



Predicted indirectly recognizable HLA epitopes presented by HLA-DR correlate with the *de novo* development of donor-specific HLA IgG antibodies after kidney transplantation

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ABSTRACT

Background: HLA class-I mismatches selectively induce antibody formation after kidney transplantation. The *de novo* development of donor-specific IgG HLA class-I antibodies may be dependent on the HLA class-II background of the patient by presenting T-helper epitopes within the recognized HLA class-I antigens.

Methods: The correlation between antibody production against mismatched donor human leukocyte antigens (HLA) class I and the number of HLA class II-restricted predicted indirectly recognizable HLA epitopes (PIRCHE-II) in the respective HLA class-I mismatches was investigated. To this end, we analyzed sera taken after nephrectomy from a cohort of 21 non-immunized individuals that received a renal transplant.

Results: Forty-nine HLA class-I mismatches were found which all contained immunogenic eplets according to HLA-Matchmaker. Donor specific HLA antibody responses were detected against 38 HLA class-I mismatches after nephrectomy. These mismatches were found to contain a larger number of PIRCHE-II when compared to mismatches which did not induce donor specific HLA antibodies. Most PIRCHE-II (68%) were not part of an eplet as defined by HLA-Matchmaker.

Conclusions: Our data suggest that presentation of donor-derived HLA class-I peptides by recipient HLA class-II molecules plays a significant role in *de novo* development of donor-specific IgG HLA antibodies.

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1. Introduction

Matching for human leukocyte antigens (HLA) significantly improves the outcome of kidney transplantation (reviewed in [1]). However, as a result of the high level of polymorphism of the various HLA loci and the limited number of donors, HLA mismatches between donor and recipient exist in approximately 85% of cadaveric kidney transplantations (Eurotransplant database; <http://www.eurotransplant.org>, accessed April 24, 2012). Evidently, these HLA mismatches frequently lead to production of HLA-specific antibodies, which shorten graft survival [2]. In order to prevent

antibody formation against HLA, the optimal kidney grafts are either HLA identical to the recipient, or express permissible HLA mismatches, which do not induce antibody formation.

To a certain extent, these permissible mismatches can be identified with the HLA-Matchmaker algorithm. This program defines polymorphic epitopes on HLA molecules, called eplets, accessible by HLA antibodies and subtracts those eplets present on the patients' own HLA [3,4]. In case this leaves no eplets to be recognized on a kidney graft, no antibody responses are to be expected [5]. Although HLA-Matchmaker predicts which HLA-antigens can potentially induce HLA antibody formation, it does not predict T-cell reactivity towards allogeneic HLA [6].

Proliferation and differentiation of antigen-specific naive B cells into memory B cells and plasma cells and class switching from the IgM to e.g. the IgG isotype requires cognate T-cell help [7]. The first step in this process is internalization of the antigen, possibly via the antigen-specific B-cell receptor [8]. Subsequently, via a phenomenon called linked recognition, the T-helper cells recognize epitopes from the antigen, presented as peptides in the groove of

Abbreviations: CDC, complement-dependent cytotoxicity; DSA, donor-specific antibodies; HLA, human leukocyte antigens; PRA, panel-reactive HLA antibody; PIRCHE, predicted indirectly recognizable HLA epitope; PIRCHE-II, PIRCHE presented by HLA class II.

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the HLA class-II molecules of the B cell. This antigen-specific recognition leads to signals to the B cells via cytokines and CD40/CD40L interaction [9].

The HLA-DR phenotype of the responder may play a determinative role in the immunogenicity of HLA antigens [10–12]. In previous studies it was shown that the HLA-DR phenotype of the responder influences the production of Bw4-specific antibodies and class-I antibody sensitization grade [10,12]. The production of Bw4-specific antibodies strongly correlated with the presence of either the HLA-DR1 or HLA-DR3 phenotype in the responder [10]. *In vitro*, a Bw4-derived peptide bound strongly to DRB1*01- and DRB1*03-expressing cells, while the corresponding Bw6 peptide did not. Similarly, HLA-DRB1*15:01 showed an enrichment in the production of HLA-A2 antibodies in HLA-A2-mismatched transplant pairs [12]. These observations suggest a role for indirect recognition of donor-derived HLA peptides on HLA class-II molecules of the antigen-presenting cells of the patient. This phenomenon would explain the role of T-helper cell responses in leading to the production of donor-specific antibodies (DSA) of the IgG isotype [13].

Binding of peptides to HLA molecules is predictable. The differences between predicted binding affinities and experimental measurements have been shown to be as small as the differences in measurements between different laboratories [14]. Predictability is particularly high for HLA class-I molecules, as these molecules have a more strict preference for nine amino acid long peptides (9-mers) and require specific amino acids as anchor residues at clearly defined anchor positions [15]. For HLA class II molecules predictability is lower, as peptides of different length can bind using different positions as anchor residues [16]. Therefore, it is difficult to determine how a peptide aligns to the HLA class II-binding groove and which amino-acid residues in the peptide are preferred as anchors. To solve this problem, Nielsen et al. used a so-called core-predictor to estimate how a peptide positions in the class II binding groove [17]. The core-predictor enabled the development of an accurate HLA class-II predictor, called NetMHCII [18].

To investigate the role of donor HLA-derived T-helper epitopes in the *de novo* development of DSA, we used NetMHCII to identify allogeneic HLA class I-derived, predicted indirectly recognizable HLA epitopes, HLA class II-presented (PIRCHE-II). We subsequently investigated whether the lack of specific HLA class I-derived PIRCHE-II explained the lack of antibody production in a cohort of 21 non-immunized individuals who received and lost their transplant and developed HLA class-I antibodies to some but not all HLA-mismatches.

2. Recipients, materials, and methods

2.1. Transplant recipients

We analyzed the entire cohort of 869 kidney transplants that were performed between 1990 and 2008 in the University Medical Center Utrecht, Utrecht, The Netherlands. From this cohort, we selected recipients whose kidney graft was removed and had no pre-transplant alloimmunizing event (non-immunized individuals), i.e. no pregnancy, no blood transfusions, and no previous organ or stem-cell transplantation. Recipient pairs that were fully matched for the HLA-A and HLA-B antigens were excluded, as they were not informative for this study purpose. One pair was excluded because no binding algorithm was available for the recipient's HLA class-II molecules. These selection criteria resulted in 21 analyzable recipient-donor pairs. For all donor-recipients combinations T-cell cross-match assays were performed using the basic NIH technique on unseparated peripheral blood mononuclear cells in the presence of dithiothreitol before transplantation. All cross-match results in were negative.

2.2. Samples

Serum samples were obtained at two time points. First, pre-transplant sera used for cross-matching was analyzed. Second, post-transplant sera were used which were obtained three months after transplantectomy. The reason for the latter time point is that at that time immune suppression was absent and antibody analysis was no longer influenced by any antibody filtering effect of the donor kidney. All sera of the recipients were obtained for purposes of regular panel-reactive HLA antibody (PRA) screening.

2.3. HLA typing

For each recipient, two independently collected samples were typed with different methods; one sample was typed serologically, using the conventional complement-dependent cytotoxicity (CDC) procedure using commercial typing trays (Biotest, Dreieich, Germany) and one sample was typed molecularly at intermediate resolution for the HLA class-I and -II alleles based upon the PCR-SSO technique in combination with Luminex using commercial reagents and following the instructions of the manufacturer (OneLambda Inc., Canoga Park, CA, USA). For donor typing, only one sample was available locally to perform both serological and molecular typing, following the identical procedure as for recipient typing. In all cases, donor typing in our center confirmed the HLA typing provided by the donor center. An additional high-resolution typing was performed for all recipients and donors of whom DNA was still available. For the remaining 5 individuals, all typing results were converted to the most likely high resolution typing based upon the reported HLA frequencies within the observed NMDP multiple allele codes [19]. In one case, this approach led to multiple options with a frequency of more than 10%; a B*44 could be converted into either a B*44:02 or a B*44:03 (Table 1). For this pair, data were analyzed for both possibilities and the results were averaged.

2.4. HLA antibody screening and characterization

Tests were performed to determine the presence or absence of HLA antibodies to HLA-A and -B using the Labscreen Single Antigen kits (OneLambda Inc.) following the standard manufacturer's guidelines. Beads were analyzed on a Luminex 200 flow cytometer (Luminex Inc., Austin, TX, USA). Results with an MFI of >1000 were scored as positive.

2.5. Identification of HLA class I-derived PIRCHE-II

For all mismatched HLA class-I molecules, the number of PIRCHE-II was examined to explain a potential antibody response to the epitope-containing HLA class-I antigen. PIRCHE-II were defined as recipient HLA class-II binding epitopes within the mismatched donor-derived HLA class-I molecule, that were not covered by any of the other HLA class-I antigens of the recipient. HLA class I-derived PIRCHE-II were predicted using the HLA class-II binding predictor NetMHCII-1.0, portable version [17]. This predictor is based upon the SMM-align predictor [18] to predict how a potential ligand aligns to the binding groove of an HLA class-II molecule, and it subsequently predicts how well the aligned ligand is expected to bind. If the predicted binding affinity was high ($IC_{50} < 1000$ nM) [20], the nine amino acids that aligned to the binding groove were defined as an HLA class-II epitope. We only distinguished identical versus non-identical peptides based upon the exact nonamer sequence that was predicted to occupy the binding cleft. Similar, but non-identical, peptides were considered as non-identical.

Table 1

Description of the transplant study group matching our inclusion criteria, extracted from a cohort of 869 kidney transplant pairs.

Nr.	HLA class I mismatch	HLA-DRB1 typing of recipient	Immunogenic alleles	Non-immunogenic alleles
1	A*32:01, B*15:17	DRB1*03:01, –	A*32:01, B*15:17	–
2	A*03:01, B*18:01	DRB1*03:01, –	A*03:01	B*18:01
3	A*02:01, B*41:01	DRB1*04:01, DRB1*07:01	A*02:01, B*41:01	–
4	A*01:01, A*24:02, B*39:06	DRB1*03:01, DRB1*15:01	A*01:01, A*24:02	B*39:06
5	A*03:01, B*07:02	DRB1*07:01, DRB1*13:01	B*07:02	A*03:01
6	A*02:01, B*57:01, B*49:01 ^a	DRB1*07:01, DRB1*15:01	A*02:01, B*49:01	B*57:01
7	A*01:01, B*08:01	DRB1*04:01, DRB1*15:01	A*01:01, B*08:01	–
8	B*44:02	DRB1*04:01, DRB1*11:01	B*44:02	–
9	B*40:01	DRB1*01:01, DRB1*04:01	B*40:01	–
10	A*24:02, B*18:01, B*35:03	DRB1*04:01, DRB1*07:01	A*24:02, B*18:01	B:35:03
11	A*03:01, B*07:02, B*15:01 ^a	DRB1*15:01, DRB1*03:01	A*03:01, B*07:02, B*15:01	–
12	A*24:02, B*44:02, B*39:06	DRB1*04:01, DRB1*11:01	–	A*24:02, B*44:02, B*39:06
13	A*24:02, B*39:06, B*51:01	DRB1*04:01, –	A*24:02, B*39:06, B*51:01	–
14	A*02:01, B*27:05	DRB1*09:01, DRB1*15:01	A*02:01, B*27:05	–
15	A*02:01, B*51:01	DRB1*12:01, –	A*02:01	B*51:01
16	A*01:01, B*07:02	DRB1*03:01, DRB1*15:01	A*01:01, B*07:02	–
17	B*51:01	DRB1*03:01, –	B*51:01	–
18	A*01:01, A*02*01, B*08:01, B44 ^b	DRB1*08:01, DRB1*11:01	A*01:01, A*02*01, B*08:01, B44	–
19	A*02:01, B*07:02	DRB1*07:01, DRB1*13:01 ^a	A*02:01, B*07:02	–
20	A*32:01	DRB1*03:01, –	A*32:01	–
21	B*57:01	DRB1*03:01 – DRB1*13:01 ^a	B*57:01	–

^a No DNA available for high resolution typing. Typing has been extrapolated from the serological broad typing.^b No DNA available for high resolution typing. Number of eplets/peptides calculated as average of B*44:02 and B*44:03.

2.6. Matchmaker analyses

HLAMatchmaker eplets were assigned to the HLA antigens based on HLAMatchmaker version 2.1 (<http://www.HLAMatchmaker.net>) [21]. Only the HLA-A, and -B loci were included in these analyses. The number of mismatched eplets was determined as the number of donor eplets that were absent in the recipient's HLA-A and -B locus.

2.7. Location of T-helper ligands and eplets

Different polymorphic residues within the HLA molecule may contribute to the different types of mismatches, i.e. as determined by either the eplet- or the PIRCHE-II method. To identify the polymorphic residues that were involved in eplets and/or PIRCHE-II, we analyzed the data obtained from our study cohort and a cohort of randomly generated virtual recipient-donor pairs. For the latter cohort, a virtual population reflecting the HLA-A/B/C/DR-haplotype frequencies in Caucasians was generated. These haplotype frequencies were obtained from previous studies [19]. To simulate a recipient-donor combination matching our local match profile, we accepted a maximum of three mismatches on the combined HLA-A and -B loci, and one on the HLA-DR B1 locus. The generation of virtual individuals was stopped when a total number of 10,000 virtual recipient-donor combinations that fitted these requirements, was reached. Subsequently, the mismatched HLA class-I antigens were assessed using the eplet method and the PIRCHE-II method as described above. Relative frequency plots were constructed based upon the location of eplets and PIRCHE-II within the HLA molecule.

To measure the overlap in position usage between the PIRCHE method and the eplet method, the usage of each amino acid by either methods was determined at those positions that are variable among MHC-I molecules. For both methods, the usage counts were normalized such that they sum up to 100%. The overlap between the counts was determined as the overlap between these normalized usage counts.

2.8. Statistical analyses

All mismatched HLA from the donor were separated into a group for which DSA were detected (immunogenic group) and a

group for which no DSA could be demonstrated (non-immunogenic group). Between these two groups, the number of non-self HLA class I-derived PIRCHE-II and/or the number of HLAMatchmaker triplets and eplet were compared using the Mann-Whitney *U* test (GraphPad Prism 5.03, GraphPad Software, Inc., La Jolla, CA).

3. Results

3.1. Overview of specificities

A total of 22 recipients matched the inclusion criteria (Table 1). In sera of 21 of them, donor-specific antibodies could be detected. A total of 38 immunogenic (18 HLA-A and 20 HLA-B) and 11 non-immunogenic HLA (3 HLA-A and 8 HLA-B) could be identified. These numbers were equally distributed (Chi-square test, data not shown).

3.2. Immunogenic HLA contains more PIRCHE-II

For all mismatched HLA we predicted the number of non-self HLA class I-derived PIRCHE that can bind to the HLA-DR molecules of the recipient. As shown in Fig. 1A, the immunogenic group contains a higher number of PIRCHE-II as compared to the non-immunogenic group ($p < 0.01$ in the Mann-Whitney *U* test). The mean values were 3.0 and 1.2, respectively. These differences were not observed when the mismatched donor-derived HLA antigens were analyzed against a scrambled recipient DR background (Fig. 1B), indicating that the DR background of the specific recipient plays a crucial role in the PIRCHE-II analyses.

3.3. Immunogenic HLA has increased numbers of triplets and eplets

The immunogenic group and the non-immunogenic group were compared for their number of triplets and eplets as determined by HLAMatchmaker. Both the number of triplets (Fig. 2A) and the number of eplets (Fig. 2B) are significantly higher in the immunogenic group than in the non-immunogenic group (triplets: $p < 0.005$; eplets: $p < 0.0005$ in Mann-Whitney *U* tests).

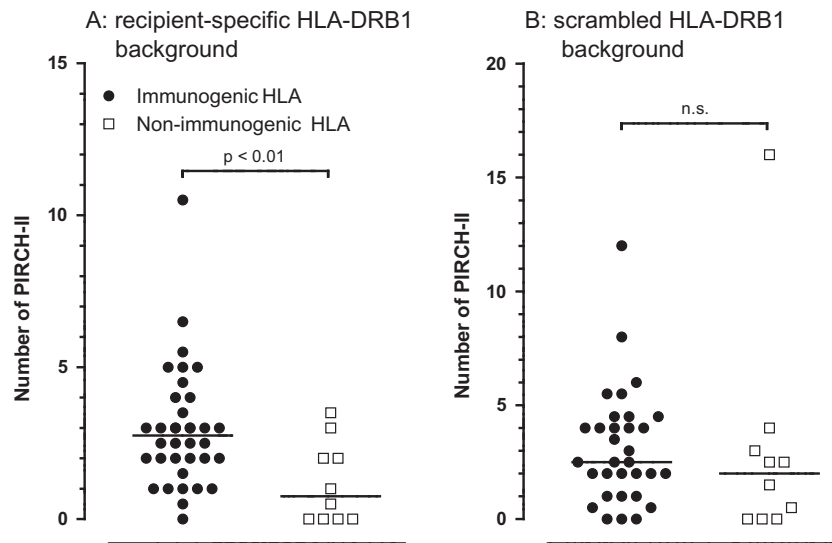


Fig. 1. Comparison of the number of PIRCHE-II in immunogenic (dots) and non-immunogenic (open boxes) HLA class-I molecules. (A) Higher numbers of PIRCHE-II were observed in the immunogenic HLA as compared to the non-immunogenic HLA. (B) No differences were observed when the mismatched donor-derived HLA antigens were analyzed against a scrambled recipient DRB1 background. The reported *p*-values are derived from the Mann–Whitney *U* tests.

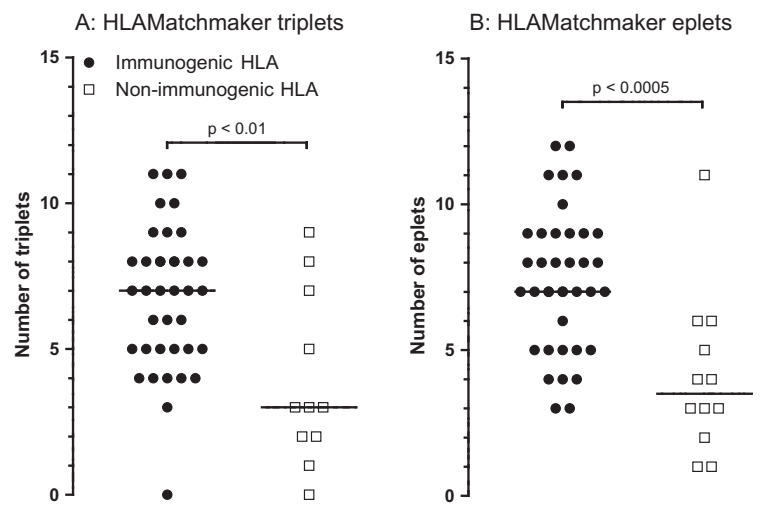


Fig. 2. Comparison of the number of HLAMatchmaker triplets (A) and eplets and (B) in immunogenic (dots) and non-immunogenic (open boxes) HLA class-I molecules. The reported *p*-values are derived from the Mann–Whitney *U* tests.

3.4. Eplets do not co-localize with PIRCHE-II

Based upon the shared biologic origin of eplets and PIRCHE-II, a correlation between the number of eplets and the PIRCHE-II is to be expected. To address this issue, we plotted the number of eplets against the number PIRCHE-II and performed correlation analyses. Correlations were observed both for the non-immunogenic HLA ($R^2 = 0.83$; significance of the slope: $p < 0.0005$) and the immunogenic HLA ($R^2 = 0.14$; significance of the slope: $p < 0.05$; Fig. 3A). The slopes of the two groups were overlapping each other.

We subsequently analyzed the topographic location of the PIRCHE-II in our study cohort, indicated by the position of the involved amino acids. The locations of these PIRCHE-II were compared to the locations of the eplets. The polymorphic amino acids of PIRCHE-II are highly overrepresented in the β -plated sheet and in the alpha-3 domain of the HLA protein (Fig. 3B), whereas the eplets are located on surface residues of the HLA protein, accessible to antibodies. These data were confirmed by simulation experi-

ments on 10,000 virtual transplant pairs (Fig. 3C), showing that a significant number of HLA class I-derived polymorphic amino acids (62%) can be identified as PIRCHE-II, while not being part of an eplet recognized by DSA.

4. Discussion

In the present study we applied a computational approach for HLA binding with subtraction of the self-HLA to explain donor-specific HLA antibodies on a strictly selected cohort; only those recipients were included that had no pre-transplant immunizing event and whose kidney graft was removed before analyzing the HLA antibodies. Although this selection led to a smaller study population, we could confine our analyses to a single immunizing event, i.e. the kidney transplantation. Moreover, this selection excluded influence of absorption effects of a residual donor organ, which

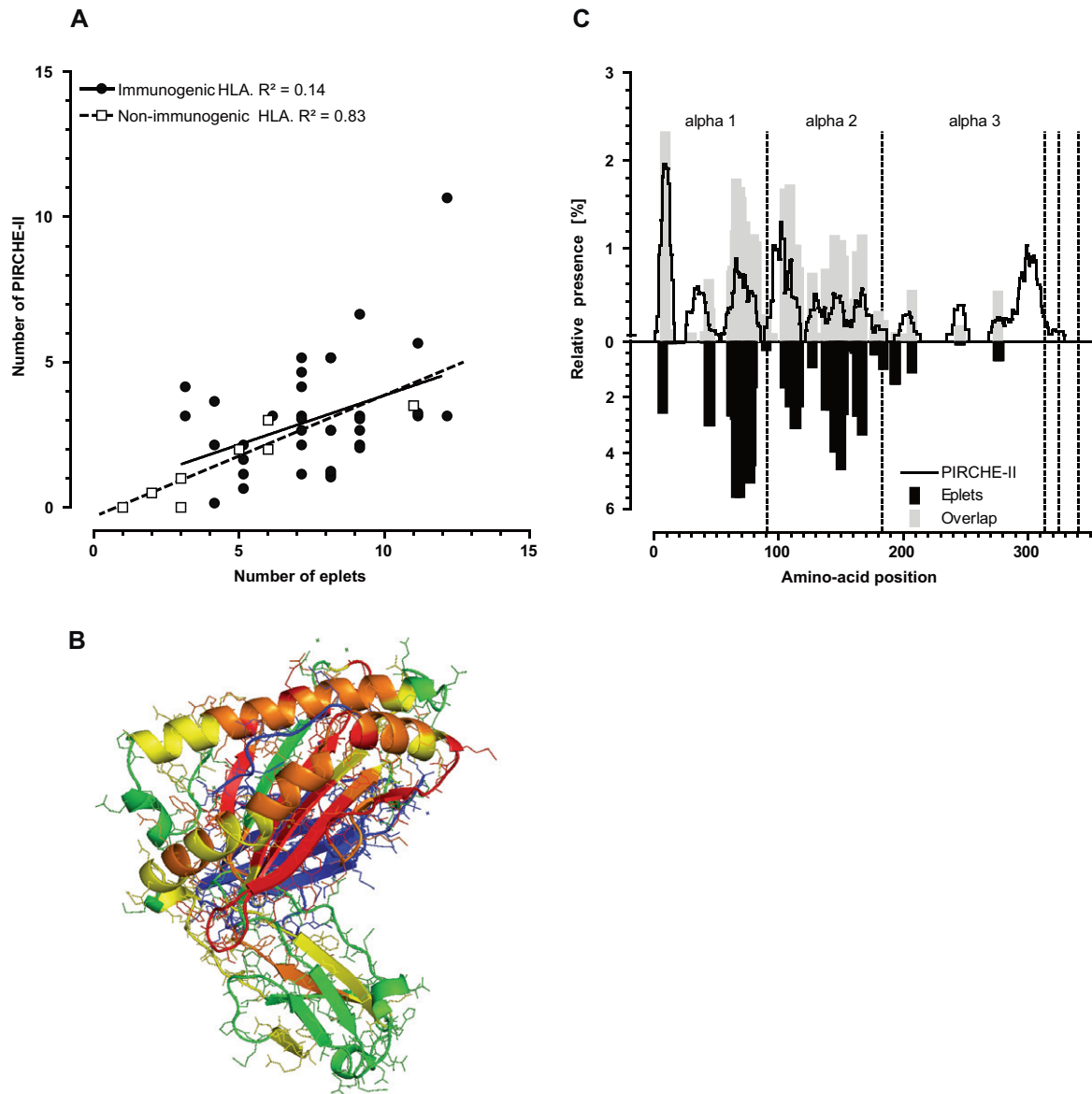


Fig. 3. Identification of eplets and PIRCHE-II as two separate entities. (A) Correlation between the number of eplets and the number of PIRCHE-II. Immunogenic HLA have been depicted as dots; non-immunogenic HLA as open boxes. The resulting regression curves have been depicted by solid lines and dotted lines respectively. The regression coefficient is based upon combined analysis of the two groups. Overlapping data have been shifted 0.1 units for visualization purposes only. (B) Location of PIRCHE-II on the HLA class-I molecule, as observed in the studied kidney transplant cohort. Colors indicate the relative presence of an amino acid in immunogenic HLA class-I antigen; (Green = 0, yellow = 1–4, orange = 5–9, and red = >10). The non-polymorphic beta-2m molecule has been depicted in blue. (C) Location of the PIRCHE-II (solid line) versus eplet-related residues (black bars) in the HLA class-I molecule, as observed in a virtual transplantation cohort of 10,000 simulated transplants. The eplet-related residues were defined as polymorphic residues present within 3.0 Angstrom eplet patches [4]. Overlap (grey bars) was calculated as the percentage of situations where an amino acid residue was present in both an eplet and a PIRCHE-II.

can hamper correct detection of donor-specific HLA antibodies in serum [22].

We demonstrate that immunogenic donor-derived HLA class-I antigens, defined as antigens towards which DSA are detectable, contain a higher number of epitopes that can be presented by HLA class-II molecules, PIRCHE-II, from the recipient. Evidently, the level of similarity between donor's and recipient's HLA class-I antigens may affect the number PIRCHE-II; the lower the similarity, the higher the chance to find non-self epitopes. To test the hypothesis that the recipient's HLA-DR rather than the level of similarity was explaining our observations, we counted the number of PIRCHE-II using a scrambled HLA-DR background. Scrambling was performed by pooling all HLA-DRB1 alleles in the study and assigning them randomly to the recipients. In these analyses, immuno-

genic and non-immunogenic HLA showed a similar number of peptides in the context of the scrambled HLA-DR background (Fig. 1B). Thus, the actual HLA-DR background of the recipient has to be taken into account to explain why certain mismatches are immunogenic or not. We therefore conclude that the recipient-specific HLA-DR background is essential in predicting the chance of developing DSA after transplantation and that the observed differences are due to differences in antigen presentation and not in similarity between the mismatches. These data also strongly suggest that the HLA mismatch effect and the PIRCHE-II effect are two independent parameters, both supporting the development of DSA.

Two factors may improve the outcome of our analyses; the quality of the HLA class II-binding prediction and the resolution

of HLA typing of recipient and donor. In NetMHCII, the HLA binding motifs are well-defined for 9 HLA-DR antigens including 11 different HLA-DR molecules. Thus, for a number of HLA-DR molecules the peptide binding characteristics have not been determined. Given the high level of diversity in HLA-DRB1 alleles, characterization of each individual HLA class-II binding motif via peptide-screening binding assays is not feasible. Therefore, an alternative algorithm, NetMHCIIpan, has been developed via a computational approach [23]. NetMHCIIpan can define binding motifs on the basis of the primary amino-acid sequence, providing information for DR molecules for which limited experimental binding data have been reported [23]. For our data set, NetMHCIIpan would only provide a better prediction for the HLA-DRB1*13:01-encoded molecule. As such, analyses with NetMHCIIpan did not enhance the performance of our models (data not shown). Apart from these two improvements, the analyses may well benefit from future studies on a better definition of which similar peptides should be considered as immunologically identical.

In the present retrospective study, low resolution typing data could not be extrapolated to high resolution HLA typing for 3 donors and 2 recipients. In these cases, the results from all likely options were averaged (in case of donor typing) or both subtracted (in case of recipient typing). This approach may have led to an incorrect assignment and subsequently to an underestimation of the effect the number of T-helper ligands on the induction of specific antibodies. Thus, the effect of PIRCHE-II on the production of anti-HLA IgG antibodies may be stronger than currently reported. Extended analyses on high-resolution typed recipient-donor are required to estimate the magnitude of the effect in more detail.

In the kidney transplant situation, the HLA antigens are derived from an external source and not from within the recipient cells. As such, the concept of linked recognition is the most plausible model. Nevertheless, T-helper cells may be activated by HLA alloantigens via bystander helper effects. Thus, B cells may present T-helper epitopes from one mismatched HLA antigen while recognizing and producing antibodies directed towards another mismatched HLA antigen. This model may explain why low numbers of PIRCHE-II were observed for mismatched HLA antigens while these mismatched antigens still caused HLA-specific antibodies. The additional implementation of this aspect in our predictive model, may lead to a better clarification of antibody production. Extension of the cohort is essential to address this issue.

Both the PIRCHE-II described in this study and the eplets as determined by HLA-Matchmaker are based upon the same phenomenon; mismatched amino acids in the HLA antigens of recipient and donor. As such, these two parameters cannot be fully dissected. However, although immunogenic HLA show higher numbers of eplets/triplets than the non-immunogenic HLA, various aspects of our analyses indicate that PIRCHE-II act, at least partly, independently from the number of eplets/triplets. First, we show that the actual HLA-DRB1 background is essential; when using a scrambled HLA-DRB1 background, no correlation with immunogenicity was found (Fig. 1B). Second, while there is a strong correlation between the number of PIRCHE-II and the number of eplets when analyzing the non-immunogenic group, this correlation is much weaker in the immunogenic group (Fig. 3A). Third, the physical locations of amino acids that are included in potential T-helper ligands are differently distributed when compared to the locations of eplet-involved amino acids (Fig. 3B–C); the alpha-3 domain and the N-terminal part of the alpha-1 domain seem to be enriched for PIRCHE-II, while they rarely result in eplets. The region around amino acid position 300 is for example containing only PIRCHE-II, but no eplets (Fig. 3C). Based upon the 10,000 simulated transplant pairs, the overlap between eplets and PIRCHE-II is only 38%. Taken together, we conclude that these two parameters are

complementary to each other when predicting the chance of DSA development.

Indirect recognition of donor HLA class I presented by recipient HLA class II in the production of post-transplant DSA led to a better predictive value in our retrospective study. The fact that both parameters complement each other for better predictability of developing post-transplant DSA, is in agreement with immunobiological concepts on IgG antibody formation. The finding of separate physical locations for antibody epitopes (eplets) and polymorphic class I-derived T-helper epitopes may have relevance for our understanding of 'linked recognition'. To our knowledge, the present study is the first report on this issue regarding clinically relevant allo-antigens and requires a functional confirmation. Although still hard to produce and validate for all HLA class-II molecules, HLA-tetramers would be the ideal reagents to follow-up the development of T-helper cells that indirectly recognize these HLA-derived epitopes. Prospective collection of blood samples of kidney transplant recipients has started recently to facilitate such analyses.

In summary, we show that the *de novo* development of donor-specific HLA IgG antibodies correlates with the number of HLA class I-derived PIRCHE-II and with the number of HLA-Matchmaker eplets in the mismatched HLA class-I molecules of the donor. Topographic analyses and scrambling of the HLA-DRB1 background strongly suggest that these two phenomena result from two in part independent entities. Therefore, presentation of donor-derived HLA class-I peptides by recipient HLA class-II molecules may be an important mechanism in IgM-to-IgG isotype switching of donor-specific HLA antibodies. As this concept may lead to a better definition of permissible HLA mismatches in organ transplantation, large clinical studies have been initiated to evaluate the effect of this mechanism on graft survival.

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