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14th IHIWS Report on the structural basis of HLA compatibility

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Abstract

Under auspices of the 14th International Human Leukocyte Antigen (HLA) and Immunogenetics Workshop, multilaboratory collaborative studies were organized addressing the following projects: (1) identification of structurally defined class I and class II HLA epitopes by human monoclonal antibodies, (2) assessment of the relative immunogenicity of structurally defined donor HLA mismatches in kidney transplantation, and (3) evaluation of the usefulness of structurally based HLA matching in platelet transfusion support of alloimmunized thrombocytopenic patients. This progress report describes a preliminary HLAMatchmaker-based data analysis in each project. The findings support the clinical usefulness of structural HLA matching.

Introduction

This workshop component addresses structurally based approaches to determine human leukocyte antigen (HLA) compatibility. It focuses on the humoral immune responses because HLA-specific antibodies represent important risk factors for transplant failure and platelet transfusion refractoriness. Detailed information about the structure of HLA molecules and amino acid sequences of HLA alleles has provided opportunities to define the structural basis of HLA antigenicity and immunogenicity. HLAMatchmaker is a computer algorithm to determine structurally based HLA compatibility and to identify acceptable HLA mismatches for highly sensitized patients (1–4). The original HLAMatchmaker programs used triplets, i.e. linear sequences of three amino acid residues (1). Triplets have proven to be useful to determine the potential immunogenicity of a mismatched HLA molecule but provide, however, an incomplete description of the HLA epitope repertoire. For the latter purpose, an expanded criteria must be used including longer sequences and polymorphic residues in discontinuous positions. Such criteria should consider the structural basis of antibody–antigen interactions including contact areas and binding energy, the essence of antigenicity.

A recent analysis has led to the concept that HLA antigens like other antigenic proteins have structural epitopes consisting of 15–22 residues that constitute the binding face with alloantibody (5). Each structural epitope has a functional epitope of about two to five residues that dominate the strength and specificity of binding with antibody. Functional epitopes have one or more nonself residues and the term 'eplet' is used to describe polymorphic HLA residues within 3.0 Å of each surface-exposed polymorphic position in the molecular sequence. Many eplets represent short linear sequences identical to those referred to as triplets but others have residues in discontinuous sequence positions that cluster together on the molecular surface. Serologically defined HLA determinants correspond well to eplets. The eplet version of HLAMatchmaker represents a more complete repertoire of structurally defined HLA

epitopes and provides a more detailed assessment of HLA compatibility. The new class I and class II HLAMatchmaker programs can be downloaded from the web site http://tpis.upmc.edu.

There are three collaborative projects.

Project 1: structural basis of antibody-defined epitopes

This project addresses the structural basis of HLA epitopes defined by human monoclonal antibodies (mAbs). Two investigators, Arend Mulder (Leiden University Medical Center) and Maria Pistillo (National Institute for Cancer Research, Genoa, Italy) submitted a total of 39 class I and 21 class II specific mAbs. Most of them have been tested by enzyme-linked immunosorbent assay (ELISA) and Luminex as well as flow cytometry with single antigen preparations (done by Medhat Askar at the University of Pittsburgh Medical Center). The availability of HLA typing information of antibody producer and immunizer has been useful in the analysis of the reactivity patterns of these mAbs. The eplet version of HLAMatchmaker has been used to determine antibody specificities and Table 1 shows preliminary results of representative mAbs. Two are specific for eplets defining private DRB1 antigens and the other two react with eplets corresponding to public DRB epitopes.

MP12 came from an antibody producer exposed to a DR5 mismatch. This mAb was specific for 58DE, an eplet uniquely present on all DRB1*11 alleles. Although no HLA information was available for the immunizer BVK6D6 was monospecific for DR3. All DRB1*03 alleles have a distinct 73GRDN eplet that is also present on DRB3*01. The lack of reactivity of DRB3*01 with BVK6D6 can be explained with the two-patch concept (5, 6) whereby DRB3*01 lacks a second contact site necessary for binding with this antibody.

MP14 was induced by DRB1*0801 and reacted with DRB1*08 + DRB1*12; all these alleles share a unique eplet14GEY. The HLA information for MP10 was obtained only serologically and the immunizing epitope appeared to be present on a DR2 haplotype. This mAb showed, however, no reactivity with DR15 or DR16 in flow cytometry assays with single antigens but it reacted with DR1, DR9, DR10, and DR51. These alleles share 53IYN and it seems that this eplet on DR51 of the immunizing DR2 haplotype might have induced this antibody.

These findings show the usefulness of HLAMatchmaker in the analysis of the epitope specificity of human mAbs. A full report is being prepared for publication.

Project II: relative immunogenicity of structurally defined HLA mismatches

During humoral immunization, the antibody producer is often exposed to multiple HLA incompatibilities but the specificities of the antibodies are generally limited to a few epitopes. The goal of this project is to determine how often structurally defined epitopes induce specific antibodies in patients with rejected kidney transplants. This information would provide an assessment of the relative immunogenicity of an HLA mismatch.

This project deals with a serum analysis of patients whose kidney transplant from an HLA mismatched donor had failed and had been surgically removed while the patient was awaiting another transplant. Several studies have shown that following allograft nephrectomy (alloNx), many patients exhibit increased serum reactivity due to the detection of circulating donor-specific HLA antibodies (7–10).

The goal was that participating laboratories would submit a total of at least 200 informative alloNx cases so that reliable estimates about epitope immunogenicity following kidney transplantation could be obtained. These cases should have no pretransplant sensitization and the following information should be provided: (1) class I and II HLA types of patient and kidney donor so that structurally defined epitope mismatches can be determined, (2) information about graft survival and causes of transplant failure, and (3) alloNx dates and serum samples drawn at appropriate time intervals before and after.

Table 1 Examples of eplet specificity of human monoclonal antibodies (mAbs)

mAb	Contributor	Class II type of mAb producer[a]	Immunizing molecule[a]	Specific eplet[b]	Antigens with eplet
MP12	Pistillo	DRB1*1303,1401 DRB3*0101,0202 DQB1*0503,0301	DR5	58DE	DR11
BVK3D6	Mulder	DRB1*11,15 DRB3*02, DRB5*01 DQB1*0301,0602/0611/0615	Unknown	73GRDN	DR3
MP14	Pistillo	DR7,-	DRB1*0801	14GEY	DR8,12
MP10	Pistillo	DR5,7	DR2 haplotype	32IYN	DR1,9,10,51

[a] HLA typing information provided by contributor of mAb.

[b] Detailed descriptions of eplets and their corresponding alleles are shown in the HLAMatchmaker programs on the web site http://tpis.upmc.edu.

Serum screening methods with HLA-typed panels include complement-dependent lymphocytotoxicity (CDC and AHG) and in antigen-binding assays such as flow cytometry, ELISA, and Luminex. HLAMatchmaker analysis of serum reactivity would then determine which mismatched donor eplets had elicited specific antibodies and which ones did not. Table 2 shows two examples of high sensitization, both cases had been screened by single HLA antigen Luminex.

Case 1 was a 3 A,B antigen mismatch and seven of 25 mismatched eplets were present on serum reactive HLA antigens in the panel. There were 12 mismatched eplets on A1 and four of them were reactive: 44K (present on A1 and A36), 76ANT (on A1, A26, A29, A36, and A80), 113YR (on A1, A3, A11, A29, A34, A66, and A80), and 163R (on A1, A25, A26, A29, A36, and A80). Thus, although this antibody response to A1 involved a limited number of eplets, it can readily be seen why a considerable number of A locus antigens were reactive, all of them should be considered unacceptable. The donor's A2 had 12 mismatched eplets, two of them were reactive: 62GR (on A2 and B17) and 66RK (unique to A2). Only one eplet of the donor's B8 was reactive: 9D is shared between B8 and Cw7.

Case 2 was also a 3 A,B antigen mismatch but serum analysis showed no reactivity with the donor's A3 and A68 antigens. On the other hand, five B60 eplets appeared to react with the patient serum: 41T (on B12, B13, B21, B40, B41, and B47), 45KE (on B12, B21, B40, and B47), 147SL (on B48, B60, and B81), 177DT (on B8, B41, B42, B48, and B81), and 180E (on B7, B8, B41, B42, B48, B60, and B81). These findings can readily explain why so many B locus antigens react with patient serum and how to identify which antigens would be acceptable mismatches.

The following participants have contributed a total of 120 alloNx cases: Lena Absi (St Etienne, France), Ella van den Berg-Loonen (Maastricht, The Netherlands), Beth Colombe (Philadelphia, PA), Debra Crowe (Nashville, TN), Amy Hahn (Albany, NY), Malek Kamoun (Philadelphia, PA), Velta Lazda (Elmhurst, IL), Bill Lefor (Tampa, FL), Andrew Lobashevsky (Indianapolis, IN), Lorita Rebellato (Greenville, NC), Constanze Schoenemann (Berlin, Germany), Agathi Varnavidou (Nicosia, Cyprus), and Adriana Zeevi (Pittsburgh, PA). Many cases, however, had limited information such as the serum was screened by only one method and no samples were available for additional testing, no data on prealloNx sera, inappropriate timing of postalloNx sera, incomplete HLA typing information of patient and donor, and evidence of prior sensitization.

Nevertheless, we have been able to do a class I HLA-Matchmaker analysis of 44 alloNx cases with informative sera. Table 3 summarizes the results for 45 eplets that were mismatched in at least six cases. The frequencies of antibody responses to mismatched eplets showed a wide range. Highly immunogenic eplets correspond to common private antigens such as A2 and A3 and public antigens corresponding to cross-reactive groups such as the A2 and B7 CREGs.

This study addressed the relation between eplet expression and eplet immunogenicity. As described elsewhere (5), the number of an eplet represents the sequence position of the polymorphic residue in the center of that eplet. Molecular viewing has shown that these residues are located on the top (mostly α-helices), the side (often the α3 domain), the underside (under the peptide-binding groove), and the bottom (close to the cell membrane) of the HLA molecule. Molecular surface expression of polymorphic residues has been graded as prominent (++), readily visible (+), and somewhat visible (−/+). Table 3 shows that immunogenic eplets are generally well expressed on the top of the

Table 2 Examples of reactive donor eplet analysis of postallograft nephrectomy sera

Patient	Donor	Mismatched donor eplets	Reactive donor eplets	No. of reactive donor eplets
Case 1 (Lefor, Tampa)				
A23	A1	9F, 44K, 62QR, 65RN, 76ANT, 79GR, 94TI, 113YR, 116D, 163R, 275EL	44K, 76ANT, 113YR, 163R	4/11
A24	A2	9F, 62GR, 65RK, 76VDT, 79GR, 105SW, 142MTT, 144TKH, 145HAA, 183DA, 193AV, 207S	62GR, 65RK	2/12
B7	B7			
B62	B8	9D, 113HN, 177DT	9D	1/3
			Total	7/25
Case 2 (Lazda, Elmhurst)				
A3	A11	150AHA, 163R		0/2
A32	A68	127K, 62RR, 142MTT, 144TKH, 145HAA, 150AHV, 245VA		0/7
B27	B35			
B35	B60	41T, 45KE, 113HN, 116Y, 147SL, 177DT, 180E	41T, 45KE, 147SL, 177DT, 180E	5/7
			Total	5/16

Table 3 Frequencies of antibody responses to mismatched donor eplets in allonephrectomized patients

Eplet	Molecular location	Surface exposure	Number of mismatches	Number of reactive sera	Reactivity frequency (%)	Eplet carrying HLA antigens
113HD	Underside	+	9	1	11	B7B17B18B35B46B53B62B71 B72B76Cw15
207S	Side	+	9	1	11	A2A10A28A29A31A32A33A43A74A80
9F	Side	±	8	1	13	A1A2A3A32A36A74A80Cw1
116Y	Underside	±	7	1	14	A2A9A69B5B7B8B40B41B42B48B70B71B78B81 Cw*12
45EE	Side	+	6	1	17	B7B8B14B16B27B42B48B55B56B59B67B70B73B81B82
113HN	Underside	+	10	2	20	B8B13B16B22B40B41B42B48B51B52B59B67B7B78B81
193AV	Bottom	++	9	2	22	A2A10A28A29A31A32A33A43A74
9S	Side	±	8	2	25	A9A30Cw4Cw14
253Q	Bottom	++	7	2	29	A2A10A28A29A31A32A33A43A74B73Cw7Cw17
113YN	Underside	+	6	2	33	B14B21B37B45B73Cw4Cw5Cw8Cw17Cw18
113YQ	Underside	+	6	2	33	A25A26A31A32A33A43A66A74
150AHE	Top	+	6	2	33	A3A10A43
245AS	Side	+	6	2	33	A10A29A31A32A33A43A74
76VDT	Top	++	9	3	33	A2A3A11A3A31A33A34A66A68A69A74
9H	Side	±	6	2	33	B18B27B37B41B45B49B50B60B61B73
163E	Top	+	11	4	36	A80B7B13B27B47B48B60B61B73B81 Cw2Cw17
183DA	Side	+	8	3	38	A2A25A26A29A32A34A43A66A68A69A74
66IA	Top	++	8	3	38	B7B27B42B54B55B56B67B73B81B82
275EL	Bottom	++	7	3	43	A1A3A11A3A36
30DTL	Underside	+	7	3	43	B12B21B27B40B41B47
76ENI	Top	++	7	3	43	A23A24B38B49B51B52B53B57B58B59B63B77
113YR	Underside	+	9	4	44	A1A3A11A29A34A36A68A8
41T	Side	++	9	4	44	B13B41B44B45B47B49B50B60B61
113YH	Underside	+	14	7	50	A2A23A24A69B27B47B63
45KE	Side	+	8	4	50	B41B44B45B47B49B50B60B61
69AQA	Top	++	8	4	50	B7B42B54B55B56B67B81B82
166DG	Top	++	11	6	55	A1A23A24A80
81ALR	Top	++	7	4	57	A9A25A32B5B13B17B38B44B49B53B59B63B77
163R	Top	+	8	5	63	A1A11A25A26A43A66
45TE	Side	+	8	5	63	B18B35B37B51B52B53B58B78
62QR	Top	++	8	5	63	A1A3A11A3A31A32A36A74
127K	Side	++	15	10	67	A2A23A24A68A69
79RL	Top	++	6	4	67	A9A25A32B5B13B17B27B37B38B44B47B49B53B59B63B77
177DK	Side	++	8	6	75	B7
180E	Side	++	8	6	75	B7B8B41B42B48B60B81
142MTT	Top	++	17	13	76	A2A68A69
145HAA	Top	++	17	13	76	A2A68A69
150AHV	Top	+	17	13	76	A2A24A68A69
66KA	Top	++	14	11	79	A2A23A24
105SW	Side	++	15	12	80	A2A69
161D	Top	++	6	5	83	A3
166ES	Top	++	6	5	83	B44B45B82
62EG	Top	++	12	10	83	A23A24
62GR	Top	++	14	13	93	A2B57B58
65RK	Top	++	15	14	93	A2

molecule, whereas eplets with low immunogenicity reside on other less accessible positions, i.e. underside the groove or at the bottom of the molecule, and have lower surface expression.

This project on eplet immunogenicity will continue and the laboratories are requested to submit new alloNx cases. We would like to have as much recipient and donor HLA typing as possible at the four-digit allelic level including HLA-C and DR, DQ, and DP antigens all of which have been shown to induce specific antibody responses in transplant recipients. Pre- and postalloNx serum screenings need to include more sensitive methods preferably with single HLA antigens. Participating laboratories may also provide specimens for typing and antibody screening.

Project III: matching for structurally defined HLA polymorphisms in platelet transfusions of alloimmunized patients

Many transfusion services apply the serological cross-reactivity method developed almost 30 years ago (11) to identify platelet donors in different mismatch groups (B1U, B1X, B2X, B2UX, etc.) for alloimmunized thrombocytopenic patients who are refractory to random donor platelets. This project addresses the question whether structural HLA matching would be useful in the donor selection strategy. So far, four participants Christine Navarrete (London, UK), Ciaran Dunne (Dublin, Ireland), Bert Tomson (Leiden, The Netherlands), and Frank Christiansen (Perth, Australia) have submitted data. At this time, the total number of cases is insufficient for a comparative analysis and other investigators plan to submit data.

At the workshop meeting, Colin Brown and Delordson Kallon (UK National Blood Service) and Ciaran Dunne (Irish Blood Transfusion Service) reported their experience of finding more platelet donors with HLAMatchmaker. Recent publications by the National Institutes of Health (NIH) Clinical Center and the UK National Blood Service describe the usefulness of HLAMatchmaker in providing platelet transfusion support of refractory patients (12, 13) Both consider matching for triplets with low immunogenicity as described elsewhere (14) and one includes ELISA screening data to identify acceptable mismatches (13). The overall consensus was that a combination of serum antibody analysis and HLAMatchmaker may become the basis of a more effective strategy of finding platelet donors for HLA-sensitized thrombocytopenic patients.

Conclusion

The three projects have generated preliminary information supporting the clinical usefulness of HLA matching at the structural level. All of them will continue and investigators are invited to contact us for specific details regarding participation.

Acknowledgments

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