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A Short History of B-Cell HLA Epitopes

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Abstract
Background: HLA epitopes are currently in the focus of transplantation immunogenetics. The main reason is the complexity of the HLA system with >38,000 alleles, the number of which increases steadily. These alleles are determined by the current state-of-the art typing methods like second- and third-generation sequencing. Screening for HLA antibodies is hampered by the lack of specific target beads with all possible alleles described. ***Summary:*** A way to circumvent the problem is to define HLA epitopes. The number of antibody-confirmed epitopes, on the other hand, was found to be 72 for HLA class I and 74 for HLA class II. Here, we elaborate on the current knowledge on these HLA epitopes. Absolute definitions of these structures are not yet available. ***Key Messages:*** Making use of eplets is a comparable way allowing statistical analyses. However, one should keep in mind that the results obtained are approximative or perhaps better associative. Continuous collaboration is needed for the full understanding of the HLA epitopes. The reactivity toward epitopes remains patient-specific.

Introduction

Antibodies recognize a partial subregion of an antigen, which is termed epitope. Consequently, only a few amino acids of the antigen are "seen" and hence determine the specificity. The surrounding amino acids interact with the stabilization of the antigen-antibody complex. These few amino acids although specific are in many of the cases found on several HLA class I or II molecules. Consequently, HLA antibodies interact with numerous HLA molecules and not a single molecule [1, 2].

Historical Overview

The history of HLA [3] describes not only the finding of the different antigens but most important also the way how to describe them following strict rules of nomenclature: The first International Histocompatibility Workshop (IHW) and all other workshops following led to the establishment of the HLA nomenclature [3]. The names were based on the reactivity of specific alloantisera-recognizing protein structures on the surface of lymphocytes [4]. These structures were shown to be able to present peptides to T cells [3]. Antibodies in sera of multiparous women, patients, or planed immunization probands were used to define the HLA antigens. The analysis of the sera alloreactivity made it obvious that relationships between the different antigens exist [5]. The term cross-reactivity was used by most authors to describe HLA antibody specificities. One must keep in mind that sera contain several different antibodies recognizing a specificity. In the USA, the term CREG (cross-reactive groups) was widely used as introduced by Fuller and Rodey [6]. In Europe, Darke and his colleagues reported in several publications on the relationship between the different antigens in terms of antibody specificity [7]. For the definition of the relationships, besides extensive HLA antibody screening using the now outdated complement-dependent cytotoxicity assay, other T-cell-specific cellular assays were used [8]. The latter has applied

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Table 1. Numbers of different confirmed epitopes for HLA class I (data source: *epregistry*)

HLA-locus	Alleles, *n*	Different epitopes, *n*	Unique* epitopes, *n*
A	86	36	
B	138	39	
C	71	24	
Total amount	295	99	72

*Without "*shared*" epitopes.

Table 2. Numbers of different confirmed epitopes for HLA class II (data source: *epregistry*)

HLA-locus	Alleles, *n*	Different epitopes, *n*	Unique* epitopes, *n*
DRB1	97	33	
DRB345	11	20	
DQA1	17	8	
DQB1	25	19	
DPA1	7	3	
DPB1	56	9	
Total amount	213	92	74

*Without "*shared*" epitopes.

for the definition of subtypes of some antigen groups. Finally, adsorption elution assays were used to identify in terms of recognition of different antigens by the same antibody [9–12]. In the meantime, protein and DNA sequences of different HLA antigens became available, and comparison between the protein sequences and the antibody reactivity was observable [2].

El-Awar et al. and Kosmoliaptsis et al. [13, 14] established algorithms for the definition of these sequences. The names are triples; TerEps, as a tribute to Paul Terasaki (Terasaki defined epitopes); eplets; or single amino acid change. In general, these are humoral epitopes in contrast to the cellular epitopes [15, 16]. According to Duquesnoy [17], "Eplets are small configurations of polymorphic amino acid residues on human leukocyte antigen (HLA) molecules and are considered as essential components of HLA epitopes recognized by antibodies," while Duquesnoy et al. mention that "Epitopes are defined structurally by three-dimensional molecular modeling and amino acid sequence differences between HLA antigens" [18]. The defined eplets are the part of the antigen, which are directly specifically primarily recognized by the antibody. Amino acids in the vicinity influence the binding and contribute to the stability of the complex comprising the term epitope. Furthermore, the peptide bound in the groove of the HLA molecule additionally influences the binding [19].

Since then, many reports described such epitopes [20–25] with the intention to use them in a later stage for diagnostic purposes. Already now, the importance of these epitopes in the clinical kidney transplantation has been reported by Kardol-Hoefnagel et al. [23].

In the earlier days, cross-reactive graphs have been established to facilitate the laboratories in typing and screening, for example [26]. Nowadays, EpiArt has been developed [27] to visualize the relationship of the alleles in an allele group (manuscript in preparation).

The New Era

From the finding and describing of the first epitopes in the HLA system, the 4a/4b dimorphism – representing the Bw4 and Bw6 antigenic determinants [28] – several epitopes have been described to date and are summarized in the HLA eplet registry (epregistry) [29]. Two different types of epitopes are described: the theoretical and the antibody-confirmed epitopes. The introduction of the second-generation sequencing method for typing, the establishment of the IMGT/HLA data base [1], the allele frequencies data base [30], and the data provided by the different groups worldwide attempted to visualize the "cross-reactivity" of the alleles of the HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB345, HLA-DQA1, HLA-DQB1, HLA-DPA1, and HLA-DPB1 loci. Furthermore, we used data of Lehmann et al. [31] for DQA1, DPA1, and DRB345 frequency calculation. The Tables 1 and 2 show the antibody-defined epitopes from the eplet registry for HLA class I and II [29].

Tables 1 and 2 depict the uniqueness of the different epitopes (eplets) for the different loci. The number of different epitopes for the class I alleles analyzed here is 72, the same as the number for all class II alleles. Figure 1

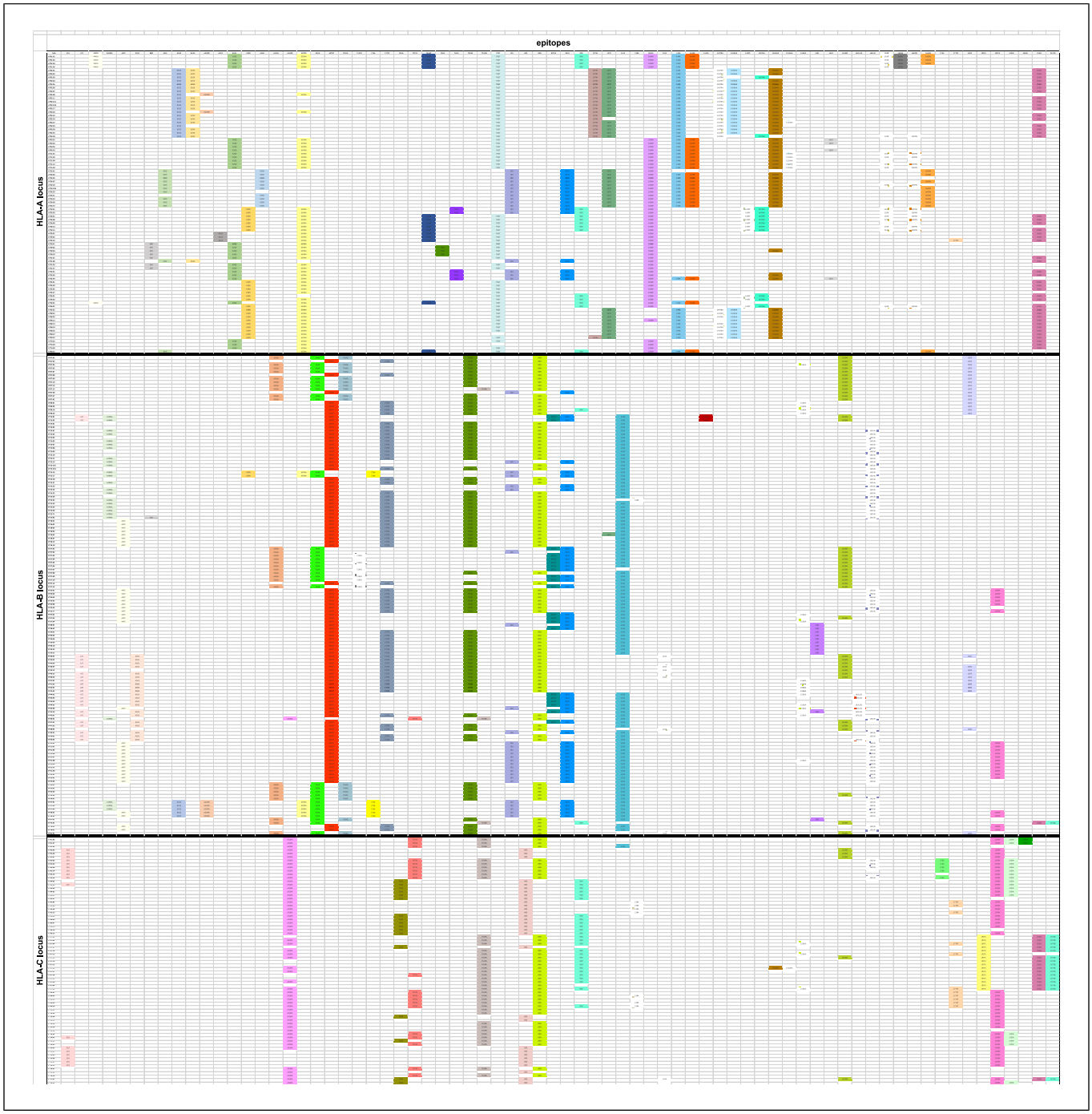

Fig. 1. Antibody-confirmed epitopes (eplets) for HLA class I generated from *epregistry*. Identical colors within a column indicate the same epitope. The epitopes of an HLA allele (frequent in German population according to [30, 31]) displayed in a row. For a more detailed view, see also online supplement Fig. 1.

visualizes the position of the different epitopes with respect to the HLA-A, HLA-B, HLA-C alleles in a German population sample of >3 million individuals. Figure 2 shows the respective epitopes for the class II determinants of HLA-DRB1, HLA-DRB345, HLA-DQA1, HLA-DQB1, HLA-DPA1, and HLA-DPB1 for the same population. Here, all alleles with a frequency over 0.00001 are included and extracted from "Allele Frequency Net database" [30] (for a total of >3 million HLA typed individuals). Following the colors, which represent the same epitope on the given position, it is obvious that many of the alleles express the same epitope, a fact already known. There are alleles of HLA-A sharing the same epitope as some HLA-B alleles, for example, 80I and 82LR; similarly, HLA-B and HLA-C (76VRN, 80N, and 193PV) and HLA-A and HLA-C (177KT, 150AAH, and 151AHA).

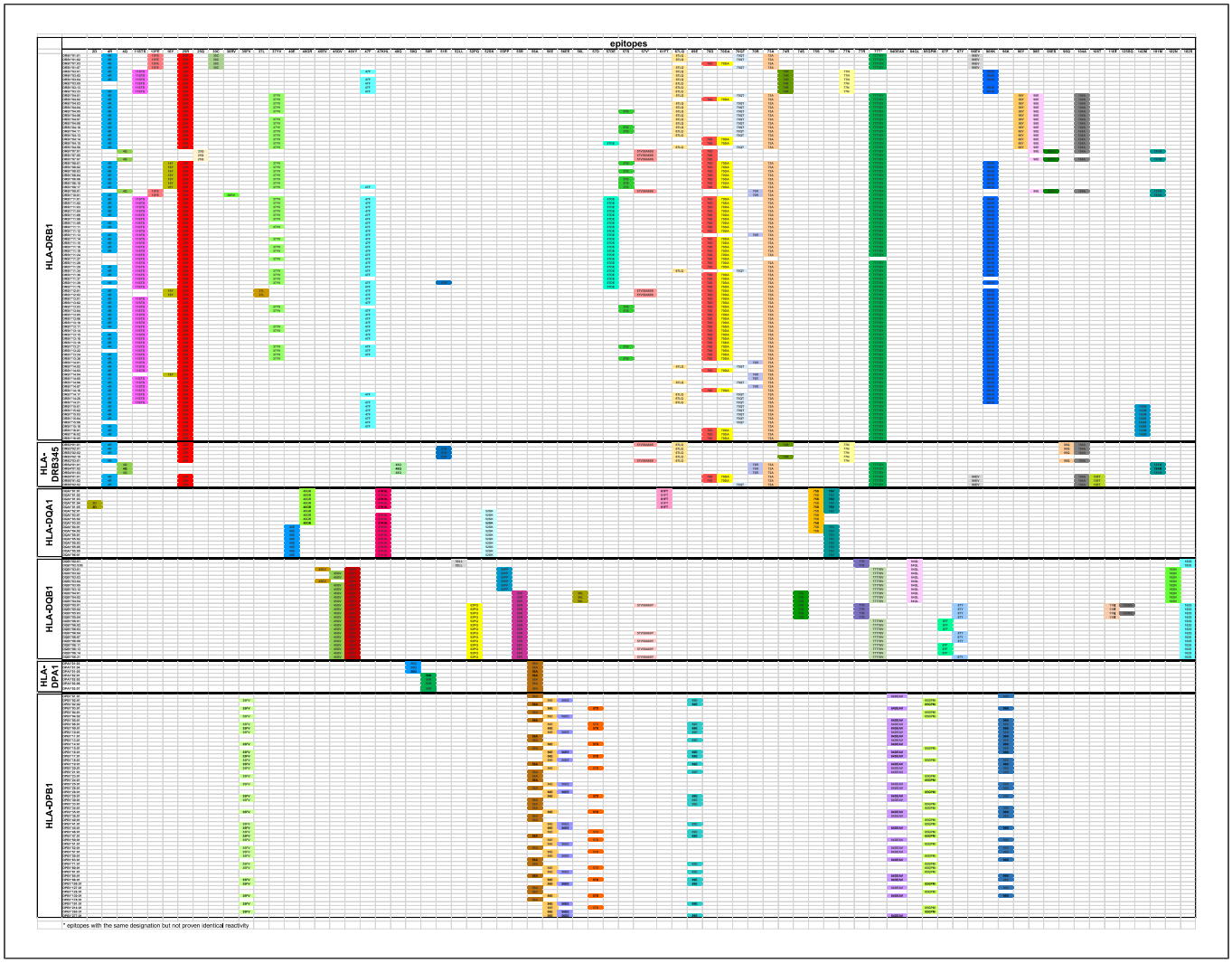

Fig. 2. Antibody-confirmed epitopes (eplets) for HLA class II generated from *epregistry*. Identical colors within a column indicate the same epitope. The epitopes of an HLA allele (frequent in German population according to [30, 31]) displayed in a row. For a more detailed view, see also online supplement Fig. 2.

The same holds true for the patterns observed in the HLA class II (Fig. 2). For practical reasons, these tables can be used for the definition of unacceptable epitopes with respect to organ transplantation (shown in online suppl. Fig. 1, 2; for all online suppl. material, see https://doi.org/10.1159/000538447). The occurrence of the same epitope expressed on different alleles allows the definition of unacceptable epitope mismatches and explains the reactivity of some alloantisera [22]. The data also show that some epitopes of the HLA class II determinants are found on different loci, for example, 57V (DRB1, DRB3, DQB1) and 77T (DRB1, DRB45, DQB1). In the past, this was known for HLA-DR and HLA-DP [32] but however not yet for HLA-DR/-DQ epitopes. The explanation is that the used nomenclature is inconsistent. The full description of the 57V eplet in DRB1 is 57V58A60S and for DQB1 57V58A60Y. Interestingly, DRB1 and DRB345 share same epitopes, though no formal proof is present that an antibody will bind to both. The Tables 1 and 2 and figures (Fig. 1, 2; online suppl. Fig. 1, 2) presented here might offer laboratories in the field of immunogenetics and transplantation, a needed tool for the daily work. It is for no saying that both eplet analysis and epregistry and also the figures shown here are subject of change depending on the ongoing testing. The eplets were defined primarily theoretically. With the introduction of the term "antibody-confirmed," a better understanding was achieved. However, with the ongoing testing, better and more accurate analyses will be done: The eplets in their definition follow the route of the HLA nomenclature. Schawalder et al. [20] and Hönger et al. [21] elaborated on the immunogenicity of selected eplets in transplantation and pregnancy. Kardol-Hoefnagel et al. [23] worked on the clinical relevance of the epitopes, although low-

resolution typing was used, and finally, Rushakoff et al. [33] presented data on the effect of homozygosity on antibody production in kidney transplantation, converting allele data in antigen data.

Conclusion

Defining HLA epitopes circumvents the problems of the ever increase in numbers of the HLA alleles worldwide. They can be used for a variety of purposes, especially for organ and stem cell transplantation since the latter is steadily using HLA mismatched donors. The accurate definition of these epitopes will soon allow better predictions with respect to their influence on patient and graft survival. However, one should keep in mind the individuality of the patient (solid organ) or donor (stem cell): Although several authors show a statistical effect of the total number of epitope mismatches in the context of transplantation [34, 35], the important role is the individual epitope which acts for the patient as the immunogen, as Dankers et al. [36] and Doxiadis et al. [37] reported earlier. This individuality of the patients might explain the differences in the results when different populations are analyzed. For the future, organ allocation organization should aim in the definition of donor-specific epitopes for the population they serve. These values would be used instead of the currently used v-PRA [38] or c-PRA [39].

In our opinion, prior to making statements on the immunogenicity of epitopes, the frequency of the epitope in the tested population (epiPRA %) should be considered and for which patient phenotypes the epitope is prone to antibody production. With state-of-the-art HLA typing methods and a more advanced understanding of epitopes, a much more individualized organ allocation would be possible in the future.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

C.L. and I.D. had substantial contributions to the conception or design of the work. C.L., H.L.-W., N.L., and I.D. performed the acquisition, analysis, or interpretation of data for the work and contributed to drafting the work or reviewing it critically for important intellectual content. Furthermore, C.L., H.L.-W., N.L., and I.D. agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved and gave the final approval of the version to publish.

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