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Antibody-defined epitopes on HLA-DQ alleles reacting with antibodies induced during pregnancy and the design of a DQ eplet map

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ABSTRACT

The concept that HLA antibodies recognize epitopes is leading to new approaches of HLA matching at the epitope level. HLA-DQ plays an important role and many studies have identified structurally defined DQ epitopes specifically recognized by antibodies; they have been recorded in the International HLA Epitope Registry http://www.epregistry.com.br but the list is still incomplete.

Pregnancy offers an attractive model to study antibody responses to HLA epitopes. The current analysis was done on 42 DQ-reactive post-pregnancy sera tested in binding assays with a panel of DQ heterodimers. The reactivity of 29 sera corresponded fully to the presence of antibody-verified DQA and DQB epitopes recorded in the Registry. Analysis of the remaining 13 sera led to the identification of additional antibody-defined DQB and DQA epitopes.

We have designed the first version of an eplet map for DQ alleles which includes antibody-defined DQA and DQB epitopes and shows sequence positions with polymorphic residues which can be used in HLA epitology studies to identify new antibody-defined DQ epitopes.

1. Introduction

More than 35 years ago, matching for HLA-DQ (then called MB) was shown to have a beneficial effect on kidney transplant survival [1] and subsequent reports support this concept [2–7]. Antibody-binding assays with single antigen beads have resulted in many reports describing the role of donor-specific HLA-DQ antibodies in transplant rejection and failure [8–16]. It is now well recognized that the antibody response to HLA-DQ (referred to below as DQ) plays a significant role in transplant rejection [7,17,18] and many transplant programs have added the polymorphisms of DQB and DQA in determining donor-recipient compatibility.

A new understanding has slowly emerged that HLA antibodies recognize epitopes rather than antigens [19–22] and they have led to the concept of epitope-based HLA matching for transplantation [20,23]. Accordingly, the specificities of DQ antibodies should be determined at the epitope level and a several studies have described DQB and DQA epitope specificities of antibodies in sera from transplant patients [9,24] and associations of DQ epitope matching with transplant outcome [25–27].

HLAMatchmaker offers a theoretical tool for the structural definition of predicted DQ epitopes [28]. In 2013, the International HLA Epitope Registry http://www.epregistry.com.br was established as a primary resource for HLA epitope information [29]. It provides structural descriptions of eplets as essential components of HLA epitopes and for each HLA locus there is a list of epitopes that have been experimentally verified with informative antibodies. Sixteen antibody-verified (AbVer) DQB and three AbVer DQA epitopes have been recorded as of January 1, 2016 [30]. This list is incomplete and more studies with informative DQ epitope-specific antibodies are needed.

Transplantation, blood transfusions and pregnancy are the principal causes of HLA antibodies in patient sera. Two recent studies demonstrate how pregnancy offers an attractive model to study antibody responses to mismatched class I and DRB epitopes which can be readily determined from the HLA types of child and mother [31,32]. This report describes our analysis how DQ reactivities of post-pregnancy sera correlate with the presence of AbVer DQ epitopes and if there were sera reacting with informative DQ heterodimers expressing newly antibody-defined epitopes. The data have led to the first map of AbVer DQ eplets identified so far.

2. Materials and methods

This study was done on sera from 301 healthy women giving full-term live birth at the University Hospital Basel between September 2009 and April 2011. All women had either their first full-term pregnancy or had previous children from the same partner as the current live birth. The number of previous live births and miscarriages was recorded as indicated by the women. Only 3/301 (1%) had prior blood transfusions and none had prior transplants. A blood sample was drawn from the mother between day 1 and 4 after delivery and antibody testing was done with single antigen beads (SAB) with DQ heterodimers (LabScreen SA class II, lot 8; One Lambda, ThermoFisher). Normalized mean fluorescence intensity (MFI) values were determined for each bead as previously described [33].

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We have identified 42/301 (14%) post-pregnancy sera with MFI greater than 1000 values that were considered DQ-reactive and all of them were selected for epitope analysis. Mother's blood and cord blood of the child were DNA-typed for four-digit HLA-DQB and -DQA alleles using next-generation sequencing (NGS) on a Illumina MiSeq platform (histogenetics.com). The remaining sera were either weakly reactive (11%) or essentially non-reactive (75%).

The HLAMatchmaker analysis of DQ reactivity patterns consisted of two steps. First, we determined which mismatched AbVer eplets are shared between the reactive alleles in the panel and the immunizing DQ-haplotype of the child. We considered the reactivities towards DQA and DQB eplets with informative DQ heterodimers in the panel. We also compared amino acid differences between DQB and/or DQA eplet-carrying heterodimers with reactivities ranging from high to low MFI values to identify additional residue configurations important for the expression of epitopes reacting with a specific antibody. Such epitopes might be defined by eplet pairs similar to those identified for class I epitopes [34,35]. This analysis also distinguished between child-specific antibodies and so-called third-party antibodies induced by previous pregnancies/miscarriages; for the latter, no HLA typing information was available for the child.

The second step was the analysis of reactive DQ heterodimers that lack specific AbVer DQ epitopes and therefore might carry other antibody-defined epitopes which have not been recorded in the HLA Epitope Registry. The successful outcome of such analysis depends on the identification of "informative" alleles which lack child-specific AbVer eplets and structural descriptions of newly identified epitopes included also an analysis of amino acid differences in polymorphic sequence positions. Such epitopes could be equivalent to eplets alone or eplets together with other residue configurations which according to the structural epitope model [36] and as determined by Cn3D molecular modelling [37] cannot be more than 15 Ångstroms away.

3. Results

All 42 DQ-reactive post-pregnancy sera with MFI greater than 1000 values were selected for epitope analysis. The first group consisted of 29 sera with DQ reactivity which could be fully explained with of AbVer epitopes. A second group of 13 sera had reactivity patterns that included additional antibody-defined DQB and DQA epitopes.

3.1. Antibody reactivities with AbVer DQ epitopes

Table 1 shows the DQB types of the mothers and the mismatched haplotype of the children for the first group. It also shows the country of origin of these women and the numbers of previous live births/miscarriages, and which epitopes were specifically on reactive alleles. For each serum it shows the mean ± SD MFI values of these reactive alleles as well those alleles generally considered as non-reactive. The data have been ordered according to sequence position of the most reactive DQB epitope. This table is intended as a summary and Supplemental Table 1 has details of each antibody reactivity pattern and the identification of informative alleles. For instance, serum #1 reacted only with 45EV-carrying DQ-heterodimers with exclusively DQB1*03:01 (MFI = 1159 ± 517) whereas for the 25 non-reactive heterodimers the MFI = 16 ± 21. In several cases, one group of reactive heterodimers had two AbVer DQB epitopes whereas another group had only one of them. In such cases we concluded two different epitopes if the first group had a significantly higher MFI value than the second group. For instance, serum #2 reacted with alleles carrying both 45EV and 55PP (on DQB1*03:01, *03:02 and *03:03) gave statistically higher MFI values (3929 ± 1512) than the alleles with only 55PP (1967 ± 1149); Student's *t*-test: p = 0.014). Serum # 3 had a similar reactivity pattern except that the 55PP-reactive antibodies were very weak (MFI = 214 ± 159) but still significantly above the 17 negative reactions (4 ± 5). Serum #4 reacted with alleles that were informative for either 45EV (MFI = 11203 ± 930) or the DQA eplet 40GR3 (MFI = 9037 ± 344).

Altogether, the DQ antibody activity of these 29 sera showed reactive DQ alleles with AbVer DQB epitopes in 41 cases and 45EV, $45GE_3$, $52PQ_2$, 52PR, 55PP and $84QL_3$ were most often found. Three sera had antibodies reacting with informative alleles with AbVer DQA eplets, namely 47KHL (two cases) and $40GR_3$ (one case).

Table 1 shows the mean ± high standard deviation numbers of MFI values for eplet-carrying alleles; they often reflected considerable ranges from high to quite low. This should not be surprising because an eplet is only one and important part of an antibody-reactive epitope but in context with the structural epitope concept [36] other nearby residues affect antibody binding. No detailed analyses could be done because the panel lacks often informative alleles to address this issue. Nevertheless, Table 1 has two illustrating examples.

Serum #27 had DRB1*06:02-induced antibodies that reacted with all 77T-carrying DQB alleles (MFI = 8304 ± 4289). Alleles with 77T paired with 74EL including the immunizing allele, were more reactive than alleles with 77T paired with 74SV (9822 ± 3033 versus 1475 ± 734; p = 0.0001) thereby suggesting an influence of residues in polymorphic sequence positions 74 and 75 which are only 3–4 Ångstroms away from 77T.

Serum #23 with DQB1*03:01-induced $84QL_3$-specific antibodies (MFI = 3019 ± 2925) that reacted better with alleles carrying 84QL paired with the 54F residue on DQA than those with 84QL paired with 54L (MFI = 5086 ± 2254 versus 437 ± 185; p = 0.005). It should be noted that the $84QL_3$ eplet reflects three possible configurations on the DQ molecular surface 84QL, 52L and 125A, the latter two are too far away to pair with 54F on DQA to be recognized as part of an epitope interacting with the CDRs of antibody. On the other hand, the 84QL and 54F are only 8 Ångstroms apart which suggests that this antibody recognizes the 84QL configuration.

3.2. Serum reactivities with newly antibody-defined DQ epitopes

Table 2 summarizes the remaining DQ-reactive 13 sera with reactivity patterns that included new antibody-defined DQB and DQA epitopes; Supplemental Table 2 describes details about assigned epitopes on reactive alleles. Nine sera reacted with alleles carrying AbVer epitopes including #31 and #41 that had 55PP which was not on the predicted DQB of the child and should be considered third-party (both women had a previous live birth ± a miscarriage). Five sera reacted with alleles without identifiable AbVer epitopes and they should have antibodies specific for new epitopes.

Nine sera reacted with informative DQA alleles carrying the $47QL_4$ eplet on DQA1*03:01, *03:02 and *03:03 in the panel (Table 2). This eplet represents five different DQA molecular configurations 47QL, 25YS, 50LFRRFRR, 75IV and 187T and one might raise the question how many are actually recognized by these antibodies. Although the panel does not have other DQA alleles that might have been informative to distinguish between these configurations some sera had different reactivity levels which could be related to the only one residue difference known to exist between the DQA1*03 alleles in the panel, namely in position 160. DQA1*03:01 has 160A whereas DQA1*03:02 and *03:03 have 160D; the rest of the amino acid se-

Table 1
Identification of child antibody-verified epitopes on DQ heterodimers reacting with 29 post-pregnancy sera.

Serum Nr.	Nationality origin	Previous LiveBirths	Previous Miscarriages	Mother DQB	Mother DQA	Child DQB	Child DQA	Child or third-party (TP) antibody-verified DQ eplet (Mean ± SD MFI)	MFI and numbers of other DQ heterodimers
#1	Egypt	0	0	DQB1*02:02, 03:02	DQA1*02:01, 03:01	DQB1*05:01	DQA1*01:01	DQB: 45EV (TP) (1159 ± 517)	16 ± 21 (1–86, N = 25)
#2	Kosovo	1	1	DQB1*02:01, 05:03	DQA1*01:01, 05:01	DQB1*03:01	match	DQB: 45EV and 55PP (2777 ± 906), 55PP alone (214 ± 159)	4 ± 5 (1–15, N = 17)
#3	Switzerland	2	1	DQB1*02:01, 06:04	DQA1*01:02, 05:01	DQB1*03:01	match	DQB: 45EV and 55PP (3929 ± 1512), 55PP alone (1967 ± 1149)	53 ± 43 (4–177, N = 17)
#4	Switzerland	1	0	DQB1*02:02, 03:02	DQA1*02:01, 03:01	DQB1*03:01	DQA1*05:01	DQB: 45EV (11203 ± 930), DQA: $40GR_3$ (9037 ± 344)	63 ± 221 (1–1019, N = 21)
#5	Macedonia	1	0	DQB1*05:02, 06:03	DQA1*01:03, 01:02	DQB1*02:02	DQA1*02:01	DQB: $45GE_3$ and $84QL_3$ (14273 ± 1288), $84QL_3$ alone (6803 ± 2999)	60 ± 95 (1–280,N = 8)
#6	Turkey	0	0	DQB1*03:01, 05:01	DQA1*01:01, 05:01	DQB1*02:01	match	DQB: $45GE_3$ (6553 ± 1676)	67 ± 126 (1–549, N = 24)
#7	Turkey	0	0	DQB1*03:02, 05:03	DQA1*01:01, 03:01	DQB1*02:02	DQA1*02:01	DQB: $45GE_3$ (TP) (13684 ± 490), DQA: 47KHL(7007 ± 1908)	17 ± 18 (1–50, N = 19)
#8	Switzerland	1	0	DQB1*06:04, 06:04	DQA1*01:02, 01:02	DQB1*02:02	DQA1*02:01	DQB: $45GE_3$ (8914 ± 1298), 55PP (TP) (1199 ± 1243), DQA: 47KHL (2524 ± 403)	29 ± 46 (1–122, N = 9)
#9	Switzerland	1	0	DQB1*02:02, 03:01	DQA1*02:01, 06:01	DQB1*06:02	DQA1*01:02	DQB: 45GV (10788 ± 2123)	12 ± 16 (1–40, N = 10)
#10	Switzerland	0	0	DQB1*02:01, 02:01	DQA1*05:01, 05:01	DQB1*03:02	DQA1*03:01	DQB: $52PL_3$ (7719 ± 1448), 45GV (584 ± 328)	94 ± 198 (1–447, N = 5)
#11	Switzerland	0	0	DQB1*02:01, 03:02	DQA1*03:01, 05:01	DQB1*06:03	DQA1*01:03	DQB: $52PQ_2$ (1783 ± 714)	4 ± 11 (1–46, N = 21)
#12	Switzerland	1	0	DQB1*02:02, 03:03	DQA1*03:01, 03:01	DQB1*06:03	DQA1*01:03	DQB: $52PQ_2$ and 52PR (13148 ± 1888), 52PR alone (6768 ± 2391)	39 ± 57 (1–165, N = 17)
#13	Spain	1	0	DQB1*03:01, 03:02	DQA1*03:01, 05:01	DQB1*05:02	DQA1*01:02	DQB: $52PQ_2$ and 52PR (5601 ± 1104), 52PR alone (2883 ± 1082))	59 ± 100 (1–364), N = 17
#14	Germany	0	0	DQB1*03:01, 03:03	DQA1*02:01, 03:01	DQB1*05:02	DQA1*01:02	DQB: $52PQ_2$ and 52PR (9501 ± 1409), 52PR alone (2286 ± 1234)	38 ± 63 (1–236, N = 17)
#15	Switzerland	1	0	DQB1*03:01, 03:01	DQA1*03:01, 05:01	DQB1*06:02	DQA1*01:02	DQB: 52PR (10805 ± 2514), $140A_2$ (469 ± 222), 45GV (184 ± 105)	3 ± 4 (1–10, N = 5)
#16	Morocco	0	0	DQB1*05:01, 06:04	DQA1*01:02, 01:02	Match	DQA1*01:01	DQB: 55PP (TP) (1294 + 1208)	36 ± 115 (1–494, N = 18)
#17	Germany	1	0	DQB1*02:02, 05:03	DQA1*01:01, 02:01	DQB1*03:04	DQA1*03:01	DQB: 55PP (1648 ± 340)	67 ± 74 (8–328, N = 18)
#18	Kosovo	2	2	DQB1*02:01, 06:03	DQA1*01:03, 05:01	DQB1*03:01	Match	DQB: 55PP (1665 ± 528)	27 ± 19 (1–58, N = 17)
#19	Turkey	1	0	DQB1*05:01, 05:02	DQA1*01:02, 01:01	DQB1*03:01	DQA1*05:01	DQB: 55PP (2120 ± 589)	25 ± 28 (1–97, N = 17)
#20	USA	0	3	DQB1*02:01, 05:01	DQA1*01:01, 05:01	Match	Match	DQB: 55PP (TP) (2560 ± 1146)	49 ± 58 (2–184, N = 18)
#21	Kosovo	0	0	DQB1*02:01, 05:02	DQA1*01:02, 05:01	DQB1*03:01	Match	DQB: 55PP (904 ± 544)	55 ± 59 (2–257, N = 18)
#22	Switzerland	1	0	DQB1*05:01, 05:01	DQA1*01:01, 01:01	DQB1*06:02	DQA1*01:02	DQB: 55PP (TP) (7310 ± 1628)	20 ± 13 (1–44, N = 17)
#23	Spain	0	0	DQB1*06:03, 06:03	DQA1*01:03, 01:03	DQB1*03:01	DQA1*05:01	DQB: 55PP and $84QL_2$ (8286 ± 3676), $84QL_2$ alone (3019 ± 2925)*	7 ± 13 (1–37, N = 7)
#24	Switzerland	0	0	DQB1*03:02, 03:02	DQA1*03:01, 03:01	DQB1*04:02	DQA1*04:01	DQB: $55RL_3$ (5493 ± 2081)	47 ± 70 (1–288, N = 25)
#25	Kosovo	1	0	DQB1*02:01, 06:04	DQA1*01:02, 05:01	DQB1*05:02	Match	DQB: $74SR_3$ (6800 ± 1177), 45EV (803 ± 445)	103 ± 98 (1–325, N = 22)
#26	Switzerland	1	2	DQB1*03:02, 03:03	DQA1*02:01, 03:01	DQB1*05:02	DQA1*01:02	DQB: 77R (929 ± 286), $140A_2$ (445 ± 159)	33 ± 47 (1–123, N = 16)
#27	Switzerland	2	0	DQB1*02:01, 05:01	DQA1*01:01, 05:01	DQB1*06:02	DQA1*01:02	DQB: 77T (8304 ± 4289)**	25 ± 12 (1–43, N = 7)
#28	Switzerland	2	0	DQB1*06:02, 06:03	DQA1*01:03, 01:02	DQB1*03:01	DQA1*05:01	DQB: $84QL_3$ (11829 ± 2334)	74 ± 56 (26–183, N = 8)
#29	Switzerland	1	1	DQB1*06:02, 06:04	DQA1*01:02, 01:02	DQB1*03:01	DQA1*05:01	DQB: $84QL_3$ (12676 ± 1511)	34 ± 94 (1–267, N = 8)

* 84QL + QA:54F (5086 ± 2254), 84QL + QA:54L (437 ± 185); p = 0.005.

** 77T + 74EL (9822 ± 3033), 77T + 74SV (1475 ± 734); p = 0.0001.

Table 2
Presence of antibody-verified and newly antibody-defined epitopes on DQ heterodimers reacting with 13 post-pregnancy sera.

Serum Nr.	Nationality origin	Previous LiveBirths	Previous Miscarriages	Mother DQB	Mother DQA	Child DQB	Child DQA	Child or third-party (TP) antibody-verified DQ epitope (Mean ± SD MFI)	New DQ Epitope (MFI + SD informative alleles)	MFI and numbers of other DQ heterodimers
#30	Switzerland	1	0	DQB1*02:01, *03:03	DQA1*02:01, 05:01	DQB1*03:04	DQA1*03:01	None	DQA: $47QL_4$ (3332 ± 1844)	66 ± 101 (1–401, N = 22)
#31	Kosovo	1	1	DQB1*05:01, *05:02	DQA1*01:02, 01:01	DQB1*06:09	match	DQB: 55PP (TP)(501 ± 272)	DQA: $47QL_4$ (TP) (4206 ± 1163)	86 ± 38 (41–155, N = 15)
#32	Ukraine	0	3	DQB1*03:01, *05:02	DQA1*01:02, 05:01	DQB1*03:02	DQA1*03:01	None	DQA: $47QL_4$ (4589 ± 1888)	43 ± 39 (1–145, N = 19)
#33	Bosnia	0	0	DQB1*02:01, *03:01	DQA1*05:01, 05:01	DQB1*02:02	DQA1*02:01	DQA: 47KHL (11452 ± 1932)	DQA: $47QL_4$ (TP) (838 ± 332)	179 ± 116 (43–422, N = 15)
#34	Switzerland	0	0	DQB1*02:01, *03:01	DQA1*05:01, 05:01	DQB1*03:03	DQA1*03:01	DQB: 45GV (10580 ± 1660), DQA: 47KHL(TP) (4858 ± 1833)	DQA: $47QL_4$ (13235 ± 158), $75I_2$ (1831 ± 542)	7 ± 13 (1–37, N = 7)
#35	Germany	0	1	DQB1*02:01, *06:03	DQA1*01:03, 05:01	DQB1*03:01	DQA1*03:01	None	DQA: $47QL_4$ (4154 ± 1209), DQB: 55PP + QA:51F (878 ± 511)	10 + 17 (1–61, N = 21)
#36	Switzerland	0	0	DQB1*02:01, *03:01	DQA1*05:01, 05:01	DQB1*03:02	DQA1*03:01	DQB: 45GV (12212 ± 1121)	DQA: $47QL_4$ and 40ERV (12420 ± 176), 40ERV (3901 ± 1938)	245 ± 231 (1–499, N = 5)
#37	Columbia	1	0	DQB1*05:01, *06:04	DQA1*01:02, 01:01	DQB1*04:02	DQA1*04:01	DQA: $40GR_3$ and 52PL + s56P (5597 ± 2000)	DQB: 52PL + s56P only (2025 ± 652)	87 ± 134 (2–515, N = 14)
#38	Bosnia	0	0	DQB1*05:01, *03:01	DQA1*01:01, 05:01	DQB1*02:01	match	DQB: $45GE_3$ (10509 ± 1011)	DQB: 56PA + QA:160A (1502 ± 559)	15 + 44 (1–148, N = 20)
#39	Switzerland	1	0	DQB1*06:03, *06:02	DQA1*01:03, 01:02	DQB1*05;01	DQA1*01:01	DQB: $71SR_3$ and 77R (10246 ± 2445), 77R alone (3099 ± 1663)	DQB: 56PV (2140 ± 264)	89 ± 57, (14–179, N = 20)
#40	Germany	1	0	DQB1*04:02, *06:02	DQA1*01:02, 04:01	DQB1*06:04	match	DQB: 55PP (TP) (730 ± 384)	DQB: 56PV (6850 ± 2208)	64 ± 97 (1–359, N = 14)
#41	Switzerland	1	0	DQB1*03:01, *06:03	DQA1*01:03, 05:01	match	DQA1*03:01	None	DQB: 56PV (TP) (2968 ± 1302) DQA: $47QL_4$ (2116 ± 1057)	102 ± 115 (1419, N = 19)
#42	Switzerland	1	0	DQB1*03:03, *06:04	DQA1*01:02, 02:01	DQB1*06:02	match	None	DQB: 87F (841 ± 832)	13 + 17 (1–67,N = 25)

quence 1–200 is identical. At least three sera showed significantly higher MFI values for DQA1*03:02 and DQA1*03:03 than for DQA1*03:01, namely, #30: 5000 ± 1486 vs 2081 ± 680 ($p = 0.008$), #32: 6562 ± 154 vs 3102 ± 391 ($p = 0.0002$) and #42: 3192 ± 307 vs 1310 ± 382 ($p = 0.005$). Position 160 is more than 20 Ångstroms away from all $47QL_5$ configurations except 187T. This suggests that 160D is important for the structure of the 187T-defined epitope recognized by these antibodies, the presence of 160A is associated with less reactivity but it did not lead to a negative reaction.

Table 2 shows also three sera (#39, #40 and #41) that reacted with alleles carrying the newly antibody-defined 56PV eplet on DQB1*05:01, *06:04, *06:05 and *06:09. Three other newly-antibody defined eplets, 40ERV (on DB1*02:01, *03:01/02/03), 87AF (on DQB1*06:01/02/03) and $75I_2$ (on all DQA alleles except DQA1*05:01/03/05) were identified with one serum.

Two weak reactivity patterns corresponded with the presence of heterodimers with distinct combinations of DQB and DQA eplets; no other combinations could be distinguished with this panel. Serum #35 reacted weakly with 55PP + 51F on DQA (878 ± 511) whereas the 55PP + 51L carrying alleles (57 ± 6) were considered non-reactive. Serum #38 reacted with heterodimers carrying 56PA (which is unique on DQB1*03:02) together with 160A on DQA (15012 ± 559) but the 56PA + 160D-carrying DQB1*03:02, DQA1*03:01 had an MFI = 1. However, the DQB:55-DQA:51 and DQB:56-DQA:160 sequence positions are more than 25 Ångstroms apart which is way too far for a structural epitope interpretation.

3.3. Design of a DQ eplet map

Complete descriptions of HLA epitope repertoires are important for the clinical application of HLA epitope-based matching for transplantation. This report shows that most of the DQ antibody reactivity of post-pregnancy can be explained with AbVer and newly antibody-defined eplets but of course, the repertoire is still incomplete. We

have created eplet maps that show the sequence locations of [1] eplets that have been shown experimentally to serve as recognition sites for HLA antibodies and [2], distinct polymorphic residues that are uniquely shared by different groups of alleles; specific reactivity of such groups with a given antibody could lead to structural characterization of newly antibody-verified eplets. Such maps would be useful for an epitope-based interpretation of otherwise unexplained serum reactivity pattern with allele panels.

Fig. 1 shows the eplet map for 15 DQB alleles commonly used in SAB panels. At present there are 16 antibody-verified eplets and they are displayed in boxes. 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 Fig. 1 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 and 67I? Moreover, can an epitope defined by the alternative 66EV 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.

Fig. 2 shows the DQA map with five antibody-verified eplets including 47KHL on DQA1*02:01 and 40ERV on DQA1*02:01, DQA1*03:01, DQA1*03:02 and DQA1*03:03. Three eplets have subscripts which means multiple unique residues in three or four sequence locations. They are $40GR_3$: 40G, 47C, 50V, 51L and 53Q; $75S_3$: 75S, 107I, 161E, 163S and 175K; $47QL_4$: 26S, 47Q, 56R, 76V and 187T but the compositions of current SAB panels do not permit a distinction which residues react with antibody. Therefore, the map shows just the eplets whereas the associated residues have been removed to make it easier to read.

Multiple polymorphic residues are distinctly shared by the same group 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 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 DQA1 alleles except DQA1*05. They have the unique 75I, 156F, 161D and 163I residues and if experimentally verified the corresponding eplet will be subscripted. The DQA map has become easier to read with the postulated EpX, EpY and EpZ eplets after removing their corresponding residues.

The DQA map has 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.

Sequence	3	9	13	14	23	26	30	37	38	40	Ep	Ep	45	Ep	Ep		56	57	66	67	70	Ep	Ep	71	74	75	Ep	Ep	86	87	116	125	126	130	135	Ep	167	185
Surface	+	-	-	-	+	-	-	±	±	±			++				+	+	++	++	+			+	+	±			±	+	+	+	+	+	+		++	+
DQB1*02:01	S	Y	G	M	R	L			V	F	$45GE_3$		G				P	A	D	I	R						77R	$84QL_3$			V		Q	R	D	$140A_2$	R	T
DQB1*02:02	S	Y	G	M	R	L			V	F	$45GE_3$		G				P	A	D	I	R						77R	$84QL_3$			V		Q	R	G	$140A_2$	R	T
DQB1*03:01	S	Y	A	M	R	Y	Y	Y	A	Y	45EV	$47VY_3$		$52PL_3$	55PP		P	D	E	V	R			T	E	L	77T	$84QL_3$			V		Q	R	D		H	T
DQB1*03:02	S	Y	G	M	R	L	Y	Y	A	Y	45GV	$47VY_3$	G	$52PL_3$	55PP		P	A	E	V	R			T	E	L	77T	$84QL_3$			V		Q	R	D		R	I
DQB1*03:03	S	Y	G	M	R	L	Y	Y	A	Y	45GV	$47VY_3$	G	$52PL_3$	55PP		P	D	E	V	R			T	E	L	77T	$84QL_3$			V		Q	R	D		R	I
DQB1*04:01	S	F	G	M	L		Y	Y	A	Y	45GV	$47VY_3$	G	$52PL_3$	52PR	$55RL_2$		D	D	I			74SV$_2$			V	77T	$84QL_3$			V		Q	R	D		R	I
DQB1*04:02	S	F	G	M	R		Y	Y	A	Y	45GV	$47VY_3$	G	$52PL_3$	52PR	$55RL_2$		D	D	I			74SV$_2$			V	77T	$84QL_3$			V		Q	R	D		R	I
DQB1*05:01	S	Y	G	L	R		H	Y	V	F	45GV	$47VY_3$	G	$52PQ_2$	52PR	56PV	P		E	V	G	71SR$_3$	74SV$_2$			V	77R		A	Y			Q	R	D	$140A_2$	R	T
DQB1*05:02	S	Y	G	L	R		H	Y	V	F	45GV	$47VY_3$	G	$52PQ_2$	52PR		P	S	E	V	G	71SR$_3$	74SV$_2$			V	77R		A	Y			H	R	D	$140A_2$	R	T
DQB1*06:01	P	L	A	M	R	Y	Y	D	V	F	45GV	$47VY_3$	G	$52PQ_2$	52PR		P	D	D	I	R			T	E	L	77T	87F	A		V	G	Q	R	D	$140A_2$	H	T
DQB1*06:02	S	F	G	M	R	L	Y	Y	A	Y	45GV	$47VY_3$	G	$52PQ_2$	52PR		P	D	E	V	G			T	E	L	77T	87F	A		V	G	Q	R	D	$140A_2$	R	T
DQB1*06:03	S	Y	G	M	R	L	H	Y	A	Y	45GV	$47VY_3$	G	$52PQ_2$	52PR		P	D	E	V	G			T	E	L	77T	87F	A		V	G	Q	R	D	$140A_2$	R	T
DQB1*06:04	S	Y	G	M	R	L	H	Y	A	Y	45GV	$47VY_3$	G	$52PQ_2$	52PR	56PV	P		E	V	R			T	E	L	77T		G	Y	V	G	Q	Q	D	$140A_2$	R	T
DQB1*06:05	S	Y	G	L	R	L	Y	Y	A	Y	45GV	$47VY_3$	G	$52PQ_2$	52PR	56PV	P		E	V	R			T	E	L	77T		G	Y	V	G	Q	Q	D	$140A_2$	R	T
DQB1*06:09	S	Y	G	M	R	L	Y	Y	A	Y	45GV	$47VY_3$	G	$52PQ_2$	52PR	56PV	P		E	V	R			T	E	L	77T		G	Y	V	G	Q	Q	D	$140A_2$	R	T

Eplet	Alleles	Unique Residues
$45GE_3$	DQB1*02	28S,30S,37I,46E,47F,52L,55L,71K,74A
$46VY_3$	DQB1*03/04/05/06	28T,46V,47Y,52P
$52PL_3$	DQB1*03/04	53L,140T,182N
$52PQ_2$	DQB1*05/06	53Q,84E,85V,89G,90I

Fig. 1. Provisional HLA-DQB map of antibody-defined eplets and polymorphic residues as potential epitopes.

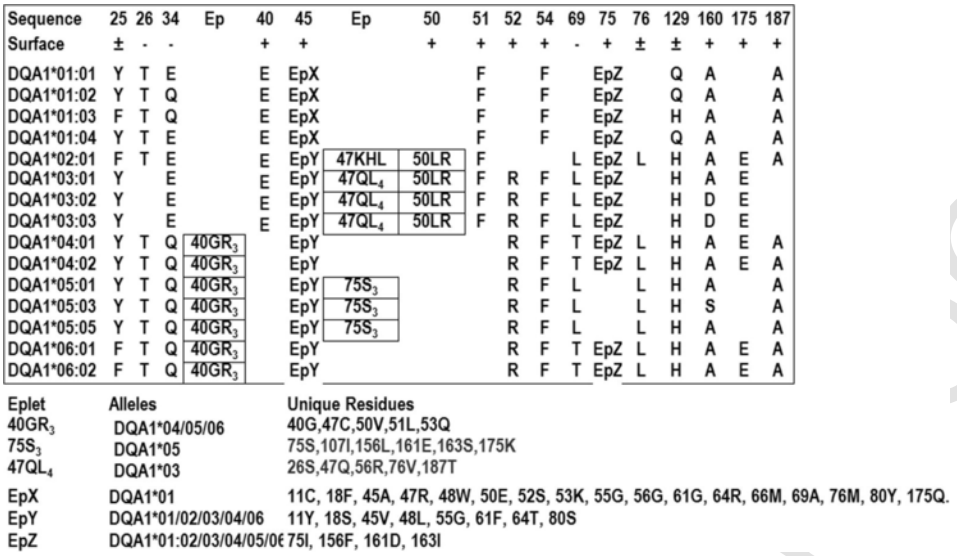

Fig. 2. Provisional HLA-DQA map of antibody-defined eplets and polymorphic residues as potential epitopes.

The DQA map shows the sequence positions of polymorphic residues where newly antibody-verified epitopes can be located. Sequence positions 26 and 34 are below the molecular surface. They are considered less likely candidates for eplets but may exert conformational effects on antibody-reactive eplets defined by residues on the molecular surface.

4. Discussion

This analysis of 42 post-pregnancy sera with DQ antibodies demonstrated that the reactivity of alleles in the panel correlates well with the presence of AbVer and newly defined DQA and DQB epitopes. This analysis involved women with a considerable variety of nationalities, but the data showed generally rather clear-cut epitope-specific reactivities of these sera. It must be recognized that the DQ epitope repertoire is still incomplete and that more studies not only on pregnancy cases but also transplant patients are needed. The post-pregnancy serum analysis offers an attractive model because antibodies are the result of immunization by a single haplotype mismatch in a healthy individual. The current study had a potential limitation that several women had previous live births and/or miscarriages, which as the data show in a few cases might have led to antibodies reactive with third-party epitopes. Fortunately, these antibodies did not seem to interfere with the identification of child-specific epitopes on reactive DQ alleles. Previous studies have shown that many HLA-ABC epitopes correspond to eplets paired with other residue configurations [34,38–40]. Such pairs are uncommon for DRB [32] and one reason is that the monomorphic DRA has no polymorphic residues that can be demonstrated as critical contact sites for antibody. Since both chains of HLA-DQ are polymorphic one might expect many DQ epitopes comprised of eplet pairs on DQA, DQB or DQA-DQB combinations. It raises the concept that a DQ epitope analysis of antibody reactivity should be based on the DQ heterodimers rather than the DQA and DQB chains alone [18]. There is little information about DQ epitopes defined by eplet pairs [41] and the HLA Epitope Registry has none [30].

This analysis looked into the possibility that some pregnancy sera had antibodies reacting with heterodimers with distinct DQA-DQB eplet pairs. Only serum #23 appeared to have such antibodies as demonstrated by strong reactions of heterodimers with DQB:84QL + DQA:54F and very weak reactions of heterodimers with DQB:84QL + DQA:54L. These residues are 8 Ångstrom apart a sufficient distance for contact by separate CDRs of antibody. Serum #35 and #38 reacted with heterodimers with DQB:55PP + DQA:51F and DQB:56PA + DQA:160A, respectively. These combinations cannot describe any structural epitope defined by a pair because the residues are too far away from each other.

Access to a complete repertoire of antibody-verified epitopes is essential to the successful application of epitope-based HLA compatibility determination in the clinical setting. This study permitted for the first time the design of a DQ epitope map that in conjunction with HLAMatchmaker can be used as a resource for antibody specificity analysis and the identification of newly antibody-defined epitopes. HLA Epitology studies in histocompatibility laboratories will enhance our understanding of epitopes and eventually will lead to complete repertoires of antibody-verified epitopes essential to this HLA matching approach in the clinical setting.

Disclosures

This study was approved by the local ethics committee and written informed consent was obtained from women enrolled in the study. Dr. Schaub is supported by the Swiss National Foundation (grant 32473B_125482/1) and the Nora van Meeuwen-Häfliger foundation. The authors of this manuscript have no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2016.06.021.

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