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Towards the identification of the relative immunogenicity of individual HLA antibody epitopes

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ABSTRACT

Recent data suggest that HLA epitope matching is more predictive for the formation of donor specific antibodies (DSA) after transplantation than classical HLA antigen matching.

A proper definition of the epitopes and their immunogenicity is crucial for a broad clinical application of epitope matching in solid organ transplantation.

For that purpose, a novel epitope component was started during the 17th International Histocompatibility and Immunogenetics Workshop. Only a limited number of antibody data was available, resulting in a preliminary analysis confirming the potential relevance of epitope matching for the development of donor specific antibodies. During the actual workshop sessions the participants mainly discussed the future strategy to collect and analyze a large number of data in the framework of the 18th International Histocompatibility and Immunogenetics Workshop, which will take place in 2021. All practical issues can be found on the website www.ihiw18.org.

Data collection will already start in early 2019 in order to have sufficient time for detailed analyses using several different algorithms.

1. Introduction

For many years HLA matching has been successfully applied to enhance graft survival in renal transplantation. Due to the introduction of more efficient immunosuppressive drugs, the impact of HLA matching on graft survival has diminished. Nevertheless, the effect on graft survival is still significant and especially patients who develop de novo donor specific HLA antibodies (DSA) after transplantation have a significantly poorer graft survival [1,2]. While the degree of HLA antigen mismatching plays a determinative role in the chance that a patient starts to make DSA, not every HLA mismatch will lead to the induction of DSA. Some HLA mismatches are more immunogenic than others [3]. High resolution molecular HLA typing has been instrumental in providing insight in the immunogenicity of the different HLA antigen mismatches. Comparison of the amino acid composition of the HLA alleles with the reactivity of human monoclonal antibodies has led to the actual identification of the antibody epitopes [4]. It is clear now that an HLA allele can be considered as a unique set of antibody epitopes while the individual epitopes are shared between different HLA alleles [5]. As a consequence, an individual HLA mismatch can carry many or only a few, or even no foreign epitope compared to the epitopes present on the patient's own HLA alleles. Recent data show that matching for epitopes expressed on the HLA molecules is a better strategy to prevent the development of de novo DSA compared to classical HLA antigen matching [6]. The number of foreign epitopes is a determinative factor with respect to the chance that patients start to make antibodies against an HLA allele mismatch. So far, epitopes on HLA alleles have mainly been defined as eplets, a cluster of a few amino acids near to each other located on an antibody accessible site of the HLA molecule [7]. However, a similar effect on the immunogenicity of a mismatched HLA allele has also been observed for the number of amino acid mismatches or the difference in physico-chemical properties [8]. Importantly, it is to be expected that not every individual amino acid polymorphism or eplet mismatch has the same impact on the immune response of the recipient. This could be due to different, not mutually exclusive mechanisms. Firstly, the physico-chemical properties of the amino acid substitutions can lead to situations in which the amino acids involved are very much alike or are very different with regards to charge and hydrophobicity, amongst others. Secondly, in order to produce IgG antibodies, B cells require T cell help through linked recognition. If a foreign HLA antigen contains a B cells epitope, but lacks the accompanying T cell epitope, no IgG DSA formation will occur. The PIRCHE software enables the prediction of the number of potential epitopes derived from a mismatched HLA antigen that can be

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presented to the CD4+ T cells of the potential antibody producer [9]. The aim of this workshop component was the determination of the relative immunogenicity of individual eplet and aminoacid mismatches. Preliminary data suggest that the immunogenicity of individual polymorphisms may differ [10,11]. In order to be able to address this question in a more solid way, the development of DSA in a large number of high resolution typed donor-recipient combinations should be studied.

2. Materials and methods

Two approaches were described as a basis to define the immunogenicity of HLA epitopes:

2.1. Project 1: differential immunogenicity of HLA epitopes

Aim: Not every epitope mismatch has the same impact on the induction of antibodies. Some mismatches seem to be more immunogenic than other ones.

Aim of this component is to define the immunogenicity of the individual epitopes.

Study group: Recipients of a first renal transplant, who are non-reactive in solid phase assays before their transplantation and who have rejected their graft and need a re-transplantation. In case of female patients, pregnancy is an exclusion criterion, irrespective of antibody status.

Information needed: The serum sample after graft rejection must be tested in luminex using single antigen beads with exclusion of a prozone effect. Raw data of the screening results must be available. HLA typing information (High Resolution) of both donor and recipient (linked to the NGS project of the workshop).

Molecular HLA typing of donor and recipient should include Class I – A, B, C and Class II – DRβ1/3/4/5, DQα/β, DP α/β.

Analyses: HLA mismatches will be translated in (antibody verified) "epitopes" and the frequency of antibody formation against these epitopes will be analysed.

2.2. Project 2: acceptable epitopes

Aim: Characterization of epitopes toward which highly sensitized patients do not make antibodies

Study group: Highly sensitized patients with a cPRA > 98% based on an MFI > 2000 and taking care of the prozone effect.

Information needed: The serum of the highly sensitized patient must be tested in luminex against single antigen beads and the raw data of this screening and high resolution HLA typing of the patient must be available (NGS project).

Analyses: The HLA type of the patient and the non-reactive alleles on the beads will be translated in "epitopes". The incidence of non-reactivity against individual epitopes will be analysed.

3. Preliminary data

Unfortunately, only a limited number of donor-recipient combinations were available for analysis, which is the reason why several sessions of the epitope group in Asilomar were dedicated to the development of strategies to be used in the 18th workshop, which will take place in 2021 in Amsterdam.

In total 233 donor-recipient combinations had been submitted for project 1. After an inventory on the quality of HLA typing and availability of antibody (DSA) data, 191 couples were eligible for analyses. All 191 couples were analysed for the induction of antibodies against HLA-ABC and 145 couples for the induction of antibodies against HLA-DR. For the analysis on the immunogenicity of HLA-DR epitopes, couples with no DRB3/4/5 typing were excluded, as no distinction could be made between absent DRB3/4/5 alleles or unavailable typing.

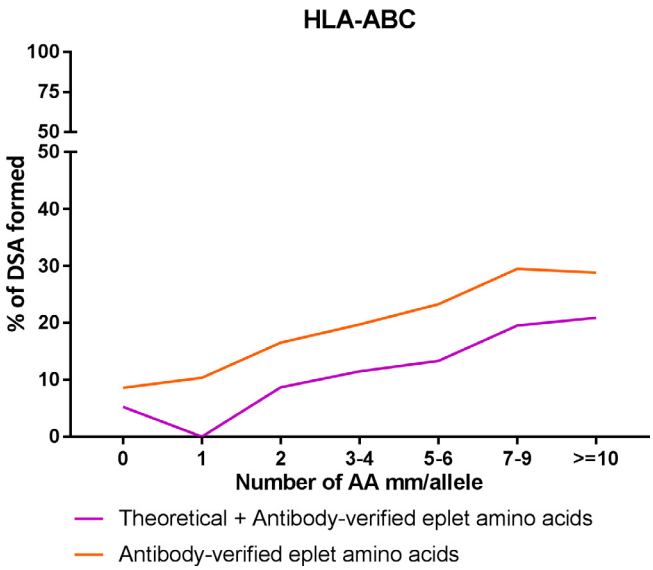

Fig. 1. Impact of the number of amino acid differences on the induction of DSA against HLA-A, -B and -C.

Per donor HLA allele mismatch, the number of amino acid mismatches were defined intralocus using the computer algorithm EMMA (Epitope MisMatch Algorithm) developed in Leiden, details of which will be published separately. Basis of this analysis are the amino acids as described for all theoretical and antibody-verified eplets in the HLA Epitope Registry (www.epregistry.ufpi.br). One analysis included all amino acids, both derived from theoretical and antibody-verified eplets, whereas a second analysis only included amino acids derived from antibody-verified eplets.

Per donor HLA allele mismatch, DSA was assigned if the donor allele in SAB assay, irrespective of the vendor, had an MFI of ≥1000. In case the mismatched donor HLA allele was not present in SAB assay, no assignment was made. Next, the number of amino acid mismatches was correlated to DSA formation by defining how often DSA formation was observed with a specific number of amino acid mismatches. For example, DSA formation was observed for 28.8% (17 out of 59) donor HLA-ABC allele mismatches with ≥10 antibody-verified eplet amino acid mismatches (Fig. 1). A similar correlation was found for the induction of antibodies against HLA-DR (Fig. 2). These data confirm the results of other studies showing an effect of the number of eplet and amino acid mismatches on the induction of donor specific HLA antibodies [12]. Unfortunately, the number of data is currently too limited for a proper analysis of the relative immunogenicity of individual epitopes or amino acid mismatches. It appeared after the workshop that more data were submitted but these were at that time not available for analysis. The same holds true for the data on the absence of antibodies against specific epitopes in highly sensitized patients. In the meantime, action has been taken in order to transfer all data of the epitope component of the 17th workshop towards the database for the 18th workshop. Furthermore, many groups from all over the world have been approached and they all expressed their interest to participate by contributing data on many donor-recipient combinations.

4. Discussion

It is clear that the number of antibody epitopes is more predictive for the induction of de novo DSA in comparison to the classical HLA antigen mismatches [6]. The aim of this workshop was to get insight in the differential immunogenicity of individual antibody epitopes. Unfortunately, insufficient data were available for a proper analysis but all participants present at the workshop were convinced that this is a crucial question to be answered in the next workshop. Several sessions

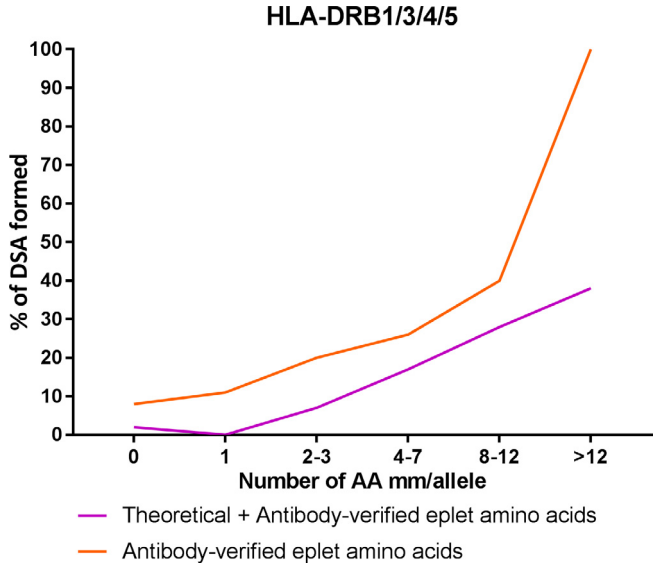

Fig. 2. Impact of the number of amino acid differences on the induction of DSA against HLA-DR.

were dedicated to the different approaches available to define the immunogenicity of HLA mismatches. Possible candidate parameters to be included in future analyses are the eplets as described in the epitope registry [13], the physico-chemical properties of amino acid mismatches [14,15], and the number of amino acid differences as described by Kosmoliaptsis et al. [15], which also form the basis of the EMMA algorithm used in the present preliminary studies on the workshop data. As T cell help is crucial for the induction of IgG DSA, it was decided that in the next workshop epitope analyses should also include T cell epitopes as described in the PIRCHE II algorithm [9,16,17]. Importantly, also the companies involved in HLA antibody detection expressed their interest in an active participation in this component. This is important as recent data clearly show differences in the sensitivity and specificity of the available antibody detection assays [18]. Furthermore, their software may be instrumental for submitting antibody data to the Workshop database.

In order to answer the question on the immunogenicity of individual epitopes, an analysis on a large database of high resolution typed donor-recipient combinations is essential. In order to make such an analysis a reality, it is essential that collection of the data starts as soon as possible. In the meantime, many groups all over the world have expressed their interest to participate and the organizers of the 18th workshop, Eric Spierings and Sebastiaan Heidt, have agreed to take the lead in this component. All data on the epitope component of the 17th workshop will be transferred to the database of the 18th workshop, and additional data collection will start in early 2019. This should enable the availability of a proper analysis during the next workshop in Amsterdam, which will take place in May 2021. A study on the definition of acceptable epitopes for highly sensitized patients will also be part of this epitope component.

In conclusion, the workshop in Asilomar has provided us only with limited data but can be considered as an excellent trigger of the epitope component of the upcoming 18th workshop.

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References

[1] A. Loupy, S.C. Jordan, Transplantation: donor-specific HLA antibodies and renal allograft failure, Nat. Rev. Nephrol. 9 (2013) 130.
[2] N. Lachmann, P.I. Terasaki, K. Budde, L. Liefeldt, A. Kahl, P. Reinke, et al., Anti-human leukocyte antigen and donor-specific antibodies detected by luminex post-transplant serve as biomarkers for chronic rejection of renal allografts, Transplantation 87 (2009) 1505.
[3] F.H. Claas, M.K. Dankers, M. Oudshoorn, J.J. van Rood, A. Mulder, D.L. Roelen, et al., Differential immunogenicity of HLA mismatches in clinical transplantation, Transpl. Immunol. 14 (2005) 187.
[4] R.J. Duquesnoy, M. Marrari, L. Jelenik, A. Zeevi, F.H. Claas, A. Mulder, Structural aspects of HLA class I epitopes reacting with human monoclonal antibodies in Ig-binding, C1q-binding and lymphocytotoxicity assays, Hum. Immunol. 74 (2013) 1271.
[5] A.R. Tambur, F.H. Claas, HLA epitopes as viewed by antibodies: what is it all about? Am. J. Transplant. 15 (2015) 1148.
[6] C. Wiebe, D. Pochinco, T.D. Blydt-Hansen, J. Ho, P.E. Birk, M. Karpinski, et al., Class II HLA epitope matching-A strategy to minimize de novo donor-specific antibody development and improve outcomes, Am. J. Transplant. 13 (2013) 3114.
[7] R.J. Duquesnoy, The eplet load concept in clinical transplantation, Pediatr. Transplant. 20 (2016) 884.
[8] V. Kosmoliaptsis, L.D. Sharples, A.N. Chaudhry, D.J. Halsall, J.A. Bradley, C.J. Taylor, Predicting HLA class II alloantigen immunogenicity from the number and physiochemical properties of amino acid polymorphisms, Transplantation 91 (2011) 183.
[9] H.G. Otten, J.J. Calis, C. Kesmir, A.D. van Zuilen, E. Spierings, Predicted indirectly recognizable HLA epitopes presented by HLA-DR correlate with the de novo development of donor-specific HLA IgG antibodies after kidney transplantation, Hum. Immunol. 74 (2013) 290.
[10] D.P. Lucas, M.S. Leffell, A.A. Zachary, Differences in immunogenicity of HLA antigens and the impact of cross-reactivity on the humoral response, Transplantation 99 (2015) 77.
[11] C. Kramer, M. Israeli, A. Mulder, I. Doxiadis, G. Haasnoot, S. Heidt, et al., The long and winding road towards epitope matching in clinical transplantation, Transpl. Int. (2018).
[12] C. Wiebe, V. Kosmoliaptsis, D. Pochinco, C. Taylor, P. Nickerson, A comparison of HLA molecular mismatch methods to determine HLA immunogenicity, Transplantation (2018).
[13] R.J. Duquesnoy, M. Marrari, A.R. Tambur, A. Mulder, L.C. Sousa, A.S. da Silva, et al., First report on the antibody verification of HLA-DR, HLA-DQ and HLA-DP epitopes recorded in the HLA epitope registry, Hum. Immunol. 75 (2014) 1097.
[14] V. Kosmoliaptsis, A.N. Chaudhry, L.D. Sharples, D.J. Halsall, T.R. Dafforn, J.A. Bradley, et al., Predicting HLA class I alloantigen immunogenicity from the number and physiochemical properties of amino acid polymorphisms, Transplantation 88 (2009) 791.
[15] V. Kosmoliaptsis, D.H. Mallon, Y. Chen, E.M. Bolton, J.A. Bradley, C.J. Taylor, Alloantibody responses after renal transplant failure can be better predicted by donor-recipient HLA amino acid sequence and physicochemical disparities than conventional HLA matching, Am. J. Transplant. 16 (2016) 2139.
[16] K. Geneugelijk, G. Honger, H.W. van Deutekom, K.A. Thus, C. Kesmir, I. Hosli, et al., Predicted indirectly recognizable HLA epitopes presented by HLA-DRB1 are related to HLA antibody formation during pregnancy, Am. J. Transplant. 15 (2015) 3112.
[17] N. Lachmann, M. Niemann, P. Reinke, K. Budde, D. Schmidt, F. Halleck, et al., Donor-recipient matching based on predicted indirectly recognizable HLA epitopes independently predicts the incidence of de novo donor-specific HLA antibodies following renal transplantation, Am. J. Transplant. 17 (2017) 3076.
[18] M.H. Ravindranath, V. Jucaud, S. Ferrone, Monitoring native HLA-I trimer specific antibodies in Luminex multiplex single antigen bead assay: evaluation of beadsets from different manufacturers, J. Immunol. Methods 450 (2017) 73.