Predicting HLA Class I Alloantigen Immunogenicity From the Number and Physiochemical Properties of Amino Acid Polymorphisms

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Background. Knowledge of the human leukocyte antigen (HLA) amino acid (AA) sequence combined with crystallographic structural data may enable prediction of the relative immunogenicity of individual donor/recipient HLA mismatches.

Methods. Multiple sera from 32 highly sensitized patients awaiting kidney transplantation were screened using Luminex/single-antigen beads to determine the HLA-specific antibody levels against mismatched HLA class I specificities. A computer program was developed to allow intralocus and interlocus comparison of mismatched HLA-A and -B specificities with corresponding recipient HLA class I type, and to determine the number, position, and physiochemical disparity (hydrophobicity and electrostatic charge) of polymorphic AA.

Results. HLA-specific antibody was detected against 1666 (85%) of the 1964 mismatched HLA specificities evaluated, with a close correlation between increasing number of AA polymorphisms and the presence and magnitude of the alloantibody response ($P<0.0001$). Hydrophobicity and electrostatic charge disparity scores were independent predictors of alloantibody production (adjusted $P=0.0009$ and $P=0.0005$, respectively). Mismatched specificities with physiochemical scores within the first decile of the scale led to weak alloantibody responses (median fluorescence intensity 2330), whereas those with scores above the sixth decile led to strong alloantibody production (median fluorescence intensity >10,000).

Conclusion. Differences in AA number, hydrophobicity, and electrostatic charge between HLA class I specificities enable prediction of donor HLA class I types with low immunogenicity for a given recipient.

Keywords: HLA immunogenicity, Hydrophobicity, Electrostatic charge.

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National and international renal transplant registries show that matching for human leukocyte antigen (HLA) remains an important determinant of long-term graft and patient survival (*1–5*). Organ allocation algorithms for cadaveric donor kidneys are often designed to promote equity of access to transplantation but most algorithms also incorporate matching for HLA in an attempt to maximize transplant outcome (*6–8*). Inevitably, because of the extensive polymorphism of HLA, both within and between different ethnic groups, the majority of recipients receive grafts that are mismatched at one or more of the three most important histocompatibility loci, HLA-A, -B, and -DR. The degree of HLA disparity between a given donor/recipient pair is often expressed as an HLA-mismatch grade, in which the number of incompatible donor antigens at each loci (HLA-A, -B and -DR) are counted. A major limitation of this approach is that within a given locus, mismatches are assigned equal relevance, even though it is well known that the relative immunogenicity of a particular donor HLA mismatch varies according to the recipient HLA type.

Attempts have been made to assess the relative immunogenicity of individual donor HLA mismatches and define,

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in the context of recipient HLA type, mismatches that are nonimmunogenic (permissible HLA mismatches) and less likely to contribute to allograft rejection (*9*, *10*). A pragmatic attempt to identify permissible HLA mismatches through large scale registry analysis of graft outcome according to recipient HLA in transplants undertaken in the presence of a single donor mismatch has been disappointing (*11*, *12*). Now, however, the availability of amino acid (AA) sequence data for all HLA alleles together with crystallographic information that allows prediction of AA position within the tertiary structure of the HLA molecule provides an alternative and more rational approach for predicting the immunogenicity of HLA alloantigens. Computer algorithms (HLAMatchmaker) are now available that examine HLA compatibility at the structural level through interlocus and intralocus comparison of AA polymorphisms in antibody accessible positions of the HLA molecule (*13*, *14*). These are based on counting the number of AA residues at continuous and discontinuous sequence positions that cluster together to form potential immunogenic epitopes (*15*). This approach is useful for determining the HLA compatibility in renal transplantation (*16*) and for predicting the magnitude of antibody response to alloantigen (*17*), which is known to be a major determinant of transplant outcome (*18–20*).

However, the immunogenicity of an HLA alloantigen depends not only on the number but also on the physiochemical nature of polymorphic AAs compared with the HLA type of recipient. The specificity and stability of antibody binding to target antigens is strongly influenced by the electrostatic and hydrophobic interactions between the two molecules (*21–23*). The initial association of antibody with antigen occurs through nonspecific long-range electrostatic forces, and the resulting antibody/antigen complex is stabilized through hydrogen bonding, formation of salt bridges, and van der Waals forces between opposing hydrophobic surfaces (*24–29*).

In this report, we describe a detailed analysis of HLA class I alloantibody response in a group of highly sensitized patients (HSPs) awaiting kidney transplantation, alongside a physiochemical analysis of HLA alloantigens. We show that the number of AA polymorphisms between mismatched HLA-A and -B specificities and patient HLA class I type correlate strongly with the generation and magnitude of alloantibody response. We also demonstrate for the first time that physicochemical disparities (hydrophobicity and electrostatic charge) between mismatched HLA molecules strongly influence HLA class I immunogenicity.

METHODS

Study Design

As a clinical readout for the relative immunogenicity of individual HLA alloantigens for a given recipient HLA type, we determined the maximum HLA class I specific antibody response, using single-antigen HLA antibody detection beads (SABs), in sera obtained from HSP awaiting renal transplantation. A computer program was developed (written in visual basic for applications [Microsoft Excel 2003, Microsoft Co, Redmond, WA]; available on request) to compare each mismatched HLA-A and -B specificity represented on the SAB with the corresponding recipient HLA-A, -B, and -C type, and to identify the number, position, and physiochemical disparity of polymorphic AA. This information was then used to examine the association between predicted HLA immunogenicity and the presence and strength of the alloantibody response. HLA-C locus was not included in the antibody analysis because of the low frequency of antibody response to this locus in HSP.

Selection of Highly Sensitized Patient Sera and Characterization of HLA-Specific Antibody Profiles

Serum samples obtained routinely at 2-month interval from all patients awaiting renal transplantation at Addenbrooke's Hospital, Cambridge, were screened by a combination of complement dependent cytotoxicity against an HLA-typed lymphocyte panel and solid-phase binding assays (enzyme-linked immunosorbent assay and Luminex) as previously described (*16*). Of 488 patients on the transplant waiting list in February 2008, 32 (6.6%, 13 men and 19 women with a median age of 46 yrs [range 27 to 66 years]) were identified as being highly sensitized, defined as more than or equal to 85% IgG lymphocytotoxic panel reactivity, and selected for study. Most (69%) had become sensitized as a result of a failed renal transplant and blood transfusion and the remainder after pregnancy or blood transfusion.

For each HSP, the serum sample with the highest IgG HLA class I specific antibody reactivity, representing the peak period of sensitization, was screened (neat, 1 in 50 and 1 in 100 dilution) using LABScreen HLA class I single-antigen antibody detection beads (LS1A04 lot 001, One Lambda Inc., Canoga Park, CA) as described previously (*30*). Antibody binding was assessed on a Luminex LABScan 100 (One Lambda Inc.), and the median fluorescence intensity (MFI) value obtained was adjusted for background signal using the formula ([sample N bead−sample negative control bead]−[negative control N bead−negative control negative control bead]) (*31*). MFI values were normalized to account for variation in the number of HLA molecules on each HLA class I-coated bead population, using the monomorphic HLA class I specific monoclonal antibody W6/32 (AbD Serotec, Oxford, UK), as described by El-Awar et al. (*31*). The highest normalized MFI value for IgG binding to individual HLA specificities was used for analysis of immunogenicity. As in our previous studies, IgG MFI values more than or equal to 1500 were considered positive (*17*).

Determination of Mismatched HLA Alloantigen Amino Acid Sequence Polymorphisms

The HLA-A, -B, and -C type of the 32 HSP was determined by low resolution polymerase chain reaction using sequence specific primers and each HLA class I specificity assigned to the most common corresponding four digit HLA allele as previously described (*17*). A computer program was developed to perform intralocus and interlocus AA sequence comparisons between the HSP HLA-A, -B, and -C type and all of the mismatched HLA class I specificities represented in the SAB and identify the position and nature of all disparate AAs. The conformational location of each disparate AA within the HLA class I molecule was considered according to whether or not they were positioned in an antibody accessible region of the molecule (*32*). Analysis to determine the number of AA

mismatches (and assignment of scores for their physiochemical properties) was performed for the entire α1 and α2 domains of HLA class I and for the α1 and α2 domains, excluding AA polymorphisms predicted to be located in antibody inaccessible regions of the peptide-binding groove (*32*).

Assignment of Hydrophobicity Scores for Mismatched HLA-A and -B Specificities

Each AA was assigned a hydrophobicity value using the Hopp-Woods scale (*33*). For each intralocus and interlocus AA disparity, a hydrophobicity mismatch value was determined as the difference in AA hydrophobicity values. In cases where the HSP HLA class I type carried several AA polymorphisms at the same position, the lowest AA hydrophobicity mismatch value was used in the analysis of immunogenicity. For each mismatched HLA class I specificity, the AA hydrophobicity mismatched values were summed to give a total hydrophobicity mismatch score (HMS).

Assignment of Electrostatic Scores for Mismatched HLA-A and -B Specificities

For each intralocus and interlocus AA disparity, the difference between the isoelectric points (pI) of each mismatched AA was determined. When more than one AA polymorphism was present at the same position in the HSP HLA class I type, the lowest pI mismatch value was used in the subsequent analysis. For each mismatched HLA class I specificity, the AA pI-mismatched values were summed to give a total electrostatic mismatch score (EMS).

Statistical Analysis

The two outcomes of interest were the magnitude of alloantibody response (MFI) for each mismatched HLA-A and -B specificity, expressed as a continuous outcome, and the probability of a positive antibody response (MFI$\geq$1500). Analysis focused on the association between the presence and magnitude of alloantibody response and the three explanatory variables (number of AA mismatches, HMS, and EMS). Exploratory analysis of MFI showed that the distribution was variable, positively skewed, and had a peak at zero. Therefore, MFI was summarized using the median and interquartile range (IQR), and the relationship between MFI and its predictors assessed using median regression. Logistic regression was used to model relationships between the probability of an antibody response and the explanatory variables. In both analyses, a log transformation was applied to the explanatory variables to account for their observed nonlinear relationship to the outcomes of interest. Initially, each explanatory variable was modeled separately; further models investigated the additional value in incorporating HMS or EMS into models including AA mismatches. HMS and EMS were highly correlated (P=0.91), and so could not be entered into the same model. For presentation, HMS and EMS were grouped in deciles, but for regression models, the absolute value was used. Statistical significance was assessed using likelihood ratio tests for nested logistic regression models and Wald tests for median regression models. All analyses were completed in the statistical software package Splus version 7.0 for Windows (Insightful Corporation, Seattle, WA).

RESULTS

Serial sera from 32 HSP (defined as $\geq$85% IgG lymphocytotoxic panel reactivity) were obtained, and the serum for each patient with the highest panel reactivity was selected for further analysis and screened using SAB. The SAB panel included 21 HLA-A and 44 HLA-B specificities. The number of mismatched HLA-A and -B specificities for each HSP ranged from 61 to 63 (depending on whether the patient was homozygous at HLA-A and/or -B loci). For the 32 HSP and up to 63 mismatched HLA-A and -B specificities, a total of 1964 mismatched combinations were identified. Of the total number of mismatched combinations, the presence of IgG HLA-specific antibody (defined as maximum normalized MFI$\geq$1500 using titrated sera) was detected against 1666 (85%) combinations, confirming that this HSP cohort was highly sensitized, with antibodies to most of the HLA-A and -B specificities expressed on the SAB.

Relationship Between the Number of Amino Acid Mismatches and HLA-Specific Antibody Responses

For each of the 32 HSP, their HLA-A, -B, and -C type along with all of the 63 possible mismatched HLA-A and -B specificities expressed on the SAB panel were entered into a computer program to perform intralocus and interlocus AA sequence comparisons. This allowed identification of the position and nature of all polymorphic AA for each mismatched HLA-A and -B specificity.

For the interlocus comparison, the number of AA polymorphisms between the recipient HLA-A, -B, and -C types and the mismatched HLA-A and -B specificity ranged from 0 to 17 (median 5, Fig. 1). The relationship between the number of AA mismatches and HLA-specific antibody response was examined both in terms of whether or not an antibody response occurred and the magnitude of the antibody response detected. Logistic regression analysis (Fig. 2a) showed

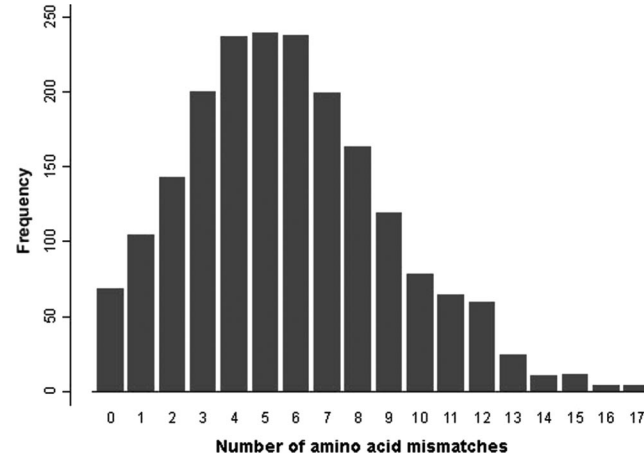

FIGURE 1. Number of amino acid mismatches present within mismatched human leukocyte antigen (HLA)-A and -B specificities. For each of the 32 HSP, the HLA-A, -B, and -C type were entered into a computer program and the number of amino acid mismatches, following interlocus subtraction, for the 65 single HLA-A and -B specificities represented on HLA class I single-antigen antibody detection beads, calculated.

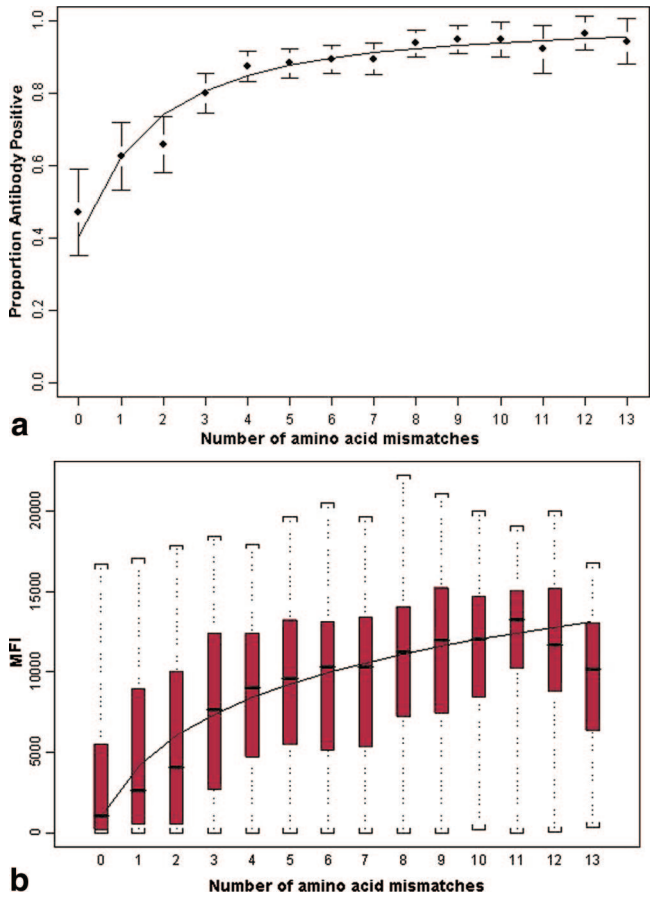

FIGURE 2. Relationship between the presence or absence, and levels of human leukocyte antigen (HLA)-specific antibody and the number of amino acid (AA) mismatches. Sera obtained from 32 highly sensitized patient were assessed for the presence or absence of antibody binding (a) and levels of antibody binding (b) to each of the mismatched HLA-A and -B specificities expressed on single-antigen beads. (a) shows point estimates and 95% confidence intervals at each level of AA mismatch and the fitted logistic regression curve. (b) shows *box* and *whisker plots* at each level of AA mismatch and the fitted median regression curve. AA mismatches more than or equal to 13 are grouped together.

that as the number of AA mismatches within a mismatched HLA specificity increased, so did the likelihood of an antibody response, and this increased from 47% (32/68) for zero AA mismatches to 91% (1315/1449) with four or more AA mismatches ($P<0.0001$). The magnitude of the antibody response detected also increased markedly as the number of AA mismatches increased (Fig. 2b), rising from a median MFI of 1048 (IQR 189 to 5415), when there were no AA differences, to 10,395 (IQR 6463 to 13,643), when there were four or more AA differences ($P<0.0001$). The small number of HLA-A and -B specificities that HSP did not have antibodies against were mostly confined to specificities with two or less AA mismatches, and when such antibody responses did occur, they were usually weak. A comparable analysis of data using intralocus instead of interlocus comparisons also showed a good correlation between the number of AA mismatches and alloantibody response ($P<0.0001$, data not shown).

Relationship Between the HMS and EMS Disparity and the Presence of HLA-Specific Antibody Responses

The physiochemical properties (hydrophobicity and electrostatic charge) of mismatched AA between each HLA-A and -B allospecificity represented on SAB and the HSP HLA class I type (following interlocus sequence subtraction) were next evaluated, and their relationship with the presence and magnitude of the HLA-A and -B alloantibody response was examined. Values for hydrophobicity and for electrostatic charge for each mismatched AA were calculated, and then summed to determine the total HMS and EMS for each mismatched HLA-A and -B specificity.

The differences in HMS between mismatched HLA-A and -B specificities and HSP HLA class I type ranged from 0 to 45.2 (median 10.0, IQR 5.5–15.6). Regression analysis was undertaken to examine the relationship between HMS and the presence and magnitude of the alloantibody response (Figs. 3a and 3b respectively). As the HMS of mismatched HLA-A and -B specificities increased, the frequency of antibody response rose from 56% (111/199) for the lowest decile of HMS to 97% (189/195) for the highest decile ($P<0.0001$). After adjusting for the number of AA mismatches, the association between HMS and antibody response remained significant ($P=0.0009$). The magnitude of alloantibody response increased from a median MFI of 2328 (IQR 362 to 8324) for the lowest HMS decile to 11,711 (IQR 7822 to 14,627) for the highest MFI decile ($P<0.0001$). However, when adjusted for the number of AA mismatches, the magnitude of the response was no longer related to HMS ($P=0.689$). In contrast to the number of AA mismatches, HMS data generated after intralocus instead of interlocus comparisons showed no correlation with the alloantibody response even when antibody inaccessible regions of the HLA class I molecule were excluded from the analysis (data not shown).

The differences in EMS between mismatched HLA-A and -B specificities and HSP HLA class I type ranged from 0 to 50.7 (median 8.8, IQR 4.6–14.7). Regression analysis showed that increasing EMS was associated with both the presence and the magnitude of alloantibody responses (Figs. 4a and 4b). As the EMS increased, the frequency of antibody response increased from 57% (112/198) for the lowest decile to 94% (186/197) for the highest decile ($P=0.0001$ unadjusted and $P=0.0005$ after adjustment for the number of AA mismatches). Similarly, increasing EMS correlated with increasing antibody levels from a median MFI of 2330 (IQR 299–8324) for the lowest decile to a median MFI of 10,941 (IQR 6670–14,316) for the highest decile ($P<0.0001$). However, as for HMS, when this was adjusted for the number of AA mismatches, the magnitude of the response was no longer related to EMS ($P=0.918$). Analysis of EMS data using intralocus comparisons (both for antibody accessible and inaccessible AA polymorphisms) did not show a correlation with either the presence or the magnitude of alloantibody responses (data not shown).

Having shown that the number of AA mismatches and physiochemical scores (HMS/EMS) correlate independently with the presence of an antibody response, we next considered the extent to which the three variables in combination

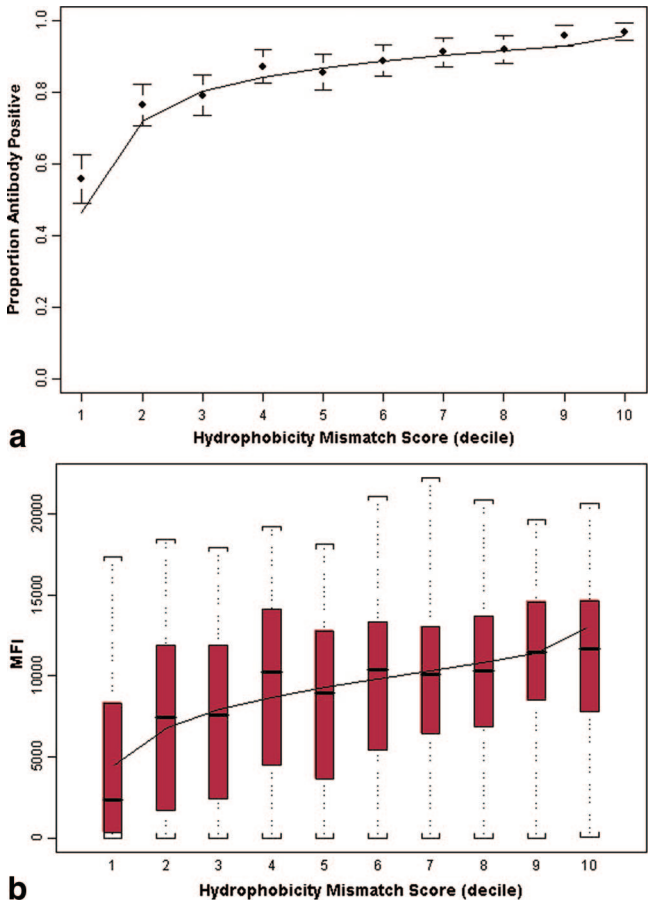

FIGURE 3. Relationship between the presence or absence, and levels of human leukocyte antigen (HLA)-specific antibody and the hydrophobicity mismatch score (HMS). Sera from highly sensitized patient were assessed for the presence of HLA class I antibody binding (a) and antibody levels (b) to mismatched HLA-A and -B specificities. Hydrophobicity mismatch scores are ranked in deciles. (a) shows point estimates and 95% confidence intervals at each decile of HMS and the fitted logistic regression curve. (b) shows *box* and *whisker plots* at each decile of HMS and the fitted median regression curve.

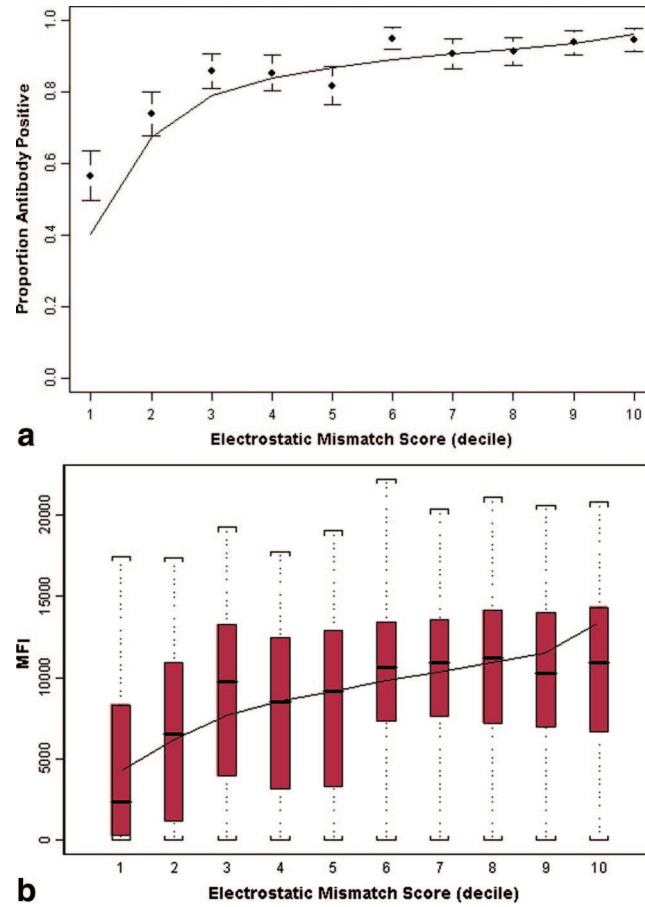

FIGURE 4. Relationship between the presence or absence, and levels of human leukocyte antigen (HLA)-specific antibody and the electrostatic mismatch score (EMS). Sera from highly sensitized patient were assessed for the presence of HLA class I antibody binding (a) and antibody levels (b) to mismatched HLA-A and -B specificities. Electrostatic mismatch scores are ranked in deciles. (a) shows point estimates and 95% confidence intervals at each decile of EMS and the fitted logistic regression curve. (b) shows *box* and *whisker plots* at each decile of EMS and the fitted median regression curve.

might better predict HLA class I alloantigen immunogenicity. Although HMS and EMS are fundamentally different physical characteristics, both are dictated in large part by the nature of AA side chain substitutions. Consequently, it might be expected that AA differences, which give rise to a high HMS, also give rise to a high EMS, and this was found to be the case in the present analysis where HMS and EMS correlated closely ($P=0.91$). Because of the close relationship between HMS and EMS, there was no merit in entering the two variables into the same predictive model. When either HMS or EMS was entered into a combined regression model with the number of AA mismatches to predict alloantibody production, the strength of the association increased as depicted in Figure 5, emphasizing the value of including physiochemical and AA sequence data when defining HLA alloantigen immunogenicity. However, both regression models had equal predictive power, and no advantage was demonstrated for combining HMS and EMS together with the number of AA mismatches.

The analyses described earlier were performed with data that considered all AA polymorphisms in the $\alpha 1$ and $\alpha 2$ domains of the HLA class I molecule. When AA sequence polymorphisms predicted to be located in antibody-inaccessible regions of the peptide-binding groove were excluded from analysis, the association between presence of alloantibody response and the three variables (AA mismatches, HMS and EMS) remained significant, but it was less strong and provided less discrimination between all three variables and the presence of alloantibody ($P<0.0001$ for AA mismatches, adjusted $P=0.0008$ for HMS, adjusted $P=0.03$ for EMS). This observation suggests that polymorphic AA located in antibody inaccessible regions of HLA class I may still influence immunogenic epitopes on the molecular surface.

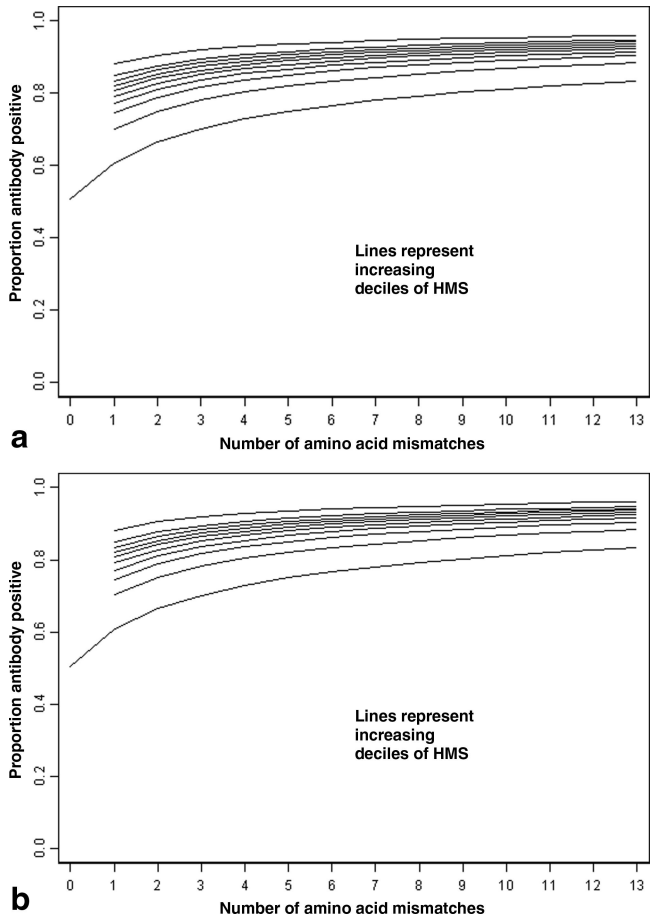

FIGURE 5. Relationship between the presence or absence of human leukocyte antigen (HLA)-specific antibody and the number of amino acid mismatches at different levels of hydrophobicity mismatch score (HMS) and electrostatic mismatch score (EMS). Sera from highly sensitized patient were assessed for the presence of HLA class I antibody binding, and the results are shown separately for HMS (Fig. 5a) and EMS (Fig. 5b). *Individual lines* are ranked in order of increasing deciles for HMS and EMS, respectively, with lowest line corresponding to lowest decile (amino acid mismatches ≥13 are grouped together). Regression lines corresponding to the second or higher deciles start from one amino acid mismatch because, by definition, HLA specificities with zero amino acid mismatches also have zero HMS and EMS scores.

DISCUSSION

The immunogenicity of a given donor HLA alloantigen varies according to recipient HLA type, but the ability to predict the magnitude of the likely alloimmune response has, until recently, been relatively limited. AA sequence analysis of HLA molecules now enables comparison of donor and recipient HLA type at the molecular level and has shown that the number of polymorphic AA differences at continuous and discontinuous sequence positions (as determined by HLAMatchmaker computer algorithms) predicts the immunogenicity of HLA mismatches, in terms of both alloantibody response (*14*,*17*,*34*) and renal transplant outcome (*35*). In the present study, we have shown for the first time that, in addition to the number of AA polymorphisms between mismatched HLA-A and -B specificities and patient HLA class I type, physiochemical disparities (hydrophobicity and electrostatic charge) between mismatched HLA molecules strongly influence HLA class I immunogenicity.

Duquesnoy (*15*) initially compared HLA compatibility at the structural level as determined by polymorphic triplet AA sequences (triplets) in antibody accessible positions of HLA molecules, and more recently refined this approach to incorporate polymorphic AA residues at discontinuous sequence positions (eplets) that are colocated within 3.5 Å on the surface of the tertiary protein structure of HLA. An integral assumption in these analyses is that an AA polymorphism at a given position in each potential mismatched donor HLA allele that is shared by any one of the different recipient HLA alleles will not invoke an alloimmune response (designated as interlocus AA sequence subtraction). We have shown previously that the structural information provided by the triplet and eplet HLAMatchmaker program enables prediction of acceptable mismatches and the magnitude of the antibody response as defined by antibody screening using the full repertoire of single-antigen HLA class I specificities in HSP awaiting renal transplantation (*16*, *17*). It is important that such studies evaluate not only the presence but also the magnitude of alloantibody response, because the magnitude of alloantibody response to donor HLA mismatches in recipients of a kidney allograft is closely related to both cellular and humoral rejection and the development of chronic allograft nephropathy (*18–20*, *36–38*).

The finding in the present study that simply enumerating the number of AA mismatches following interlocus AA subtraction provided a strong correlation with both the presence and magnitude of alloantibody to mismatched HLA-A and -B specificities was unexpected, and we had previously assumed that more sophisticated algorithms based on antibody binding models were required. The focus of our analysis is on alloantibody responses in HSP as a surrogate for HLA immunogenicity, and interestingly the predictive value of counting AA mismatches in this study was comparable with that observed in our previous studies using triplet and eplet mismatches to predict HLA class I immunogenicity (*16*, *17*).

The principal new finding from this study is that HLA immunogenicity, as determined by alloantibody response, is dependent not only on the number of AA mismatches but also on the physiochemical properties of the mismatched AA. Hydrophobicity and electrostatic charge were selected for analysis because of their fundamental importance in stabilizing the protein-protein interactions required to generate an immune response, and in particular, their role in determining the specificity and stability of antibody binding to target antigens (*21–23*). Antibody-antigen interfaces are composed of relatively large protein surfaces, and the binding energy of the interaction is directly related to the buried hydrophobic area (*39–42*). Electrostatic forces increase the rate of antibody-antigen complex formation by facilitating the initial association and aiding stability of the antibody-antigen interface (*24*, *25*, *43*, *44*). Within the interface, hydrogen bonds between juxtaposed polar residues and salt bridges are crucial for the specificity of binding (*45*, *46*).

Assignment of overall scores for differences in AA hydrophobicity and electrostatic charge for each mismatched HLA class I specificity, and incorporating interlocus subtrac-

tion, revealed that in addition to the number of AA mismatches between HLA class I and recipient HLA type, both the hydrophobicity and the electrostatic charge of mismatched AA influenced HLA class I immunogenicity. The hypothesis underpinning interlocus AA subtraction was supported here by the finding that intralocus instead of interlocus subtraction abrogated the correlation between the physiochemical scores and the alloantibody response. Intralocus sequence comparisons do not consider the presence and nature of AA polymorphisms located at the same sequence position across different HLA class I loci, and this may account for these observations. It should be noted, however, that when using interlocus AA subtraction, alloantibody responses were still occasionally observed against some HLA specificities with a zero AA mismatch. Similarly alloantibody responses were present against approximately 50% of HLA-A and -B specificities, where the physiochemical scores were in the first decile for HMS and EMS, although antibody levels were low overall and generally below the threshold considered as clinically relevant in terms of humoral rejection when undertaking kidney transplantation (*36*, *38*). Taken together, these observations suggest that AA identity at a particular sequence position, but on a different HLA-A, -B, or -C allele (although assumed to be nonimmunogenic) can still constitute an immunogenic epitope in the tertiary structure of an HLA class I alloantigen.

In conclusion, the number of AA mismatches between HLA-A and -B alloantigens and patient HLA class I type (HLA-A, -B, and -C) correlate with the development and strength of an alloantibody response. In addition, differences in AA hydrophobicity and electrostatic charge between HLA class I specificities provide additional predictive value for antibody responses that is independent of the number of AA mismatches. Mismatched HLA-A and -B specificities with a large number of AA mismatches and a high hydrophobicity or electrostatic charge mismatch score are more immunogenic and likely to invoke an alloantibody response, so may be best avoided. The two physiochemical mismatch scores (HMS and EMS) are equally predictive of humoral immune responses, and hence both parameters can be used to assess HLA class I immunogenicity. Further studies are being undertaken to determine whether the number and physiochemical properties of mismatched AA s correlate with kidney allograft survival.

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