicating that it is not appropriate to assume a common logistic regression model for each HSP.

To determine the extent to which HLAMatchmaker was able to predict the presence of HLA-specific antibody for individual patients, we therefore performed logistic regression analysis for each HSP (Fig. 2). This revealed considerable heterogeneity in the antibody response according to TAA mismatch category. Of the 24 HSP studied, there was a statistically significant positive association between the presence of HLA-specific antibody and the number of TAA mismatches in 16 patients. Of the other eight patients (patient numbers 1, 5, 7, 15, 17, 18, 19, and 24), seven showed a positive association, but this was not statistically significant. Only 1 of the 24 HSP showed a negative association, providing strong evidence of an overall association between the number of TAA mismatches and the presence of antibody ($P<0.0001$ using the sign test). Analysis of patient HLA type did not reveal any differences in either the occurrence of rare HLA specificities or HLA class I homozygosity that are likely to explain this heterogeneity.

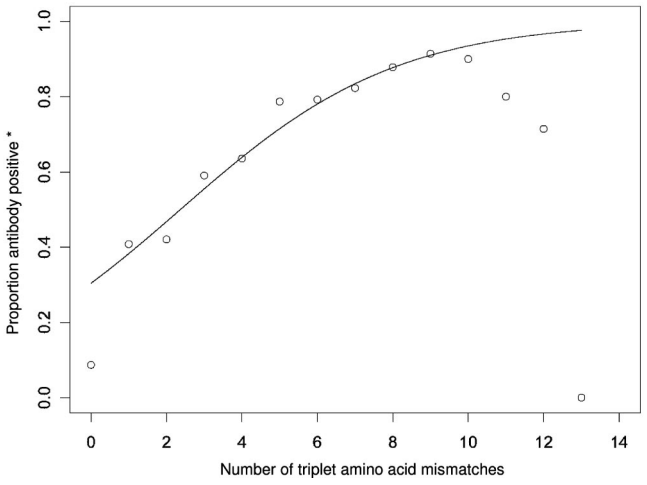

FIGURE 1. Logistic regression analysis of antibody binding to single human leukocyte antigen (HLA) specificities and the number of triplet amino acid (TAA) mismatches. Sera from 24 highly sensitized patients (HSP) were screened to detect antibody binding to single HLA-A and –B specificities (n=64; LABScreen) and TAA determined by the HLAMatchmaker program. The regression analysis was weighted for the number of observations in each TAA group. *Proportion antibody positive=proportion of positive reactions detected using LABScreen single-antigen HLA class I antibody detection beads.

DISCUSSION

Serologically defined HLA differences can be further differentiated at the TAA sequence level using the HLAMatchmaker program to identify those mismatches that, for a given donor-recipient combination, are clinically relevant in terms of renal allograft survival and the development of alloantibody responses. The relationship between the number of TAA mismatches and the presence of alloantibodies to the relevant HLA class I specificity has been validated previously in sensitized patients in whom the alloantibody response was characterized by conventional antibody screening techniques using lymphocyte or purified HLA antigen panels (*8*). However, such techniques do not enable comprehensive analysis of patient sera with broadly reactive HLA-specific antibodies because each target carries up to six different HLA class I molecules, giving complex antibody reaction patterns caused by serological cross-reactivity between HLA specificities. The use, in the present study, of single-antigen based antibody screening covering the full repertoire of HLA-A and -B specificities enabled accurate analysis of antibody specificity in a group of HSP and gave an unambiguous definition for acceptable HLA mismatches. The results of this study demonstrate that even for HSP (average IgG PRA, 96%), AMM defined on the basis of TAA mismatches correlate strongly with the likelihood of a humoral response to a particular HLA class I mismatch.

Our observations are consistent with previous studies showing that patients with high levels of panel reactive antibodies do not produce antibodies to shared triplets on mismatched HLA antigens and that HLA specificities with less than three non-self TAA-defined epitopes have relatively low immunogenicity for a given patient (*8*). We found that 87% of mismatched HLA specificities with nine or more TAA mismatches were antibody positive compared with only 9% with zero TAA mismatches (all of which had only weak reactivity), which is in close agreement with Dankers et al. (*12*) who showed that 94% of patients receiving a renal transplant with 11 to 12 TAA donor mismatches formed antibodies, whereas no patients with zero TAA mismatches formed antibodies. However, in four cases, we found antibodies against HLA specificities with zero TAA mismatches, albeit with only equivocal reactivity. This might be caused by the expression of self TAA sequences in a different tertiary conformation on the alloantigen or may reflect allele-specific antibodies, the existence of which has not been confirmed.

Despite the overall positive correlation between the number of TAA mismatches of a particular HLA class I specificity and the presence of HLA-specific antibody, our study, which is the first to report such data at the level of individual patients, revealed an unexpected degree of heterogeneity between patients. It is clear from our analysis that the HLAMatchmaker algorithm, at least in its present form, cannot

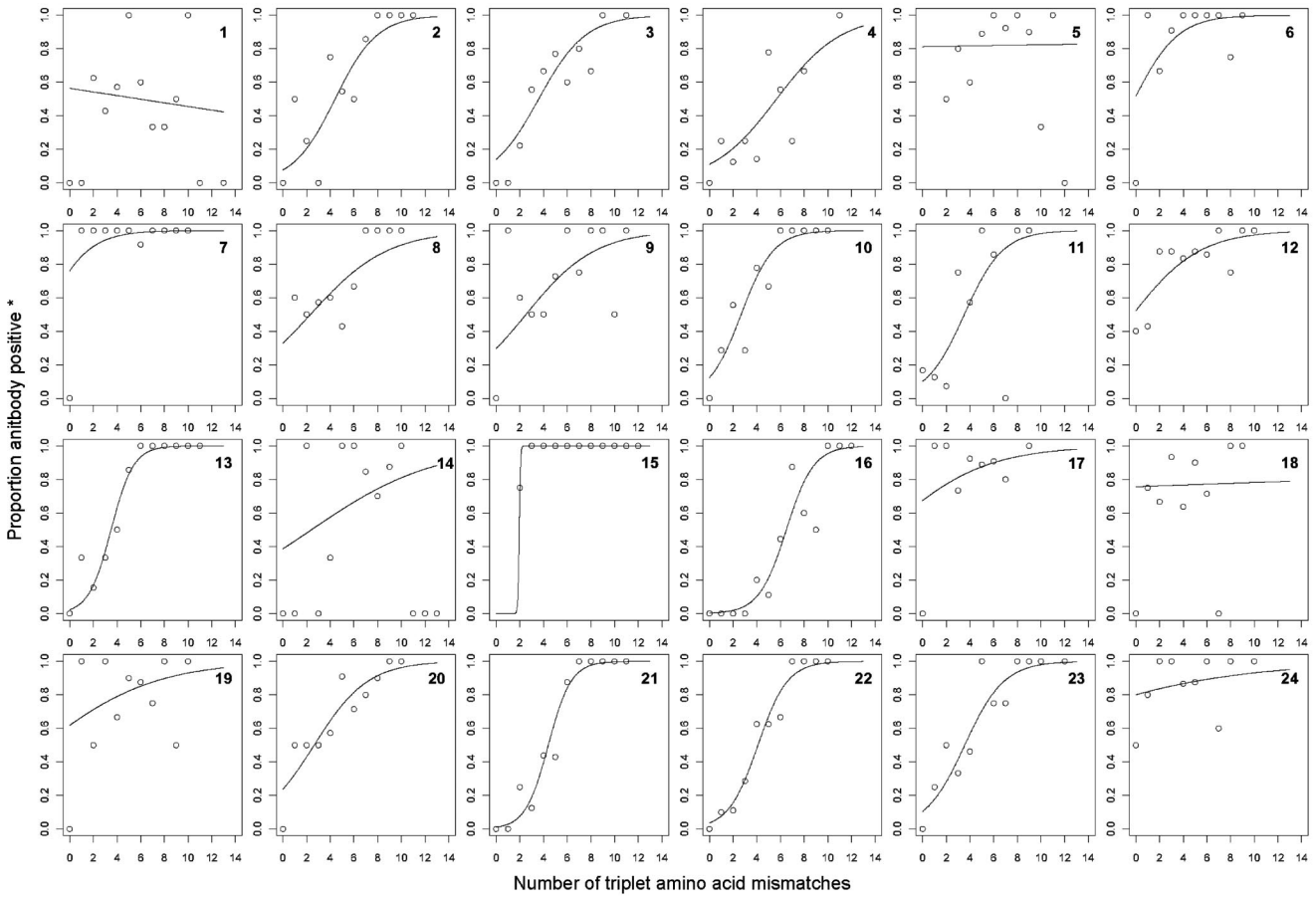

FIGURE 2. Individual patient logistic regression analysis of antibody binding to single human leukocyte antigen (HLA) specificities and the number of triplet amino acid (TAA) mismatches for each of the 24 highly sensitized patients (HSP). Sera were screened to detect antibody binding to single HLA-A and –B specificities, and TAA mismatches were determined by the HLAMatchmaker program. The regression analysis was weighted for the number of observations in each TAA group. *Proportion antibody positive=proportion of positive reactions detected using LABScreen single-antigen HLA class I antibody detection beads.

reliably predict relative HLA immunogenicity in all individuals. More than one third of the HSP in our study had high levels of antibodies to mismatched HLA specificities that contained only one or two TAA mismatches.

Individuals with rare HLA types or homozygosity at HLA class I might be expected to be more easily sensitized through exposure to common HLA class I alloantigens and, therefore, to conform less strictly to the HLAMatchmaker hypothesis. However, the presence of rare or homozygous HLA class I haplotypes did not account for the high antibody reactivity to HLA specificities with single TAA mismatches in our study. Interestingly, Duquesnoy et al. (*13*) have demonstrated that HLA class I specific antibody binding to triplet defined epitopes requires a primary contact site for the specificity-determining complementarity-determining region of the antibody and the presence of a self-sequence elsewhere in the HLA molecule that forms a critical secondary contact site for antibody binding. It seems likely, therefore, that individuals with alloantibodies to an HLA class I specificity that contained only one TAA mismatch also contain within the relevant HLA class I molecule a self-class I epitope that is required for antibody binding. Most patients with high PRA have antibodies whose specificity is restricted to a small number (typically three to five) of HLA epitopes shared by several HLA specificities. This raises the possibility that some patients with antibodies against a single triplet defined epitope present on several different HLA specificities may display broad alloreactivity and not conform to the HLAMatchmaker hypothesis. However, we were not able to determine if the TAA specificity of our HSP sera determined whether patients conformed to the HLAMatchmaker hypothesis. The requirement for both an allo-determinant and self-determinant for antibody binding makes determination of TAA specificity in an individual patient extremely complex and beyond the scope of this study. Interestingly, one could also speculate that the essential requirement for a primary and secondary (self) contact site for effective alloantibody binding might also help explain the apparent decrease in antibody binding to highly disparate antigens that express 10 or more TAA mismatches.

The ability to predict donor HLA types that will have a negative crossmatch has the potential to markedly improve access of HSP to renal transplantation. Eurotransplant have used large cell panels selected to have only a single HLA mismatch with each patient to identify AMM, and this has facil-

itated successful transplantation of HSP, but the large resources required to implement this strategy have limited its application to only a few centers worldwide (*5*). The availability of single-antigen HLA class I antibody detection beads now enables comprehensive identification of AMM and the incorporation of AMM in organ allocation policies. Based on observations in sensitized patients, Duquesnoy and colleagues (*14*) have proposed that the definition of AMM can be extended further by including donors with zero, one, and two TAA mismatches, thus markedly increasing access to the donor pool. The results from our study of HSP (≥85% IgG PRA) confirms that HLA specificities with zero TAA mismatches are AMM but demonstrates that HLA-specific antibodies are common (approximately 40%) against HLA class I specificities with one or two TAA mismatches. The high level of HLA-specific antibodies against antigens with one or two TAA in our study is likely to reflect the high level of sensitization in the patient group studied.

CONCLUSION

This study has shown, using single-antigen HLA class I antibody detection beads covering the full repertoire of serologically defined HLA-A and –B specificities, that analysis of the number and nature of TAA mismatches for a given HLA type correlates with the risk of humoral sensitization. Identification of TAA using HLAMatchmaker is a helpful approach for predicting potential donors with AMM, although the presence of a single TAA mismatch is often sufficient to invoke a strong alloantibody response.

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