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Editorial

HLA antigens to epitopes: Meeting the challenge

This special edition of Human Immunology provides a historic overview of the journey the HLA field has traveled since its inception, focusing on the prominent role of HLA epitopes in histocompatibility testing. Additionally, it also includes a sampling of current research and predictions of how HLA epitope analyses may improve the outcome of allogeneic organ transplantation.

The concept of an epitope is not new. Epitopes were presented as an explanation for cross reactive groups (CREGs) by Rodey, Fuller and colleagues in the early 1990s [1,2]. This was at a time when the amino acid sequence of HLA proteins had not been fully elucidated and, as such, CREGs were serologically characterized.

This volume begins with historical perspectives on HLA epitopes from two major contributors to the literature after sequence information was fully available. Each approached this topic from different perspectives. Subsequently, there was a meeting of the minds. Rene Duquesnoy's approach was theoretical. He identified short clusters of one to three polymorphic amino acid residues on HLA antigens (named an eplet) within 3.0 to 3.5 Angstroms of each other. Eplets were associated with antibody specificity and predicted, when mismatched, the induction of an antibody response (reviewed in reference [3]). Eplets were named, for the most part, by the position of the first polymorphic amino acid in the HLA protein with the single residue code [4]. This scheme was used separately for each HLA locus. At the same time, Nadim El-Awar and other investigators in the Terasaki laboratory took a different approach to epitope identification: They directly verified epitopes by performing adsorption-elution studies using genetically engineered cell lines expressing single recombinant HLA molecules. (An epitope, as used herein, refers to the eplet plus nearby amino acids that may influence antibody responses.) Alloantisera, monoclonal human antibodies and Luminex single antigen beads were all used in these studies. The nomenclature the Terasaki group devised for these antibody verified epitopes arbitrarily assigned numerical values of 1–1000 for Class I, 1001–2000 for HLA-DR, 2001–3000 for HLA-DQ, 4001–5000 for HLA-DP (reviewed in reference [5]). The two approaches to nomenclature were discussed at the 2009 ASHI Annual Meeting during the Scientific Affairs Committee meeting. (AJN was chair of the meeting, MK was a participant.) It was resolved that an HLA Epitope Registry would be implemented using the eplet designation of Duquesnoy as described above but with verification by techniques used in the Terasaki laboratory as well as other laboratories (https://www.epregistry.com.br/).

The remaining articles in this issue address a number of important aspects of HLA epitopes with the ultimate goal of extending the longevity of allografts. One noteworthy subject is practical efforts to provide accurate two field (high resolution) HLA typing for the unequivocal identification of a deceased donor's mismatched eplets to his/her recipient. It is now well understood that different alleles in the same serologic group may express different eplets, for example, DRB1*04:01 compared to DRB1*04:02. If a patient had antibodies to eplet 70DA they would be compatible with a kidney from a donor that was DRB1*04:01 but incompatible with a donor that typed as DRB1*04:02 [6]. At issue is the ability to achieve two field HLA typing resolution in a timely manner for deceased donors. Current HLA typing methods in widespread use are unable to routinely obtain this objective. Many articles in this issue advocate the use of imputation to obtain two field resolution and provide comparisons of different algorithms to obtain a high degree of accuracy (90–99%). [7–11]. The argument for using imputation instead of sequence based typing (SBT) is that the latter method cannot be accomplished in a timely manner for deceased donors. However, Sherwood et al. [11], review a SBT method (Nanopore technology) that can be performed in just 6 h from DNA isolation to analysis. If other clinical laboratories can routinely replicate these findings then it holds promise as a game changer. Of course, questions of cost of equipment and staffing must also be considered in placing this technology into universal practice. Also to be considered: is 100% two field resolution provided by SBT absolutely necessary or will improved imputation methods with >99% accuracy be sufficient?

Another important matter addressed by reports in this issue [8,10,12,13] is whether the number of mismatched eplets (the so-called eplet load, [12]) is sufficient criteria for assignment of donor organs or whether some eplets may induce more (or less) vigorous immune responses and should be factored into the "calculations." The identification of mismatched eplets is already in clinical practice utilizing the HLA Epitope Registry and HLAMatchmaker, as well as new software as presented in this issue [9,13]. While the number of eplet mismatches has correlated with graft loss, in certain cases the specific eplet mismatch may be more important than the total number of eplet mismatches. Of course, the greater the number of mismatches, the more likely they will include a potent immunogenic eplet mismatch.

Categorization of the immunogenic properties of eplets is complicated by the potential influence of alternative amino acids within 15 Angstroms, as shown in Figure 2 of reference [10] in this issue. Additionally, the structural basis for a human monoclonal antibody binding to HLA-A*11:01 was recently reported [14]. A 2.4 Å resolution map of the binding interface of this antibody on HLA-A*11:01 provided insight into the paratope–epitope relationship between the alloantibody and its target HLA molecule. Nota-

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bly, it showed that the eplet prediction algorithm accurately identified a key residue that forms part of the epitope. In addition to Asp90, it was shown that the fine specificity of the alloantibody was also impacted by Arg14. This finding illustrates the complexity in identifying residues that form the entire epitope without a map of the binding interface of the antibody.

A further challenge regarding the potential immunogenicity of HLA eplets is the response of CD4+ T cells in the induction of IgG anti-HLA antibodies and the generation of allospecific CD8+ cytolytic T cells [15]. At an individual level, exposure to viral pathogens shapes the immunologic repertoire, producing T cell memory to cross-reactive viral – HLA epitopes. For example, elegant studies by Kolle et al. [16], demonstrated that a T cell clone able to lyse Herpes Simplex Virus infected cells was also able to lyse uninfected HLA-B44 positive cells. In such a circumstance, even a well-matched eplet pair may, nevertheless, be overcome by T cell memory.

Another concern that may affect T cell reactivity involves epitopes in or near the peptide binding groove. They may influence whether mismatched HLA-derived peptides are able to bind to HLA class II molecules of the recipient, thereby inducing indirect T cell recognition. Algorithms that predict mismatched HLA-derived peptide binding to HLA class II molecules have shown promise in helping to predict alloreactive risk for individual patients [17].

It is likely that personalized medicine approaches to organ transplantation of individual donor - recipient pairs will require the use of novel machine learning tools to integrate many immunologic factors, of which eplet matching/mismatching is one important consideration. Until then, transplant programs will need to rely on currently available methods to evaluate eplet mismatches.

A considerable number of regulatory standards will be required regarding histocompatibility testing for HLA epitope matching. These include standards for HLA typing, assessment of sensitization, and use of cPRA/listing of unacceptable antigens for kidney transplant candidates. HLA eplets are likely to be included as factors in CMS standards. In fact, as a practical matter, the process began on June 17, 2021, (https://optn.transplant.hrsa.gov/media/3839/2020-06_histo_policy_notice.pdf). Specifically, in the Waitlist and KPD unacceptable antigens sections of the UNOS, there is an option to select HLA-DPB1 eplets. Selection of an unacceptable DPB1 eplet results in the listing of the corresponding unacceptable donor HLA DPB1 alleles. The use of eplet analyses for pretransplant donor specific antibody determination and crossmatching are also under CMS guidance.

Clearly, the reports in this special issue of *Human Immunology* underscore the many additional studies needed to optimize the potential benefits of HLA epitope matching in transplantation. Furthermore, the recent ground-breaking pig to human heart xenotransplant is now on the radar of the histocompatibility community, which will undoubtedly begin to develop tools to assess antibodies to porcine MHC epitopes.

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