A single amino acid polymorphism in α2 domain of HLA DQα regulates the assembly and expression of HLA DQ alleles and the potential clinical impact

Thoa Nong, N. Remi Shih, and Jar-How Lee Terasaki Innovation Center, Los Angeles, California

TERASAKI INNOVATION CENTER

Abstract

Background: The current practice in preforming virtual crossmatch (vXM) or determining the calculated panel reactive antibody (cPRA) is based on the information of patient HLA antibody and donor HLA genotypes, the cell surface expression level of the donor antigens is not considered unless it's a known null allele. Some centers raise the cutoffs of low expression loci in assigning unacceptable antigen (UA) in order to compensate for the high amount of antigens on the Single Antigen Bead assays (SAB). However, minimum information are available for the protein expression and assembly that might affect the surface expression on individual alleles.

Methods: We have identified and characterized a panel of DQ4 allosera and used it to quantify the DQ4 antigen assembly and surface expression on EBV transformed B cell lines and transfectants of DQ4 variants.

Results: A single amino acid change in the α2 domain DQα chain can affect the stability of the αβ heterodimer and resulting in the low surface expression of the DQ antigens. The compounding effect of additional amino acid changes in the DQβ chain lower the surface expression of DQ antigen even further.

Conclusions: We have demonstrated that surface expression level of DQ4 variants can significantly affect the reactivity to allo-antibody. The impact of the sequence variations in α2 and β2 domains of the HLA Class II serological groups and alleles within the serological groups should be further investigated. Identifying those low surface expression antigens might benefit the donor selection of those patients that have high PRA.

Introduction

It is well established that the surface expressions of certain HLA loci are expressed at relatively low levels. The current practice in performing a virtual crossmatch (vXM) or determining the calculated panel reactive antibody (cPRA), is based on Single Antigen Bead (SAB) analyses of anti-HLA antibodies in the recipient compared with donor HLA genotypes. The cell surface expression level of the detected donor HLA antigens is not routinely considered unless it is a known null allele. Most of the polymorphic amino acids of the HLA Class II molecules are located on the α1 and β1 domains. It has been shown that cis-associated DQα and DQβ chain combinations co-evolved to from favorable heterodimers, but it was limited to the polymorphic positions in the α1 and β1 domains. How polymorphisms within the α2 and β2 domains may impact assembly and expression of the heterodimers and potential impact of polymorphic amino acid residues in the α2 domain of DQA1 alleles to affect αβ heterodimer formation has not been reported. We observed low antibody reactivity of some DQ4 specific sera to the DQB1 antigen that was associated with DQα0402 but not with DQα0401 in LS2PRA which leads us to this study.

Materials and Methods

Thirty-nine (39) of the DQ4 reactive sera showed minimum cross reactivity to the second DQ antigen in the heterozygous cell panel were selected for investigation. Purified DQ antigens from the same 7 heterozygous B cell lines that were used to produce the LS2PRA antigen panel were examined. Additional heterozygous DQα0401 and DQα0402 antigens were also used to confirm the low DQα0402 antibody reactivity. Recombinant single antigen DQβ0402 with DQα0401 or DQα0402 were created in a T2DM cells. Flow Cytometry was used to confirm the reactivities observed on the single antigen bead assay. Surface expression of the Site-directed mutagenesis DQβ0402:DQα0401 mutants were used to check the essential amino acid for the DQα04 assembling and expression.

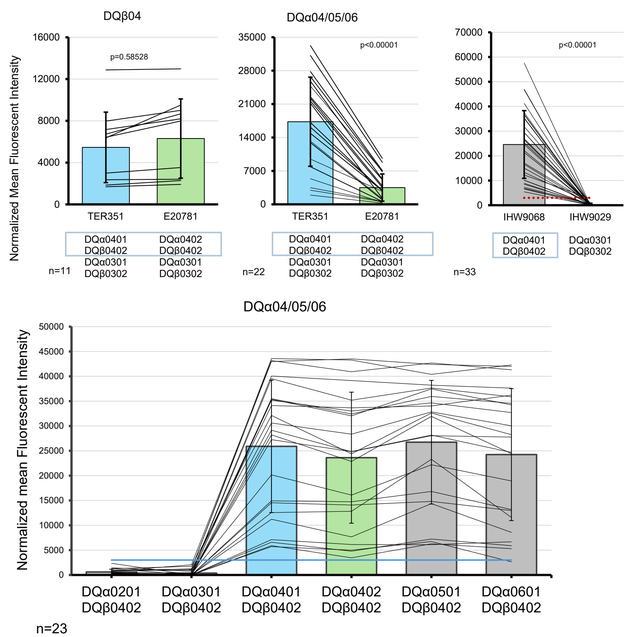


Figure 1. DQα0402 is responsible for the low DQ4 antigen surface expression in DQB1*04:02-DQA1*04:02 haplotype

Results

The serum samples that were reactive to DQβ04 chain showed similar reactivity to all 7 LS2PRA DQ4 antigen preparations while serum samples that were reactive to DQα04 showed a significant reduction in reactivity to the DQ4 antigen purified from the DQα0402 harboring antigens. All serum samples that were reactive to DQα04 recognize an epitope shared with DQα05 and 06 alleles and were considered as DQα04/05/06 positive sera. Their low DQα0402 antibody reactivity was also confirmed in another DQA1*04:02 positive B cell line. The purified recombinant DQβ0402:DQα0402 antigen was equally reactive as the purified recombinant DQβ0402:DQα0401 antigen to all the DQβ04 and DQα04/05/06 reactive sera. These results support that DQα04/05/06 positive sera recognize both DQα0401 and DQα0402 are in the same serology group.
The only difference between DQA1*04:01 and DQA1*04:02 sequence is a single nucleotide cytosine to thymine change at codon 137 resulting in an amino acid change from threonine (Thr), a polar amino acid, to isoleucine (Ile), a non-polar amino acid. This single amino acid change at residue 137 in the α2 domain of the DQα chain can significantly affect the stability of the αβ heterodimer and result in the reduced surface expression of the affected DQ antigens. Site-directed mutagenesis on codon 137 variants of DQA1*04:01 amino acid indicates that Serine and Threonine with their small molecular size and hydroxy bearing polar side chain are preferred in the DQα:DQβ complex due a hydrogen bond formation with the amide backbone of α150I predicted by AlphaFold 3. All other 18 amino acid substitutions result in varying degrees of reduction in DQ surface expression indicating the naturally evolved Thr is the optimal amino acid in the assembly of the DQ αβ heterodimer. The compounding effect of additional amino acid changes in the DQβ chain further lowers the surface expression of DQ antigen, results in changes in anti-HLA antibody reactivity.

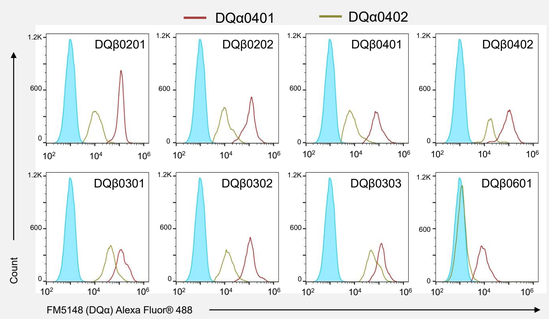


Figure 2. Inefficient assembly with DQβ chain and not poor synthesis of DQα0402 chain is responsible for the low surface expression

Discussion/Conclusion

Prediction of antibody reactivity based on their structural protein sequence relies on a fundamental assumption that all related HLA polypeptides will fold and assemble into a common functional tertiary structure and be expressed on the cell surface at similar levels. A single amino acid change at codon 137 in the α2 domain of the DQα chain, a location that is not previously believed to impact serology, can significantly change the surface expression of the antigen and can mislead to wrong diagnosis. This single amino acid residue can influence the expression of a DQ4 molecule. Low surface antigen expression might be less likely to induce complement binding or Fc receptor engagement by the DSA. It's plausible that low surface expression of HLA antigens might explain why some patients fail to show antibody mediated rejection even in the presence of high quantities of DSA as measured by SABs. As a result, amino acid sequence variations in the α2 and β2 domains of the HLA Class II serological groups and alleles within the serological groups should be further investigated to understand the scope of allele expression variability. Identifying low surface expression antigens/alleles might benefit the donor selection for highly immunized patients.

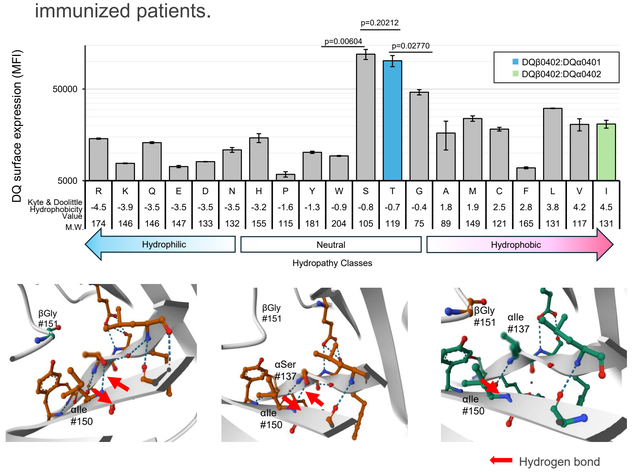


Figure 3. Additional hydrogen bonds between α137T side chain and α150I might contribute to the stability of DQ4 heterodimer

Acknowledgements

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