Five Maps for HLA Epitopia: Their Usefulness in Navigating for Newly Antibody-Defined Epitopes

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HLA antibodies are primary causes of allograft rejection and transplant failures. They recognize structurally defined epitopes in which so-called eplets play a dominant role. Many eplets have been verified experimentally with informative antibodies but more studies are needed for reaching complete repertoires that are clinically relevant. Such investigations should be conducted on antibody reactivity patterns of allele panels which cannot be explained with antibody-verified eplets but which might correlate with the presence of certain polymorphic amino acid residues that determine newly antibody-reactive eplets. Such residues have different molecular locations that can be mapped accordingly.

The world of HLA epitopes also referred to as HLA Epitopia, has five continents: HLA-ABC, HLA-DR, HLA-DQ, HLA-DP and MICA. For each one there is a provisional map that describes the sequence locations of antibody-verified eplets and polymorphic residues as potential candidates defining additional epitopes. The HLAMatchmaker website (www.epitopes.net) has a downloadable "HLA Epitopia" Excel document that can be used in searches for newly antibody-defined epitopes. These maps will be updated when new epitope information becomes available.

Each map has been designed for alleles commonly used in SAB panels used for antibody testing. The antibody-verified eplets currently recorded in the International HLA Epitope Registry (www.Epregistry.com.br) are shown in boxes. Many antibodies have been shown to be specific for epitopes that correspond solely to eplets but others recognize eplets paired with other residue configurations. This means that there are variations between epitopes defined by the same eplet. Some antibodies have been reported to react with epitopes defined so far only by eplet pairs. Such eplets have the superscript p (e.g. 62QE$^{p\cdot}$).

These maps describe the repertoires of eplets that have already been verified experimentally with informative antibodies. They show also polymorphic residues with standard single letter notations in sequence positions where new epitopes might be located. Blank spaces in such positions indicate that the residues are already accounted for with the current antibody-verified eplets. The maps do not show sequence positions for which all residues have already defined antibody-verified eplets.

The maps have information about the molecular surface expression of residues in sequence positions. Many residues are exposed on the molecular surface and being readily antibody-accessible they might be considered as good candidates for newly antibody-reactive eplets. Other residues are in locations below the molecular surface including the peptide-binding groove. Such residues might not solely define eplets but they may exert conformational effects on antibody-reactive eplets determined by nearby residues on the molecular surface.

All HLA Epitopia maps have been designed for alleles in common panels used for antibody screening. Each Excel sheet has a filter on row 3 that can be used to select a residue in a certain sequence position so one can assess how such residue is associated with an unexpected antibody reactivity with an allele panel and thereby may give a clue about a new epitope.

ABC epitope map

This map shows the locations of 78 antibody-verified eplets on 116 ABC alleles which can be found in SAB panels. All eplets are in boxes and most of them are in the α1 and α2 domains. Some eplets such as 44KM$_3$ and 62GK$_2$ have subscripted numbers indicating two or more distinct residues. Other eplets such 62QE^p and 65RNA^p are superscripted with p to indicate that they have been antibody-verified only as pairs with other residue configurations.

Several eplets correspond to serological specificities. Examples are 62GK$_2$ on A2 and 65GK on A9 (A23+A24) whereas 17RS is on A30 and 56R is on A30+A31. Certain eplets are on alleles within so-called cross-reacting groups. Examples are 44RT on a B5-CREG (B18, B35, B51, B52, B53 and B78) and 127K on a A2-CREG (A2, A23, A24, A68 and A69). Other eplets are shared by distinct groups of alleles that have not been classified as CREGs. Examples are 41T on all B13, B40, B41, B44, B45, B47, B49 and B50 alleles whereas 163DG is on A1*01:01, A*23:01, A*23:02, A*24:02, A*80:01 and B*15:12. Certain eplets such as 79GT, 80N, 131S and 138MI are shared by many alleles. A single antibody against a high-frequency epitope will dramatically lower the probability of finding an acceptable mismatch for the sensitized patient.

The ABC map has polymorphic residue information that might be useful in identifying newly antibody-verified eplets. It covers the polymorphic sequence positions in all three domains. Certain loci have in a given position the same residue for all alleles. Such monomorphic residues are always matched and therefore they are not shown on the map.

As an example, the 62-67 sequence has 13 antibody-verified eplets together with relevant polymorphic residues. Blank spaces under each sequence position indicate that the residues are already accounted for with the current antibody-verified eplets.

For instance, residues G (glycine), E (glutamic acid) and L (leucine) in position 62 have been deleted because they are already fully accounted for by the antibody-verified 62GE, 62GK$_2$, 62EE and 62LQ eplets. Residue R (arginine) has been deleted from HLA-A because of the

antibody-verified 62RR eplet. It has remained on HLA-B because it might constitute an eplet possibly recognized by antibodies produced by individuals whose HLA-B type have only B57 and/or B58 alleles which have 62G. Another possibility is an eplet defined by 62R+65Q. Since position 62 residue R is monomorphic for HLA-C it is not shown on the map.

The 62RR eplet is defined by a pair of arginine residues in positions 62 and 65. It should be noted that the map has kept R in position 65 because this residue is on many more HLA-A alleles besides those that have 62RR. In principle, individuals whose HLA types have only the 65G-carrying A23 and/or A24 alleles could make antibodies specific for an epitope solely defined by 65R. The map may also predict more HLA-A eplets involving 65R together with other nearby residues such as 65R+66K+67V, 65R+67V, 63E+65R, 63N+65R+67V, etc. These are of course theoretical considerations and specific antibodies with informative reactivity patterns are needed to verify that such epitopes really exist.

The map shows several HLA-A alleles (A1, A3, A11, A32, A36 and A74) with 62QE^{p} indicating that antibody-reactive epitopes have only verified for 62QE paired with other configurations, in this case 62QE+56G and 62QE+151H. The A30 and A31 alleles have also 62Q and 63E and they need to be included for the antibody verification of an epitope fully defined by 62QE.

The A2, A23, A24, A68 and A69 alleles share the well-known antibody-verified 127K eplet. The map has residue 66K which is shared by all of them except A68 and A69 and it should be noted that 66K is on A*34:01 but not on A*34:02 which has 66N. A specific reactivity pattern with A2, A23, A24 and A*34:01 would be informative for the antibody verification of 66K.

Several sequence locations are polymorphic for only one or two loci. The other loci have the same residue for all alleles and since such monomorphic residue is always a match it is not shown on the map. As an example, position 45 has residues E, K, M and T on HLA-B and the antibody-verified 44RMA and 45RT eplets fully account for M and T. All HLA-A alleles have 45M and all HLA-C alleles have 45G, these residues have been excluded from the map because they are always matches. This leaves us with residues E and K as potential eplets and the maps shows the HLA-B alleles that express them.

DRB epitope map

The DRB map shows for 39 DRB1/3/4/5 alleles generally used in SAB assays the sequence locations of 28 eplets that have been experimentally verified with informative antibodies. Some eplets are unique for DRB1 alleles; examples are 25Q$_3$ on DRB1*07:01 and 51R on DRB3*02:02. Other eplets such as 4Q (on DRB1*07:01, DRB1*09:01 and DRB4*01:01/03) and 16Y (on DRB1*08:01/02 and DRB1*12:01/02) are shared by small groups of alleles. On the other hand, eplets such as 4R, 25R, 73A and 77T are shared by many alleles. Highly sensitized patients may have a single antibody against a high-frequency epitope and this will lower the probability of finding an acceptable mismatch.

This map shows also polymorphic residues that might determine additional mismatched epitopes. It can serve as guide for interpreting antibody reactivity patterns specific for new epitopes. For instance, DRB1*03:01/02, DRB1*07:01 and all DRB3 alleles carry the 73G residue; the distinct reactivity pattern of this group of alleles could indicate antibodies specific for an epitope determined by 73G.

As another example, the reactivity of the 40Y-carrying DRB1*10:01, DRB3*02:02 and DRB4*01:01/03 might indicate a 40Y-specific antibody which can be in principle, produced by many patients with different DRB types. Conversely, antibodies against the high-frequency 40F can only be made by people whose DRB types have only the 40Y-carrying DRB1*10:01, DRB3*02:02 and/or DRB4*01:01/03 but this possibility is highly unlikely.

DQB epitope map

A recent publication (Human Immunology, on line, June 2016) describes the DQ epitope map. At present, 16 antibody-verified epitopes are shown for 15 DQB alleles commonly used in SAB panels. Examples are 45EV (on DQB1*03:01), 55PP (on DQB1*03:01, DQB1*03:02 and DQB1*03:03) and 87F (on DQB1*06:01, DQB1*06:02 and DQB1*06:03).

Several antibody-verified eplets have subscripted numbers ranging from 2 to 4 indicating that how many separate molecular configurations are shared between alleles with such eplets. As an example, $45GE_3$ is on DQB1*02:01 and DQB1*02:02 and these alleles have unique polymorphic residues in different locations: 46E, 47F,52L, 55L, 71K and 74A on the molecular surface whereas 28S, 30S and 37I are below the molecular surface and not readily accessible to antibody. Since current SAB panels cannot distinguish which residues define the epitope referred to as $45GE_3$, the map shows just the eplet whereas the associated residues have been removed to make the map easier to read. The legend below the table shows all eplets with subscripts and the analogous residues that have been removed from the map.

It should be noted that several antibody-verified eplets are shared by many alleles. Examples are $47VY_3$ (on all DQB alleles except DQB1*02) and 77T (on all DQB alleles except DQB1*02 and DQB1*05). Antibodies against high-frequency epitopes will adversely affect the numbers of donors with acceptable mismatches for sensitized patients.

This map shows also the polymorphic residues for which no corresponding antibody-verified epitopes have been identified. The sharing of distinct residues uniquely shared by a group of antibody-reactive alleles might give a clue about the epitope specifically recognized. For instance, DQB1*02:01, DQB1*02:02, DQB1*04:01, DQB1*04:02 and DQB1*06:01 share a unique 66D residue that is well exposed on the molecular surface. These alleles share also the nearby 67I. Can certain patients have antibodies specific for an eplet defined 66D+67I? Moreover, can an epitope defined by the alternative 66E+67V eplet induce specific antibodies?

The DQB map shows the sequence positions of polymorphic residues where newly antibody-verified epitopes can be located. Sequence positions 9, 13, 14, 26 and 30 have residues are in locations below the molecular surface including the peptide-binding groove. Such residues by themselves, are considered less likely candidates for eplets but may exert conformational effects on antibody-reactive eplets defined by residues on the molecular surface.

The map has also high-frequency residues shared between most alleles in the SAB panel. As an example, the high-frequency residue 56P is on all DQB alleles except DQB1*04. Other very high-frequency residues are 3S, 23R, 45G, 126Q, 135D and 167R. In each case, a specific antibody seems unlikely because it can only be made by patients who are homozygous for the allele with the alternate residue.

DQA epitope map

The current map has five antibody-verified DQA eplets including 47KHL on DQA1*02:01 and 50LR on DQA1*02:01, DQA1*03:01, DQA1*03:02 and DQA1*03:03. Three eplets have subscripts which means multiple unique residues in several non-overlapping sequence locations. They are (1) $40GR_3$: 40G, 47C, 50V, 51L and 53Q; (2) $75S_3$: 75S, 107I, 161E, 163S and 175K; (3) $47QL_4$: 26S, 47Q, 56R,76V and 187T but the compositions of current SAB panels cannot distinguish which residues might interact with antibody. All of them have been removed from the map.

The presence of multiple polymorphic residues seems to suggest that many more DQA eplets need to be identified experimentally with informative antibodies. However, many residues in non-overlapping sequence locations are distinctly shared by certain groups of DQA alleles. For an antibody reacting with such group we can only assign an eplet with a subscripted number.

For instance, DQA1*01:01, DQA1*01:02, DQA1*01:03 and DQA1*01:04 alleles share seventeen unique residues: 11C, 18F, 45A, 47R, 48W, 50E, 52S, 53K, 55G, 56G, 61G, 64R, 66M, 69A, 76M, 80Y and 175Q. An antibody reacting only with all DQA1*01 alleles would be specific for what we may call Epitope X which can after experimental verification, only be annotated by an eplet with a subscript. Another antibody might be specific for an Epitope Y present all DQA alleles except DQA1*01; such alleles share 11Y, 18S, 45V, 48L, 55G, 61F, 64T and 80S. Again, such epitope can be only annotated by an eplet with a subscript. As a third example we can postulate an Epitope Z present on all DQA alleles except DQA1*05. They have the unique 75I, 156F, 161D and 163I residues and if experimentally verified the corresponding eplet will be subscripted. An easier readable DQA map includes the postulated EpX, EpY and EpZ eplets; their associated residues have been removed.

There are four sequence positions whereby only one residue is on a rather uncommon allele, 2G on DQA1*01:04, 41K and 130A on DQA1*01:03 and 139R on DQA1*06:02. Conversely, the other residues 2D, 41R, 130S and 139S are on all remaining DQA alleles and they could

define high-frequency eplets. Antibodies against such eplets are extremely unlikely because they can only be produced by someone homozygous for an uncommon allele.

DPB epitope map

The DPB epitope map has 8 antibody-verified eplets on 20 DPB alleles commonly used in SAB assays. None of them have subscripted numbers because their residues are always within a radius of 3 Angstroms, the dimension of an eplet. Two DPB eplets 56E and 56EE can cross-react with eplets in similar locations on DRB1*11 alleles. There still well-exposed residue positions such as 57, 65, 69 and 170 that could define a few additional eplets that need to be verified experimentally.

DPA epitope map

This map with 8 DPA1 alleles has only two eplets 50QA on DPA1*01 and DPA1*03 and 50RA on DPA1*02 and DPA1*04 that have so far provisionally been antibody-verified. The number of polymorphic residues is much lower than for the other HLA loci and this would suggest a very limited repertoire of DPA epitopes.

MICA epitope map

Twenty antibody-verified eplets have been identified on 30 MICA alleles used for antibody analysis. This map shows the residues in positions such as 122, 129, 213 for which eplets have already been identified, such residues might be good candidates for newly verified eplets. A shown under the "Res" heading of column Y, many residues are present on only MICA allele. Specific antibodies seem unlikely for residues in such positions.

Summary

The HLA Epitopia maps describe the current repertoires of antibody-verified epitopes. In my experience with the new HLAMatchmaker Antibody Analysis Version 02, these repertoires appear to be more than 90% complete but still more epitopes need to be verified experimentally. These maps have been designed to provide directions where to look on the "terra incognita" of the HLA molecular surface for new epitopes recognized by antibodies with unexpected reactivity patterns. Periodical updates will be made after new information becomes available.

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