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HLA-DQ antibodies in alloimmunity, what makes them different?

Maria Meneghini[a,b] *and Anat R. Tambur*[a,*]

Purpose of review
De novo HLA-DQ antibodies are the most frequently observed after solid-organ allotransplantation; and are associated with the worse adverse graft outcomes compared with all other HLA antibodies. However, the biological explanation for this observation is not yet known. Herein, we examine unique characteristics of alloimmunity directed specifically against HLA-DQ molecules.

Recent findings
While investigators attempted to decipher functional properties of HLA class II antigens that may explain their immunogenicity and pathogenicity, most early studies focused on the more expressed molecule – HLA-DR. We here summarize up-to-date literature documenting specific features of HLA-DQ, as compared to other class II HLA antigens. Structural and cell-surface expression differences have been noted on various cell types. Some evidence suggests variations in antigen-presenting function and intracellular activation pathways after antigen/antibody interaction.

Summary
The clinical effects of donor-recipient incompatibility at HLA-DQ, the risk of generating de novo antibodies leading to rejection, and the inferior graft outcomes indicate increased immunogenicity and pathogenicity that is unique to this HLA antigen. Clearly, knowledge generated for HLA-DR cannot be applied interchangeably. Deeper understanding of features unique to HLA-DQ may support the generation of targeted preventive-therapeutic strategies and ultimately improve solid-organ transplant outcomes.

Keywords
alloimmunity, allorecognition, HLA antibodies, HLA-DQ

INTRODUCTION

The role of de novo humoral alloimmunity in organ transplantation, especially as directed against HLA class II antigens, has been the focus of increased interest in the past decade. Modern HLA typing technologies, and HLA antibody assays with higher sensitivity and specificity, provided information that was not available for earlier transplant cohorts [1–3,4▪]. Importantly, better control of early acute rejection by calcineurin inhibitors (CNI) based Immunosuppression resulted in larger number of patients achieving longer-term graft survival and provided insight into the high proportion of patients exhibiting circulating donor-specific antibodies (DSA) and higher rates of chronic antibody-mediated rejection (ABMR) [5]. It was further recognized that alloimmunity not only against HLA-DR but also (and maybe mostly) against HLA-DQ, plays a significant role.

The scope of this review, therefore, is to summarize recent evidence that associate HLA-DQ with increased immunogenicity and pathogenicity in allotransplantation. We will further discuss possible explanations and highlight open questions.

[a]Transplant Immunology Laboratory, Comprehensive Transplant Center, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA and [b]Nephrology and Kidney Transplantation Department, Vall d'Hebron University Hospital, Vall d'Hebron Research Institute (VHIR), Barcelona, Spain

Correspondence to Anat R. Tambur, Director, Transplant Immunology Laboratory, Comprehensive Transplant Center, Research Professor, Feinberg School of Medicine, Northwestern University, 300 E Superior Ave, Tarry Building Suite 11-711, Chicago, IL 60611-3008, USA.
Tel: +1 312 503 8069; fax: +1 312 503 3366;
e-mail: a-tambur@northwestern.edu

*A.R. Tambur is a Paul I. Terasaki Scholar.

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KEY POINTS

- Clinical data in kidney transplantation associate the development of HLA-DQ antibodies with inferior allograft outcomes.
- Physiologically, HLA class II molecules have common functions, yet HLA-DQ seems to be more associated with autoimmunity and alloimmunity.
- The unique immunogenicity of HLA-DQ can potentially be related to differences in level of expression, both constitutive and induced, on different cell types on allograft target tissue.
- The unique pathogenicity of HLA-DQ may be due to distinct Intracellular pathways following interactions with T cells or allo-antibodies.
- The structural differences of HLA-DQ heterodimers, having two polymorphic alpha and beta chains, may also contribute to its immunogenicity. Up to four heterodimers (or more?) may be expressed on the cell surface, potentially increasing the likelihood of allorecognition and subsequent immune activation.

DONOR SPECIFIC HLA-DQ ANTIBODIES IMPACT ALLOGRAFT OUTCOMES AND REDUCE ACCESS TO TRANSPLANTATION

The first definitive evidence for the role of HLA-DQ antibodies in transplant outcome came from a well designed study by Willicombe *et al.* [6]. Not only this group demonstrated increased risk of generating de-novo HLA-DQ antibodies; but they further showed that these antibodies exhibit increased pathogenicity, with higher frequencies of transplant glomerulopathy and graft loss, when compared with DSA directed at other HLA loci. More recently, and in prospective study cohorts, the associations between HLA-DQ mismatching and generation of de-novo DSA (dnDSA) were confirmed both in the United States [7] and in Europe [8] with especially deleterious impact in the settings of immunosuppression minimization [9–11].

While mounting evidence exist to document HLA-DQ DSA as the most frequently detected antibodies after transplantation, straight-forward analyses of large data repositories (e.g., Scientific Registry of Transplant Recipients (SRTR)) failed to demonstrate an association between HLA-DQ mismatching and poor transplant outcomes. We believe this is mostly due to the low typing resolution of HLA-DQ available in these repositories, (only up to seven antigens), and masking by linkage disequilibrium with HLA-DR (showing higher granularity even at the antigen-level, with twice as many antigens) necessitating use of more sophisticated analyses. For example, in [12], using the Australia and New Zealand Dialysis and Transplant Registry, an increased risk for developing any rejection, late rejection, and ABMR was associated with HLA-DQ mismatches. Importantly this was independent of HLA-ABDR mismatching, sensitization status and initial immunosuppression. Of note, though, follow-up was relatively short, graft survival was not affected, and an interaction with mismatches at HLA-DR was observed. Leeaphorn *et al.* [13], using the US SRTR registry, demonstrated a direct effect of DQ mismatching on long-term graft survival specific for recipients of deceased donor kidney-transplant with short cold ischemia ($\leq$17 h), and for recipients of living donor kidney-transplant.

Given the lack of high-resolution typing, or granular information regarding dnDSA or ABMR in the SRTR, we took a different approach. Looking at patients who lost their first organ and relisted for a second transplant, we assessed registration of all *new* unacceptable antigens. Since crossmatch prior to the first graft was negative, if the new unacceptable antigen corresponded with the first donor HLA typing, it may suggest generation of dnDSA against the first donor, possibly associated with graft loss. While not conclusive, using this approach, we demonstrated that significantly more patients returned to the waitlist with de novo antibodies against their first donor HLA-DQ antigens compared with de novo antibodies to any other mismatched locus. Furthermore, patients with new unacceptable antigens against their first donor DQ-mismatched had the highest increase in cPRA, diminishing their access to retransplantation [14▪].

Clearly, despite accumulating evidence, our community is lacking conclusive studies to support rehauling the principles guiding allocation algorithms to prioritize HLA-DQ matching. Creative approaches to mitigate the potential negative impact on equity have been proposed [15], yet a significant debate is still currently on-going in the United States and in other countries [16▪]. At this point, we are still missing definitive evidence to demonstrate unique qualities leading to increased alloimmunity associated with HLA-DQ.

We therefore continue to review aspects differentiating HLA-DQ from the other class I/II HLA molecules, levels of cell-surface expression and intracellular signaling pathways. We believe that only by uncovering these differences a better understanding of HLA-DQ alloimmunity can be gained.

HLA DQ IMMUNOGENICITY: EXPRESSION ON ALLOGRAFT CELLS

The three HLA class II molecules, expressed on professional or unprofessional antigen presenting cells

(APCs), share the function of presenting peptides derived from extracellular proteins after endosomal degradation, while class I HLA molecules present peptides derived from intracellular proteins. As compared to class I, Class II molecules are characterized by a different genetic structure, where both genes, encoding for the α and β chains are encoded within the MHC region [17].

An important difference between the three HLA class II molecules is their level of expression on the cell surface. This may be due to differences in regulatory checkpoints at the transcriptional/posttranscriptional level; the stability of the molecule on the cell surface; and turnover rates (reviewed in [18]). Differences in expression may be governed by organ specific regulatory processes [19] or even due to gender-specific differences, at least in specific conditions [20]. Understanding of these differences is critical but inconsistencies were observed depending on the assay used to define expression.

Specific to solid organ (kidney) transplantation, studies describing expression of HLA class II molecules on graft tissue focused mainly on endothelial cells, as this is the first surface where antibody/antigen interaction occurs. In biopsies, Muczynki *et al.* [21] reported that HLA-DR was constitutively expressed on endothelium of glomerular and peritubular capillaries, but not on larger blood vessels. HLA-DQ or -DP were not expressed, but mRNA transcripts were detected for HLA-DP. Later, expression of all three class II molecules was demonstrated on glomerular endothelial cells freshly isolated from normal human kidney biopsies; albeit HLA-DQ expression was lower compared with the other antigens [22]. Confirmation for this observation was also reported using single cell RNA-seq in kidney allograft biopsies [23]. In preimplantation biopsies, using gene expression microarrays, Mine *et al.* [24] associated high HLA-DQB1 RNA expression with poorer graft outcomes.

In a highly innovative study in professional antigen presenting cells, Casasola-LaMacchia *et al.* [25▪] showed that the expression of the HLA-DR/DQ/DP was highly variable between cell types, especially in response to inflammatory stimuli within different donors. Interestingly, results of mass spectrometry were not always concordant with surface flow-cytometry analysis, suggestive of posttranscriptional control mechanisms as well as difference in recycling rates of those molecules. Interestingly, measuring the relative abundance of the alpha (α) chain of the three different loci, they demonstrated the highest abundance to be that of DRα (>90% of all α chains), followed by DPα (5–10%) and lastly DQα (<1%, or even absent in more than one cell type even if traditionally classified as APC expressing class II MHC; both lines and primary human cells).

Studying the role of inflammatory cytokines [interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα)] required for in-vivo and in-vitro induction of HLA class II expression, Valenzuela described upregulation of HLA-DR mRNA and cell surface expression within the first 24 h, whereas HLA-DQ or -DP showed similar, but lower responses in different vascular endothelial cells [26]. Cross *et al.* [27▪] also demonstrated lower expression of HLA-DQ compared with that of HLA-DR on human renal glomerular endothelial cells, in response to inflammatory conditions. In this latter study, HLA expression was followed for a longer period, 10 days, demonstrating prompt expression of HLA-DR (within 18–48 h) while upregulation of HLA-DQ molecules was delayed and apparent only between days 3 and 7.

We have studied the kinetics of HLA class II antigen response to IFNγ stimulation using human primary glomerular endothelial cells. Using both flow cytometry and qPCR, we have confirmed the rapid upregulation of HLA-DR expression (within 48 h). Similar to the observation from Cross *et al.*, HLA-DQ and HLA-DP demonstrated up-regulation only around days 4–7. We further showed that HLA-DP shows higher levels of expression compared to that of HLA-DQ. Our experimental system was designed to follow mRNA and cell surface expression up to 28 days of continuous IFNγ exposure. In these conditions we have observed that HLA-DR expression reached a plateau around 10–14 days, being ∼20-fold higher than baseline. HLA-DP reached a plateau at about 21 days with ∼10 times increased expression. Finally, HLA-DQ was the slowest to respond to IFNγ stimulation, demonstrating increased expression up to day 28, with only up to 4-fold increase compared with baseline (Fig. 1).

Providing a potentially different perspective, Fersl *et al.* [28] demonstrated that different HLA-DQ alleles can be expressed at different levels. More recently, Beland *et al.* [29▪] further showed highly variable degree of cell surface expression of different HLA-DQ molecules, but not of HLA-DR or DP molecules. This variability was associated with the specific HLA-DQ genotype of the cell line tested, adding another potential layer of complexity in understanding the role of the HLA system in organ transplantation [30].

Clearly, a significant gap remains in our understanding of HLA-DQ cell surface expression (in health and pathology), specifically on cells that are the likely target for immune processes, the microvasculature of the transplanted organ.

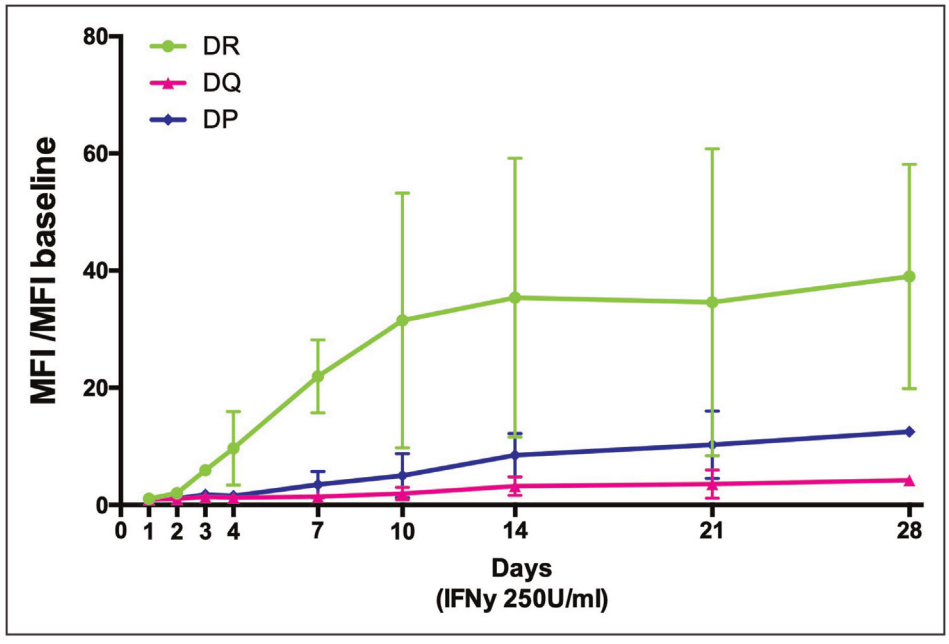

FIGURE 1. Expression of HLA-DR, HLA-DQ and HLA-DP on primary human glomerular endothelial cells over time. Cells were continuously exposed to IFNγ 250 U/ml for up to 28 days. HLA expression was assessed by flow cytometry, MFI (median fluorescence intensity) corrected for baseline MFI is shown. Results are from eight experiments.

PATHOGENICITY OF ANTI-HLA DQ ANTIBODIES

A decade had passed since Willicombe *et al.* [6] demonstrated that immune responses against donor mismatched HLA-DQ are more deleterious for graft outcome compared with responses against other HLA targets. Multiple other publications had substantiated this observation [16▪]. The dichotomy between the low levels of HLA-DQ cell-surface expression on target cells, and the vigorously high immune activation is puzzling. Some insights may be gained by studying pathways activated by antigen-antibody ligation [31]. However, as above, most of the studies performed focused on HLA-DR.

Early studies by Leveille *et al.* [32] described differences in downstream signaling pathways comparing activation of HLA-DR and -DP molecules on B cells. However, their model specifically lacks HLA-DQ expression. Haylett *et al.* [33] further identified similarities between HLA-DQ and -DP, but different from DR when studying downstream signaling. Specifically, cross-activation of HLA DQ and DP, but not of DR, led to MEK1/2 and ERK1/2 phosphorylation, a step in the AP1/NFAT cascade. This observation may be significant in response to specific drugs as it suggests differences in regulation of cell activation.

HLA-DR mediated pathways were studied in an elegant model of aortic endothelial cells genetically engineered to constitutively express class II HLA without inflammatory stimuli. Signaling pathways were found to be similar to those observed for class I (Src, FAK, PI3K/Akt, and ERK), although differences in mTOR pathways suggested regulation by mechanisms upstream to Src, FAK, and PI3K/Akt [34]. Unfortunately, this study did not permit investigation of HLA-DQ and -DP. Additional studies focusing on ligation of HLA-DR demonstrated phosphorylation of S6 ribosomal protein (S6RP), a downstream target of the PI3K/Akt/mTOR pathway. Staining for S6RP in biopsies showed significant association with histological ABMR; and with circulating HLA class II, but not class I, antibodies. Based on these observations the authors proposed that functional differences exist in pathogenicity of class I vs. class II antibodies [35]. Here again HLA-DQ specific pathways were not studied. Still focusing on HLA-DR, Le Bas-Bernardet, demonstrated differences in phosphorylation at least for PKC-α/β, when comparing mature B cells to human arterial endothelial cells, suggesting an association with resistance to apoptosis of endothelial cells [36].

Literature specific to signaling pathways initiated by HLA-DQ ligation is quite sparse. In one of the rare studies focusing on the role of HLA-DQ antibodies in modulating microvascular endothelial cell line activation, Cross *et al.* [37] showed that HLA-DQ antibodies phosphorylated Akt and S6 kinase (similar to HLA-DR). Co-culture with alloreactive lymphocytes increased interleukin 6 (IL-6) and RANTES secretion and antibody-mediated upregulation of IL-6 was Akt-dependent. In this study, the binding of HLA-DQ antibodies to endothelial cells

selectively reduced T cell allo-proliferation and Fox-P3^{high} Treg differentiation. Additional studies along these lines are required to decipher whether the pathogenicity of HLA-DQ antibodies is different from that of HLA-DR. It is, however, quite clear that the immunogenicity of HLA-DQ is higher given the increased frequency and levels of dnDSA, associated with antibody mediated rejection.

An important difference between HLA-DR and -DQ (and -DP) is the fact that the α chain for the DR locus is monomorphic, thus its contribution to the full HLA-DRα/β is mostly to stabilize the heterodimer and allow for peptide presentation. This is in difference from HLA-DQ where both the α and the β chains are polymorphic. This means that in any given moment there are two α chains and two β chains "floating" in the endoplasmic reticulum. In principle, then, pairing between the chains can occur regardless of the chromosome on which the genes encoding these chains are located. Stochastically speaking, 4 different pairs of HLA-DQα/β combinations may therefore be formed and expressed on the cell surface. This phenomenon, referred to as cis- or trans-pairing of the chains, was reported two decades ago [38]. Anecdotical report correlated such a phenomenon with allograft loss [39]. In practice, though, specific rules dictate which of the DQα/β heterodimers are functionally stable (the others will not reach the cell surface) and thus some individuals express only two DQ heterodimers on their cells while others may express up to 4 HLA-DQ heterodimers [40].

The pathologic significance of this observation has not been investigated in solid organ transplantation but a recent report from the field of hematopoietic cells transplantation demonstrated strong association with increased risk of disease relapse, in a large cohort of HLA matched and unmatched recipients [41▪]. This suggests specific role of HLA-DQ, in concert with CD4 helper T cells in mediating graft versus tumor effects. To add additional level of complexity, preliminary data from our laboratory suggest that, at least in-vitro, in CRISPR-Cas9 manipulated cells, an HLA-DRα chain (and some HLA-DPα chains) may pair up with some of the DQβ chains to form a stable HLA class II hybrid heterodimer on the cell surface (Fig. 2). In this particular system, though, the cells are genetically edited to be devoid of HLA-DRβ or DPβ expression, which may skew the likelihood of the α chains to find their most suitable β chain partner. The clinical significance of these observations is not clear and its probability in cells with functional levels of all HLA class II gene products must be documented.

Additional differences between the three HLA class II antigens were summarized in [42]. Unfortunately, we are still lacking a correlation between these observations and their potential impact on

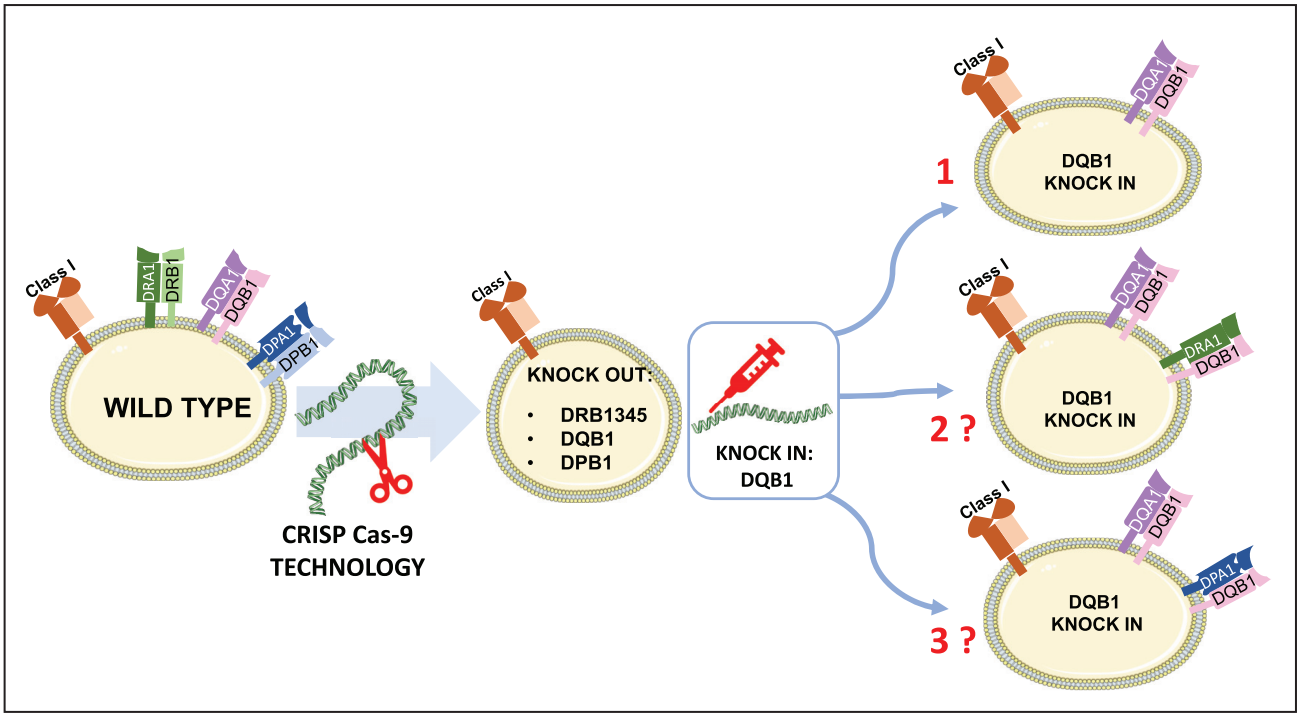

FIGURE 2. Suggested phenomenon of HLA class II hybrid heterodimers expression on CRISPR-Cas9 manipulated cells. B-Lymphoblastoid cell lines were knocked out for DRβ1345, DQβ1, and DPβ1. After knock-in of DQβ1 genes, expression of hybrid heterodimers (HLA-DRα1/ DQβ1 or HLA-DPα1/ DQβ1) was observed by flow cytometry.

the physiologic and pathologic function of HLA class II molecules. This seems to be a missed opportunity as it may explain the unique immunogenic/pathogenic role of HLA-DQ in solid organ transplantation.

CONCLUSION

The strong association between HLA-DQ mismatching in solid organ transplantation with the generation of dnDSA, reduced graft survival and high degree of sensitization after graft loss are well documented. Yet, currently it is not clear why HLA-DQ is the most immunogenic, and potentially most pathogenic, mismatch. Clearly, this is one of the most relevant open questions in transplant immunology. While some studies have attempted to address this question, much research is still required. Emphasis should be placed on generating HLA-DQ specific data rather than drawing conclusions from studies of HLA-DR. Future investigations should also address new insights into α/β pairing of HLA-DQ.

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Conflicts of interest

There are no conflicts of interest.

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