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MULTISCREEN SERUM ANALYSIS OF HIGHLY SENSITIZED RENAL DIALYSIS PATIENTS FOR ANTIBODIES TOWARD PUBLIC AND PRIVATE CLASS I HLA DETERMINANTS

IMPLICATIONS FOR COMPUTER-PREDICTED ACCEPTABLE AND UNACCEPTABLE DONOR MISMATCHES IN KIDNEY TRANSPLANTATION[1]

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A multiscreen serum analysis program has been developed that permits a determination of antibody specificity for the vast majority of highly sensitized patients awaiting transplantation. This program is based on a 2 × 2 table analysis of correlations between serum reactivity with an HLA-typed cell panel and incorporates two modifications. One implements the concept of public HLA determinants based on the serologic crossreactivity among class I HLA antigens. The other modification derives from the premise that most highly sensitized patients maintain the same PRA and antibody profiles

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over many months and even years. Monthly screening results for patients with persistent PRA values can therefore be combined for analysis.

For 132 of 150 highly sensitized patients with >50% PRA, this multiscreen serum analysis program yielded information about antibody specificity toward public and private class I HLA determinants. The vast majority of patients (108 of 112) with PRA values between 50 and 89% showed antibody specificity generally toward one, two, or three public markers and/or the more common private HLA-A,B antigens. For 24 of 38 patients with >90% PRA, it was possible to define one or few HLA-specific antibodies.

The primary objective of the multiscreen program was to develop an algorithm about computer-predicted acceptable and unacceptable donor HLA-A,B antigens for patients with preformed antibodies. A retrospective analysis of kidney transplants into 89 highly sensitized patients has demonstrated that allografts with unacceptable HLA-A,B mismatches had significantly lower actuarial survival rates than those with acceptable mismatches ($P = 0.01$). This was shown for both groups of 32 primary transplants (44% vs. 67% after 1 year) and 60 retransplants (50% vs. 68%). Also, serum creatinine levels were significantly higher in patients with unacceptable class I mismatches (3.0 vs. 8.4 mg% [$P = 0.007$] after 2 weeks; 3.9 vs. 9.1 mg% [$P = 0.014$] after 4 weeks). Histopathologic analysis of allograft tissue specimens from 47 transplant recipients revealed a significantly higher incidence of humoral rejection ($P = 0.02$), but not cellular rejection, in the unacceptable mismatch group. These results suggest that the multiscreen program can establish which donor HLA-A,B mismatches must be avoided in kidney transplantation for most highly sensitized patients.

For 18 of 150 high PRA renal dialysis patients, the multiscreen program could not define HLA-specific antibody. Most patients had >90% PRA, and many of their sera appeared to contain IgM type nonspecific lymphocytotoxins that could be inactivated by dithioerythreitol (DTE). Preliminary studies have shown that this treatment enabled the detection of HLA-specific antibodies upon subsequent screening on many occasions. These data suggest that non-HLA specific reactivity revealed by multiscreen analysis can often be removed by DTE treatment.

Multiscreen analysis offers an attractive approach to regional organ-sharing programs for highly sensitized renal transplant candidates. It enables the development of an efficient strategy for donor selection based on the computer assignment of acceptable HLA-A,B mismatches for each patient.

The highly sensitized renal dialysis patient presents an enigma to most transplant programs. Not only is it difficult to find a suitable crossmatch negative donor, but it is also apparent that a kidney transplant is generally less successful (*1–3*). The accumulation of highly sensitized patients on renal transplant waiting lists is a universal problem (*4, 5*).

Many sensitized patients have HLA-specific antibodies due to previous graft failures, blood transfusions, and pregnancies. Humoral sensitization is determined by testing patient sera in lymphocytotoxicity assays against a cell panel from HLA-typed donors. These assays are designed to primarily detect antibodies specific for the products of the HLA-A and HLA-B loci. Patients with panel-reactive-antibody activity of greater than 50% are considered highly sensitized. The higher the PRA value, the more difficult it is to find a crossmatch negative donor. The chances of a successful transplant are improved by selecting HLA-A and HLA-B identical or compatible donors, but the extensive polymorphism of HLA limits this approach. Another approach is via desensitization protocols aimed at reducing antibody levels by plasmapheresis in combination with immunosuppressive drugs (*6*) or with immunoabsorbent columns (*7*). Alternatively, several collaborating transplant programs have implemented the distribution of high PRA sera among tissue-typing laboratories to identify negative crossmatches with random potential donors by trial and error (*5, 8, 9*).

Another strategy utilizes the screening of high PRA sera with specifically selected panel cells to determine which HLA-A and HLA-B antigen mismatches might be considered acceptable because they do not cause a positive crossmatch (*10, 11*). This approach has been successfully applied in kidney transplantation but is very labor intensive and requires access to an extremely large panel of HLA typed donors.

The HLA antibody specificities of high PRA sera have been analyzed by absorption and elution studies using selected HLA typed cells (*12, 13*). These antibodies can be categorized according to specificity toward private and public determinants. Each private determinant represents a unique epitope configuration on one HLA gene product, whereas a public determinant represents an epitope shared by more than one HLA gene product (*14*). Antibodies against public determinants have been used to classify HLA antigens into several major crossreactive groups (CREGs).* Historically, high PRA sera have been assumed to be multispecific, but it has become apparent that these sera generally contain a limited number of antibodies directed against public and private determinants of high frequency (*15*). Differential absorption-elution studies will generally enable a dissection of the specific antibody subsets, thereby permitting an assessment of which mismatched HLA antigens of the donor would be acceptable to the patient (*13*). This method of serum analysis is also labor intensive and time consuming and requires considerable serologic expertise not available in most laboratories on a routine basis.

In this report we describe a computer approach for analysis of high PRA sera for antibody specificities toward private and public HLA determinants. Assignments of public markers to panel cells are based on serologic crossreactivity among HLA-A and HLA-B antigens. An algorithm then categorizes mismatched donor HLA-A,B antigens as acceptable or unacceptable to each highly sensitized patient. A retrospective analysis has shown that highly sensitized patients experience lower success rates with kidney transplants from donors with unacceptable mismatches than from those with acceptable HLA-A,B mismatches.

These findings demonstrate that this computerized analysis to determine HLA antibody specificity of high PRA sera might be valuable in strategies to identify suitable transplant donors for highly sensitized patients.

MATERIALS AND METHODS

Patients. This study was done on 150 renal dialysis patients with persistent PRA values of greater than 50%. There were 62 patients

* Abbreviations: CREGs, crossreactive groups; DTE, dithioerythreitol; DTT, dithiothreitol.

who had not received a transplant and 88 patients with one or more previous failed allografts. No attempt was made to document their blood transfusion histories or previous pregnancies. The PRA values were distributed into four groups: 50–69% (n = 36), 70–79% (n = 29), 80–89% (n = 47), and 90–100% (n = 38). Ninety-two kidney transplants were performed in this group of patients, and all 89 recipients received cyclosporine as the primary immunosuppressive drug. Transplant outcome was evaluated as actuarial graft survivals during the first 360 days. Serum creatinine levels at 2 and 4 weeks after transplantation were used to assess allograft function.

Renal histopathology was done on 108 specimens from 47 transplants during the first 120 days posttransplant although most were taken during the first month. Needle biopsies and nephrectomy specimens were obtained when clinically indicated and processed routinely for light microscopy and, where feasible, for immunofluorescence using commercial antibodies to IgG, IgM, C1q, C3, and fibrinogen as previously described (*16*). For histologic evaluation, vascular lesions were classified as due to humoral or cellular rejection. Humoral rejection was diagnosed with multifocal fibrinoid necrosis, thrombosis, and polymorphonuclear leukocytes in the microvasculature. Positive fluorescence was considered supportive evidence but was not necessary for the diagnosis. Vascular involvement in cellular rejection was characterized by endothelial cell wall adhesions or infiltrates of mononuclear cells with or without fibrinoid necrosis.

Histocompatibility testing. HLA-A,B typing was performed by the Amos modified lymphocytotoxicity technique, and HLA-DR typing was done by two-color fluorescence using standard methods. No consideration was given to HLA-C polymorphisms. The Amos wash technique was also used for antibody screening of patient sera against 45–60 cell panels from HLA typed individuals and for crossmatching against donor lymphocytes. Selected sera with non-HLA specific antibody activity were treated with dithioerythreitol (DTE) to inactivate IgM type lymphocytotoxins (*17*). This was done by treating 100 μl of serum with 5 μl of 100 mM DTE in RPMI-1640 medium at 37°C for 30 min.

Computer program for serum screening analysis. Serum reactivity and specificity against a cell panel were analyzed by computer (IBM PS/2 model 70) using custom software. This program generates a list of panel cells grouped according to their cytotoxicity scores with a patient's serum. Using 2 × 2 table statistics, antibody specificities were determined from correlations of positive reactions with the presence of private and public HLA determinants on the panel cells. This computer program generates for each serum a list of antibody specificities, generally no more than three or four with significant χ^2 values. Computer assignment of the primary antibody specificity is based on the positive correlation with the highest χ^2 value. For the remaining serum reactions, a second antibody specificity is assigned by χ^2 analysis of correlations between reactions and the HLA specificities in the cell panel excluding those cells with the marker recognized by the first antibody. A third antibody specificity is determined from serum reactivity patterns with panel cells except those with antigens recognized by the first and second antibody. This tail analysis is repeated until all positive reactions of the serum had been accounted for. Information is also generated about the undefined tail, i.e., the number of positive reactions that cannot be significantly correlated with any marker defined in the cell panel.

Assignment of public markers. This is based on generally available information on serologic crossreactivity among class I HLA antigens, reviewed by Rodey and Fuller (*14*) and derived from our own experience. Computer-assigned public markers have a "P" prefix except those with low frequencies. For instance, the public marker P01 is assigned to all panel cells typed for the private antigens A1, A3, A11, and/or Aw36; these antigens are well known for crossreactivity among themselves. P02 is assigned to cells positive for the crossreacting A2 and A28 (including the Aw68 and Aw69 subtypes). Our current list of computer-assigned public markers and their included HLA antigens is shown in Table 1. Each panel cell used for serum screening is automatically assigned by computer a set of P markers from its HLA-A,B type according to Table 1. For instance, the HLA-A1,3;B7,8(Bw6) phenotype is assigned with the following series: P01;P11;P21, P22;P32;P42,P43;P62,P63;P71;P91,P92,P93,P94,P95 whereas A2,–; B51,44 (Bw4) is assigned with P02;P12;P22;P32;P41;P51;P61, P64;P72;P81,P82;P92.

Table 1 also shows the relationship of the public markers to the major CREGs. For instance, P01, P11, P21, P31, P71, P91, and P93 belong to the A1 CREG. These P markers are all interrelated, i.e., P11 = P01 + A10 + Aw19; P71 = P01 + A10 + A9; P93 = P11 + A28, etc. Multiple P markers within each CREG have increased the efficiency of the computer analysis program in detecting antibody specificities of high PRA sera. Three markers—P21, P22, and P92—were based on specificity patterns of class I specific murine monoclonal antibodies (*18*).

This computer program also incorporates low-frequency public markers generally comprising two or a few HLA antigens. They are listed as three- or four-digit number combinations, e.g., 3031 is A30 + A31; 2532 = A25 + A32; 311 = A3 + A11, etc. As negative controls, this program also designates "nonsense" markers (N97, N98, and N99) representing combinations of HLA antigens not belonging to recognized CREGs.

Statistical analysis. Antibody-specificity assignments were based on 2 × 2 table analysis using χ^2 statistics to determine significant correlations between serum reactivity patterns and the presence of specific markers in the cell panel. Actuarial renal transplant survivals were analyzed by generalized Wilcoxon (Gehan) statistics using Stata Release 2 software (Computing Resource Center, Los Angeles, CA). Other differences between groups were analyzed for statistical significance with the Mann-Whitney *U* test, Students' *t* test, or by χ^2 analysis (Statview 512+, Macintosh microcomputer).

RESULTS

Results of monthly serum screens. Monthly serum specimens from renal dialysis patients were screened against cell panels from 45 to 60 HLA typed donors. For most sensitized patients, the serum PRA values vary only slightly from month to month. In 159 patients with greater than 50% PRA during at least 6 months, we identified 150 patients with similar reactivity patterns in their sera. For many high PRA sera, the computer analysis showed one or few antibody specificities, primarily toward public markers.

Table 2 shows typical examples of monthly PRA values and computer-assigned antibody specificities based on highest χ^2 values. Patient 21632 with serum PRA values in the 80–90% range exhibited consistent antibody specificity toward P32, a public determinant of the A2 CREG. The high PRA sera from patient 17793 reacted toward a public marker in the A1 CREG, although no exact assignment could be made. An example of a dual antibody specificity is presented by patient 7145 whose sera consistently reacted with the Bw4-associated P81 and the A1 CREG-associated P21 markers. For many patients with greater than 90% PRA, we could obtain information about possible antibody specificities, but the χ^2 values of the 2 × 2 correlations were not statistically significant due to the small size of the cell panels in the monthly screens. An example is patient 18358 whose sera seemed to react toward P81, P42, and perhaps P22. Conversely, other high PRA patients like 25064 repeatedly failed to display any tendency toward a specific antibody. The inability to assign HLA antibody specificity for some high PRA sera could be due to non-HLA specific lymphocytotoxicity or the inadequate size of our cell panel or both.

Antibody-specificity analysis of multiscreens. The data in Table 2 illustrate our experience that from month to month most highly sensitized patients show similar PRA values and pat-

TABLE 1. Computer-assigned public specificities and their corresponding HLA antigen groups

	CREG	Included HLA-A, B antigens
P01	1C	A1, A3, A11, Aw36
P02	2C	A2, A28, Aw68, Aw69
P03	1C	A25, A32, Aw33, Aw66
P11	1C	A1, A3, A10, A11, Aw19, Aw36, A25, A26, A29, A30, A31, A32, Aw33, Aw34, Aw66, Aw74
P12	2C	A2, A9, A28, A23, A24, Aw68, Aw69
P21	1C	A1, A9, A10, A11, A23, A24, A25, A26 (defined by moAb)
P22	2C	A2, A3, A28, A30, A31, A32, Aw68, Aw69
P31	1C	A26, A28, Aw33, Aw34, Aw68, Aw69, Aw74
P32	2C	A1, A2, A9, A23, A24, A28, Aw68, Aw69
P41	5C	B5, B15, B17, B18, B21, B35, Bw53, Bw70, B49, Bw50, B51, Bw52, Bw57, Bw58, Bw62, Bw63, Bw71, Bw72, Bw75, Bw76, Bw77
P42	7C	B7, Bw22, B27, B40, Bw41, B42, Bw46, Bw47, Bw48, Bw73, Bw54, Bw55, Bw56, Bw60, Bw61
P43	8C	B8, B14, B16, B38, B39, Bw59, Bw64, Bw65, Bw67
P51	5C	B5, B15, B18, B35, Bw53, B51, Bw52, Bw62, Bw63, Bw75, Bw76, Bw77
P52	7C	B13, B27, B40, Bw41, Bw47, Bw60, Bw61
P61	5C	B5, B35, Bw53, B51, Bw52
P62	7C	B7, Bw22, B27, Bw42, Bw54, Bw55, Bw56
P63	8C	B8, B14, B18, Bw59, Bw64, Bw65
P64	12C	B12, B21, B40, B44, B45, B49, Bw50, Bw60, Bw61
P71	1C	A1, A3, A9, A10, A11, A23, A24, A25, A26
P72	12C	B5, B12, B13, B17, B21, B27, B37, B38, B40, Bw47, B51, Bw63, B44, B45, B49, Bw50, Bw57, Bw58, Bw60, Bw61
P81	4C	A9, A23, A24, A32, Bw4
P82	12C	B12, B13, B21, B27, B37, B40, Bw47, Bw41, B44, B45, B49, Bw50, Bw60, Bw61
P91	1C	A1, A10, A11, A32, A25, A26
P92	8C	B8, B14, B18, B51, Bw59, Bw64, Bw65 (defined by moAb)
P93	1C	A1, A3, A10, A11, Aw19, A28, Aw36, A25, A26, A29, A30, A31, A32, Aw33, Aw34, Aw66, Aw68, Aw69, Aw74
P94	6C	A11, Bw6
P95	8C	B8, B14, B16, Bw22, B38, B39, Bw54, Bw55, Bw56, Bw64, Bw65, Bw67
Low-frequency groups:		
3031	1C	A30, A31
2532	1C	A25, A32
311	1C	A3, A11
1126	1C	A11, A26
722	7C	B7, Bw22, Bw54, Bw55, Bw56
727	7C	B7, B27
740	7C	B7, B40, Bw60, Bw61
1340	7C	B13, B40, B60, B61
1213	12C	B12, B13, B44, B45
1221	12C	B12, B21, B44, B45, B49, Bw50
814	8C	B8, B14, Bw64, Bw65
1517	5C	B15, B17, Bw57, Bw58, Bw62, Bw63, Bw70, Bw71, Bw72, Bw75, Bw76, Bw77
217	2C	A2, B17, Bw57, Bw58
1622	8C	B16, Bw22, B38, B39, Bw54, Bw55, Bw56, Bw67
"Nonsense" specificities:		
N97		A1, A23, A25, A29, B5, B40, B45, Bw60, Bw61
N98		A2, A11, A29, B7, B14, B15, B37, B44, Bw64, Bw65
N99		A24, A26, A32, B8, B13, B35, B44, Bw57

terns of panel reactivity. This suggests that such patients maintain in their sera the same antibody-specificity profile. This experience is consistent with findings reported by Oldfather et al. (*12*, *16*) and others (*19*, *20*). Therefore, it was decided to combine monthly screening results for each patient, for up to a 12-month period. The consequent larger number of panel cells in these multiscreens enabled a better statistical analysis of the specificity of antibody-reactivity patterns, yield-

ing higher χ^2 values. Table 3 summarizes the results of multiscreens on the five patients whose monthly screening data are shown in Table 2. For four patients, the antibody-reactivity patterns correlated with one or two public markers with very high χ^2 values. A multiscreen of eleven monthly serum specimens from patient 21632 indicated monospecific antibody activity toward P32, whereas a multiscreen on six monthly sera from patient 17793 showed antibody specificity against P93. Patient 7145, with a multiscreen PRA of 87%, showed two readily identifiable antibody specificities toward P81 and P21.

For these patients, the multiscreens generated essentially the same information about antibody specificity as did the individual monthly screens. However, multiscreens yield considerably higher χ^2 values, thereby enabling more definite conclusions about the antibody specificity of high PRA sera. This is even better illustrated by the multiscreen of patient 18358 whose monthly screens yielded no statistically significant correlations regarding antibody activity. A multiscreen on six monthly specimens from patient 18358 showed a well-defined dual specificity toward P81 and P42. Conversely, multiscreens on certain patients failed to reveal antibody specificity to any defined public or private HLA-A,B marker. An example is patient 25064 whose 93% PRA appeared to represent a non-HLA-specific lymphocytotoxic antibody.

Summary of multiscreen results. For 132 of 150 highly sensitized patients (or 88%), it was possible to obtain information about HLA-specific antibody in their sera. For these patients the multiscreens were done on the most recent sera (n=61) or just prior to transplantation (n=89). Figure 1 shows the percentages of patients within different PRA ranges for which one or few HLA antibody specificities were identified. Antibody assignment was based on 2×2 analysis yielding $\chi^2 > 10.9$ (or $P<0.001$). The vast majority of patients (108 of 112) with PRA values between 50% and 89% yielded specific HLA antibody

TABLE 2. Examples of screening results of monthly serum specimens from highly sensitized renal dialysis patients

Patient HLA	Serum date	PRA	AB1	++	+−	−+	−−	χ^2	AB2	++	+−	−+	−−	χ^2
21632	11/87	91%	P32	48	0	5	5	26.26	1213	4	0	1	5	6.67
A3,-	12/87	85%	P32	49	3	2	6	26.06						
B8,-	02/88	79%	P32	53	4	2	11	37.86						
DR5,8	03/88	84%	P32	52	4	4	7	21.38						
17793	05/87	80%	P21	38	3	13	7	4.95						
A2,-	08/87	91%	P11	44	0	4	5	26.99	P62	3	0	1	5	5.62
B39,44	10/87	78%	P93	42	9	3	4	5.52						
DR5,-	12/87	70%	P01	30	4	12	14	12.42						
	01/88	76%	P71	42	6	3	8	17.84						
7145	08/88	90%	P21	42	2	18	5	4.47	P81	13	1	5	4	4.48
A2,3	10/88	82%	P81	41	2	8	9	18.98	P21	6	0	2	9	10.43
B7,35	12/88	90%	P81	47	0	6	6	26.16	P21	4	0	2	6	6.00
DR2,6	02/89	89%	P81	50	2	8	5	12.97	P21	6	0	2	5	6.96
	04/89	92%	P81	46	1	8	4	12.00	P21	6	0	2	4	6.00
18358	04/88	90%	P81	41	2	14	4	4.42	P42	14	0	0	4	18.00
A2,3	11/88	97%	P42	28	0	32	2	NS	P81	32	1	0	1	NS
B8,39	01/89	97%	P81	54	0	12	2	NS	P22	11	0	1	2	NS
DR3,6	02/89	94%	P81	51	1	11	3	NS	P22	9	1	2	2	NS
	03/89	99%	P81	106	0	25	1	NS	P42	22	0	3	1	NS
25064	01/87	95%	P94	43	1	9	2	NS	217	5	0	4	2	NS
A3,-	02/87	91%	P32	41	2	10	3	NS	P52	5	0	5	3	NS
B7,-	09/87	96%	P41	32	0	19	2	NS	P42	13	0	6	2	NS
DR2,-	10/87	95%	N97	57	0	51	5	NS	P92	20	0	37	5	NS

TABLE 3. Examples of multiscreens on high-PRA patients

Patient	Monthly interval	Panel size	PRA	AB	++	+−	−+	−−	χ^2	Undefined tail
21632	10/87–06/88	568	82%	P32	430	35	31	70	209.6	7%
17793	01/87–12/87	328	79%	P93	252	48	8	20	47.9	3%
7145	07/88–04/89	491	87%	P81	356	18	73	43	84.5	6%
				P21	49	0	24	43	50.0	
18358	03/88–02/89	323	94%	P81	241	8	62	12	16.6	1%
				P42	58	3	4	9	32.6	
25064	01/87–12/87	345	93%	?						100%

information. For 24 of 38 patients (or 63%) with PRA values in the 90–100% range, it was possible to identify one or more HLA-specific antibodies.

It is evident in Figure 1 that most high PRA sera showed a limited number of antibody specificities. They were primarily toward public and the more common private antigens. Table 4 shows the distribution of public and private HLA determinants recognized in 132 high PRA patients with defined antibodies. They have been categorized into the different CREGs. There was a relatively even distribution among the antibodies specific for antigens in the combined A-locus and the combined B-locus CREGs. Public markers in the A1 and A2 CREGs were recognized 117 times, whereas in 121 instances a public marker in the combined B-locus CREGs was detected. A private antigen was detected 50 times for the A locus and 41 times for the B locus. For the A1, A2, B5, B7, and B12 CREGs, the antibody specificities were more frequently toward the public markers than the private antigens. The exception is the B8 CREG, for which antibody specificity was primarily directed toward the private antigens rather than the public markers. Also somewhat surprising was the relatively low incidence of antibody specificity toward Bw6 and its related P94 marker (Bw6 + A11) in comparison to the frequencies of antibodies toward Bw4 and its related P81 marker (Bw4 + A9 + A32). Three public markers—P21, P22, and P92—were assigned on the basis of the specificity pattern of class I specific murine monoclonal antibodies (*18*). For each of them, we identified high PRA sera with specific antibody activity. It should be noted that no sera showed specificity toward any of the "nonsense" markers N97, N98, and N99.

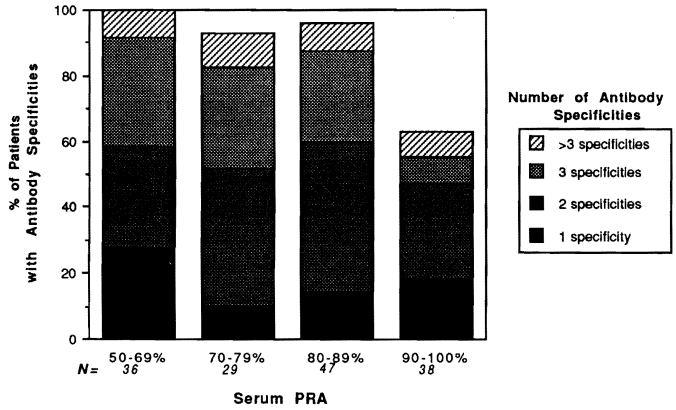

FIGURE 1. Serum antibody specificity distribution among highly sensitized renal dialysis patients within different PRA ranges.

No attempt was made to determine associations between antibody specificity toward public and private determinants and the HLA-A,B type of the patient. Similar to observations by Kreisler et al. (*21*) and others (*5, 10*), we observed an increased frequency of DR2 in high PRA patients (30% vs. 18% in nonsensitized renal dialysis patients, $\chi^2 = 6.25$; $P = 0.012$).

Undefined tail analysis of high-PRA sera. The multiscreen program also revealed how much panel reactivity of each serum had no demonstrable specificity toward any defined private or public HLA-A,B marker. The extent of this nonspecific reactivity was assessed by the length of the undefined "tail," expressed as the percentage of positive reactions with no statistically significant correlations. Figure 2 shows the distribution of undefined tails among patient sera within different PRA ranges. A tail of less than 5% was considered insignificant for nonspecific reactivity. Whereas a tail of 5–10% was suggestive

TABLE 4. Distribution of serum antibody specificities toward public markers and private HLA-A,B determinants of the various crossreactive groups in 132 high-PRA patients

Group	No. patients with antibody specificity toward:		Total No. of antibody specificities		
	Public markers	Private determinants	Publics	Privates	Total
A1 CREG	P01(14),P03(1),P11(6),P21(10) P31(2),P71(6),P91(6),P93(10) 311(8),3031(0),2532(0),1126(5)	A1(3),A3(4),A10(4) A11(2),Aw19(2),A25(1) A30(1),A32(1),A33(1)	68	19	87 (27.5%)
A2 CREG	P02(20),P12(19),P22(4),P32(10) 217(6)	A2(18),A9(9),A23(2) A24(1)	59	30	89 (27.1%)
B5 CREG	P41(15),P51(6),P61(7) 1517(1)	B15(2),B17(4),B35(1) B49(2),B53(1),B57(2) Bw70(1)	29	12	41 (12.5%)
B7 CREG	P42(9),P52(4),P62(3) 722(1),727(2),740(3),1340(1)	B7(4),B13(2),B41(1), B55(1),B60(1)	23	9	32 (9.7%)
B8 CREG	P43(0),P63(0),P92(1),P95(0) 814(0),1622(2)	B8(12),B14(3)	3	15	18 (5.5%)
B12 CREG	P64(4),P72(12),P82(8), 1213(4),1221(3)	B12(3),B44(3)	31	6	37 (11.3%)
Bw4	P81(17),Bw4(8)	—	25	—	25 (7.6%)
Bw6	P94(3),Bw6(1)	—	4	—	4 (1.2%)
Totals			338	91	329

of residual undefined reactivity, we considered undefined tails of >10% as significant evidence for non-HLA specific antibody activity.

These significant tails were grouped according to their lengths: 11–20%, 21–60%, and 100% (no sera were found with tails from 61 to 99%). An undefined tail of 100% means that no antibody specificity could be determined in the multiscreen. In the group of 38 patients with >90% PRA values, there were 14 (or 38%) whose sera were completely nonspecific as indicated by the 100% tails. The remaining 24 patients exhibited one or more antibody specificities in their sera (Fig. 1); for 20 of them the undefined tail was under 10% (Fig. 2). These data show that for 20 of 38 patients with greater than 90% PRA, we could define virtually complete specificity patterns of HLA antibodies.

Almost two thirds of patients in the 80–89% and 70–79% PRA groups showed nonspecific tails of less than 10%, suggesting that computer-determined antibody specificity accounted for more than 90% of their serum reactivity. Only a few patients in these PRA ranges had 100% nonspecific sera.

The 50–69% PRA group showed a different distribution of undefined tails. Although all of 36 patients in this group exhibited one or more antibody specificities (Fig. 1), only 12 (or 33%) showed undefined tails of less than 10%, a markedly lower percentage than that observed for the higher PRA groups. These somewhat unexpected findings suggest that a complete assessment of antibody specificity is more difficult for sera with 50–69% PRA values. Studies are currently in progress to investigate the reactivity patterns of these sera.

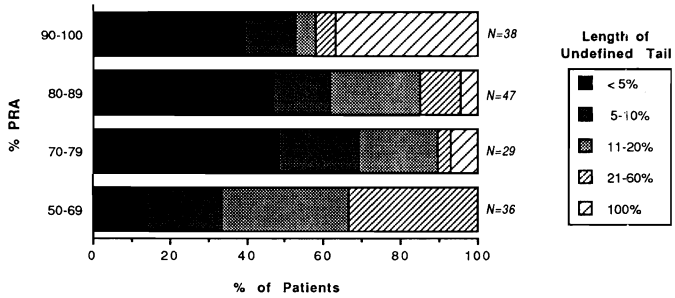

FIGURE 2. Distribution of undefined serum reactivity tails of different lengths for highly sensitized renal dialysis patients within different PRA ranges.

DTE treatment of nonspecific sera. As noted above, 18 of 150 patients (or 12%) showed only nonspecific antibody activity as indicated by the 100% undefined tails. The majority of these sera came from patients with >90% PRA values. It was postulated that many such patients may have IgM type non-HLA specific lymphocytotoxic antibodies that could be inactivated by treatment with DTE. Preliminary results of serum-screening analysis of DTE-treated sera from 13 patients are summarized in Table 5. Most sera (but not all) showed a reduction in PRA activity after treatment with DTE. Although a much smaller cell panel was used for this screening analysis, HLA antibody specificity was detected in 9 of 13 DTE-treated sera, whereas one serum (from patient 22815) became essentially nonreactive.

Renal transplant outcome in highly sensitized patients. From May 1985 to June 1989, a total of 92 renal transplants were performed on 89 of 150 highly sensitized patients. Donor selection did not depend on any information about the antibody specificity as described in this report. There were 32 primary transplants and 60 retransplants. A retrospective study was conducted to assess differences in survival of allografts from donors with computer-predicted acceptable and unacceptable HLA-A,B mismatches as determined from the serum antibody specificity defined by multiscreens. Figure 3 shows a considerably lower survival rate of grafts from donors with unacceptable than with acceptable HLA-A,B mismatches. The differences in graft survival were seen primarily during the early posttransplant period. About one half of the retransplants with unacceptable HLA-A,B mismatches showed a precipitous graft failure within 60 days. The primary allograft failure rate was somewhat more gradual but still resulted in the loss of about one half of the transplants during the first 90 days posttransplant. For the combined series of primary transplants and retransplants, the actuarial graft survival of transplants with unacceptable HLA-A,B mismatches was significantly lower than of those with acceptable mismatches ($P = 0.01$). Between these groups there were no significant differences in the PRA values (77.8 ± 2.7% vs. 73.2 ± 1.6%), crossmatch results (mean Terasaki scores: 1.41 ± 0.11 vs. 1.44 ± 0.16), number of HLA-A,B mismatches (2.36 ± 0.28 vs. 2.24 ± 0.14), and number of HLA-DR mismatches (1.03 ± 0.09 vs. 1.13 ± 0.17). These data suggest that in highly sensitized patients kidney transplants from donors with computer-predicted unacceptable HLA-A,B mismatches have lower survival rates.

TABLE 5. Reactivity and HLA antibody specificity of DTE-treated "nonspecific" high-PRA sera

Patient	No treatment		After DTE treatment			
	Panel size	PRA	Panel size	PRA	Antibody specificity	% Tail
8030	265	98%	101	92%	P11,A9	2
29825	261	98%	58	89%	Bw4	31
21068	569	97%	101	73%	P52,B35	57
22298	389	97%	58	82%	Bw6	9
16892	590	96%	101	98%	Undefined	100
11527	484	95%	101	89%	P11	10
23979	264	95%	101	89%	P81,P42	0
24560	516	95%	101	98%	Undefined	100
27100	388	95%	58	91%	Undefined	100
21357	261	94%	58	88%	Bw4	30
21476	391	94%	101	91%	P12,P42?	15
22815	326	88%	101	5%	Nonreactive	—
22694	496	83%	101	96%	P01?,P02?	15

Renal allograft function was assessed by serum creatinine levels 2 and 4 weeks after transplantation (Fig. 4). This information was collected from all patients transplanted since July 1986. Creatinine levels were significantly higher in patients with transplants from donors with unacceptable HLA-A,B mismatches. These findings suggested that kidneys with unacceptable mismatches had poorer function than kidneys with acceptable mismatches. Renal histopathology was available for 35 high PRA patients with acceptable mismatched grafts and for 12 patients with unacceptable mismatches (Table 6). Humoral rejection was significantly increased in kidneys with unacceptable HLA-A,B antigens. No differences between the groups were noted in the incidence of vascular lesions due to histologically diagnosed cellular rejection.

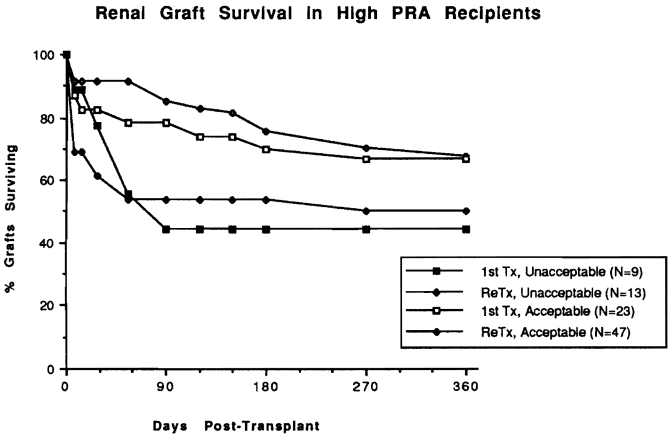

FIGURE 3. Actuarial graft survivals in highly sensitized patients transplanted with kidneys from donors with computer-predicted acceptable and unacceptable HLA-A,B mismatches. For the combined series of primary and retransplants, the difference between the two groups was statistically significant ($P = 0.01$ by generalized Wilcoxon [Gehan] rank).

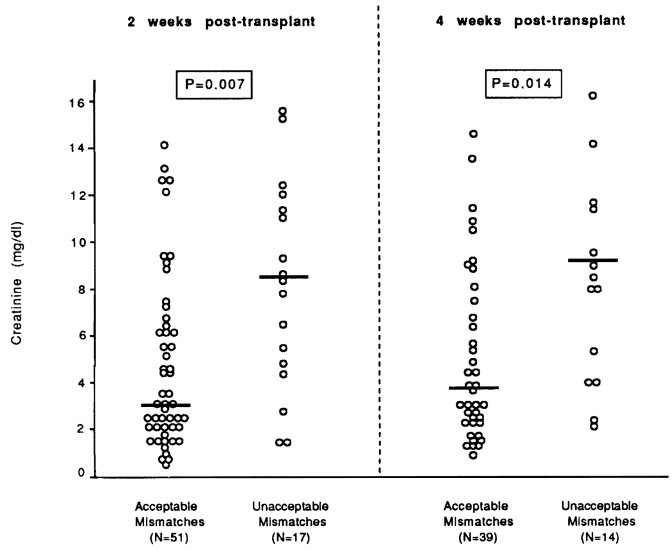

FIGURE 4. Serum creatinine levels in highly sensitized patients with kidney allografts from computer-predicted acceptable and unacceptable HLA-A,B mismatches. Differences were analyzed by the Mann-Whitney U test. Median values for each group are indicated by the horizontal bars.

TABLE 6. Incidence of histologically diagnosed humoral and cellular rejection of kidney transplants with acceptable and unacceptable HLA-A,B mismatches

HLA-A,B match	n	Humoral rejection[a]		Cellular rejection[b]	
		+	−	+	−
Acceptable	35	10	25	8	27
Unacceptable	12	8	4	3	9

[a] $\chi^2 = 7.83$; $P = 0.02$.
[b] $\chi^2 = 0.02$; P = NS.

Other aspects of the multiscreen program. As described above, this computer analysis program of serum screening yields the PRA value, antibody specificity information toward public and private class I HLA antigens, and the undefined tail of nonspecific serum reactivity. It determines what donor HLA-A,B antigens must be considered unacceptable to potential recipients with preformed HLA specific antibodies.

This program also generates information about significant *negative* correlations between serum reactivity and certain HLA antigens. As can be expected, many of these negative correlations pertain to the patient's own HLA antigens and to those that are strongly crossreactive. Frequently, significant negative correlations have also been seen with noncrossreacting mismatched HLA-A,B antigens. Examples are shown in Table 7. These findings suggest that certain acceptable HLA-A,B mismatches might be preferable over others. We are currently studying the effect of such preferred mismatches on transplant outcome.

DISCUSSION

The multiscreen serum analysis program permits a determination of serum antibody specificity for most highly sensitized transplant candidates. It incorporates two modifications into computer programs commonly used to determine the HLA specificity of allosera from multiparious women. One modification implements the concept of public HLA determinants based on serologic crossreactivity among class I HLA antigens. The second modification derives from the premise that most highly sensitized patients maintain the same PRA and antibody-specificity profiles over many months and even years and deals with combining screening results of consecutive monthly sera if the PRA values remain similar. For the majority of highly sensitized patients, rather complete information is obtained about serum-antibody specificity toward public and private HLA class I antigens. This information is critical to the selection of suitable transplant donors because distinctions can be readily made between acceptable and unacceptable HLA antigens.

It should be noted that the multiscreen program does not require any extra effort at the laboratory bench. Instead, it utilizes existing data from monthly serum screenings against HLA-typed cell panels.

The primary objective of this computer program is to determine for highly sensitized patients which HLA-A,B antigens should be avoided in the selection of a suitable transplant donor. Using the Amos wash technique for serum screening, we have obtained information about antibody specificity against public markers and the more common private class I HLA antigens for almost 90% of patients with greater than 50% PRA. This has enabled an algorithm to identify computer-

TABLE 7. Examples of significant negative correlations of high-PRA serum reactivity with mismatched private HLA-A,B antigens

Patient	HLA type	PRA	Ab specificities	Mismatched HLA-A,B with significant negative correlations	
				Antigen	χ^2
28559	A2,-;B35,63;DR5,6	74%	P01, A9, 740	A26	40.2
				Bw58	21.1
				A28	16.8
				B38	11.5
14153	A3,29;B7,44	83%	P32, B17, B8	B14	71.8
				A30	48.4
				B42	18.1
				Aw33	17.6
				A31	12.0
24714	A3,33;B42,-;DR4,8	89%	P32, P41	B7	39.5
				A30	37.6
				B14	10.1
20245	A2,3;B7,61;DR2,-	89%	P81, P41, P82	B8	91.5
				A1	27.4
				Bw22	19.9
				A26	13.5
7530	A2,11;B18,35;DR3,-	91%	P21, Aw19, P62	A28	53.9
				Bw41	27.4
				B14	24.7
				A3	16.2
				B39	7.8

predicted acceptable and unacceptable donor HLA-A,B antigens for patients with preformed antibodies. Donors with unacceptable HLA antigens can be expected to give positive crossmatches with patient serum. In a future paper we will address the predictability of the outcome of the crossmatch test from the computer-assigned acceptable and unacceptable donor antigens. Using a similar approach to analyze serum antibody reactivity toward public and private class I antigens, Turka et al. (*22*) observed a high computer predictability of positive but not of negative crossmatches. No data were given about the antibody specificities detected in their patient population. We believe that for high PRA patients with defined serum antibody specificities, the accuracy of a computer-predicted positive crossmatch is inversely correlated with the percentage of false-negative reactions in the multiscreen, whereas the accuracy of a computer-predicted negative crossmatch is reflected by the length of the nonspecific tail of serum reactivity.

The distinction between acceptable and unacceptable donor HLA antigen mismatches is relevant to transplant outcome. Our analysis of 89 highly sensitized transplant recipients has demonstrated that renal allografts with unacceptable mismatches were less successful than those with acceptable mismatches. This difference was shown not only by a lower graft survival rate but also by higher serum creatinine levels during the first month posttransplant and histopathologic evidence of humoral immune injury to the renal allograft. It should be noted that the failures of kidney transplants with computer-predicted unacceptable class I HLA mismatches occurred primarily during the early posttransplant period.

These transplant data show that many patients had received renal allografts from donors with unacceptable HLA-A,B mismatches. Since the vast majority of the final crossmatches with those donors were negative, it must be concluded that our crossmatch methodology (i.e., Amos wash technique) might not have been sensitive enough to detect these unacceptable mismatches. A more sensitive assay such as antiglobulin augmentation (*23, 24*) or the flow cytometric crossmatch (*25, 26*) might have detected these donor incompatibilities. On the other hand, more sensitive crossmatch assays are more likely to give false-positive results, thereby excluding many high PRA patients from transplantation. Relevant to this problem is the frequency of "false-negative" reactions of many high PRA sera. As illustrated in Table 3, the multiscreen of the P32 specific serum from patient 21632 showed false-negative reactions with 35 of 465 (or 8%) of P32 cells. It can be estimated that a potential donor with an unacceptable P32 mismatch would have an 8% chance of showing a negative crossmatch. Similarly, there would be a 16% chance of a false-negative crossmatch for patient 17793 when tested by conventional lymphocytotoxicity. Although there is no perfect crossmatch assay available at this time, it appears that information about serum antibody specificity is essential to the proper interpretation of the crossmatch results and the selection of suitable transplant donors for highly sensitized patients. The importance of the multiscreen program in donor-selection strategies is illustrated by the improved graft survivals of kidneys with computer-predicted acceptable HLA-A,B mismatches: 67% vs. 44% for primary transplants and 68% vs. 50% for retransplants after 1 year (Fig. 3). These survival rates are still lower than those observed for nonsensitized patients at our institution (*27*). Although further refinements in public antigen definition may improve the computer prediction of acceptable class I mismatches, we must also consider

the likely possibility that presensitization against class II HLA antigens will adversely affect transplant outcome in sensitized recipients.

This computer program also generates information about significant negative correlations between serum reactivity and HLA antigens. Although many of these HLA antigens are the same as the patient's own or strongly crossreactive splits, others are noncrossreactive mismatches. The latter deserve special consideration in strategies to increase the number of potential donors with preferred acceptable mismatches. A similar strategy has been used by Claas et al. (*10*) who performed serum screenings with selected panel cells with only one HLA antigen mismatched to the patient. Its purpose is to identify which HLA antigens would not be recognized by patient serum. This approach is labor intensive, and the determination of acceptable mismatched HLA antigens must be done by trial and error. Our computer analysis program offers the advantage of more readily identifying acceptable HLA-A,B mismatches.

For 18 of 150 patients, we could not identify HLA-specific antibody by conventional lymphocytotoxicity screening. Many of these non-HLA specific sera appear to contain IgM type antibodies that can be inactivated by DTE (or dithiothreitol [DTT]) DTT) treatment. Following the initial studies by Iwaki et al. (*17*) and Chapman et al. (*28*), it is now generally recognized that these nonspecific IgM lymphocytotoxins are clinically unimportant in humoral rejection of renal transplants (*29*). This has been demonstrated by the good graft survivals of kidney transplants from donors with conventional crossmatch positive but DTT crossmatch negative donors. However, it has also become apparent that certain patients have HLA-specific IgM-type antibodies that are sensitive to DTT treatment (Duquesnoy RJ, Marrari M, White LT, et al. Laboratory survey of serological crossmatching by the NIH, Amos wash, antiglobulin and flow cytometry procedures [manuscript in preparation]). Their clinical relevance in kidney transplantation is yet unclear, although it seems likely that some of these antibodies could induce humoral immune injury. Since our computer program can readily distinguish between HLA and non-HLA antibodies, it can be applied to determine which patient sera should be evaluated for DTT sensitivity. This pertains not only to completely nonspecific sera but also to sera with significant undefined reactivity tails.

Computer assignment of public markers to our cell panel was based on the crossreactivity among groups of HLA-A and HLA-B antigens. This approach is limited by the empirical nature of the serologic studies to establish crossreactivity. Although workshop efforts have been directed toward a better serologic definition of public determinants (*30*), there is no recognized nomenclature documenting the polymorphisms. Considerable progress has been made with the molecular studies by Schwartz and co-workers (*31, 32*) on the B5 and B7 CREGs and by Ayres and Cresswell (*33*) on Bw4/Bw6, demonstrating that public determinants represent epitopes distinct from the private determinants. More recently, the investigations by Parham and co-workers (*34, 35*) have provided detailed information about the amino-acid sequences and location of public and private epitopes on the HLA peptide, primarily on the α_1 and α_2 domains. Such studies will undoubtedly improve the definition of public epitopes on class I molecules expressing the different private HLA-A and HLA-B specificities, thereby increasing the efficiency of antibody-specificity analysis of sera from highly immunized patients.

A common strategy to increase the opportunity for transplantation of high PRA patients has been through serum exchanges between collaborating transplant centers for the purpose of finding crossmatch negative donors (*5, 8, 9*). Although this approach has led to successful transplants, several logistical problems prevent its widespread implementation. These difficulties relate to differences in sensitivity of crossmatch tests in the various laboratories, availability of current sera for final crossmatching, and delays in donor organ transportation causing prolonged ischemia time. Moreover, crossmatching is done randomly and rarely considers the antibody specificity of the patient's serum. With the computer analysis program described in this report, it would be possible for regional organ-sharing programs to develop a file of high PRA patients, each of whom will have a list of acceptable and unacceptable donor HLA-A,B antigens as determined from the antibody specificity of their sera. This approach would eliminate the need for distribution of patient sera to other tissue-typing centers. Given the HLA type of the potential donor, it would be relatively easy to determine the most suitable candidates for whom a final crossmatch is likely to be negative. Donor selection should also consider HLA-DR compatibility to increase renal allograft survival in highly sensitized patients (*36–38*).

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