ELSEVIER

Case Report: Binding of a Clinically Relevant Human Leukocyte Antigen–DQα–Specific Antibody in a Kidney Graft Recipient is Inhibited by Donor-Type Human Leukocyte Antigen–DQβ Chain

S. Mikkelsen, T. Korsholm, A. Iburg, M.S. Petersen, and B.K. Møller

ABSTRACT

In this case report, we have found what may be an immunization with donor-specific human leukocyte antigen (HLA)-DQα in combination with recipient-specific HLA-DQβ. A renal allograft recipient who did not comply with immunosuppressive therapy during pregnancy had graft failure 23 months posttransplantation with biopsy-proven humoral and cellular rejection. Sera were tested in a Luminex-based single-antigen bead assay. We compared Luminex reactivity with the degree of eplet mismatching between the recipient's own HLA-DQ chains and the HLA-DQ chains bound to the Luminex beads. Eplet calculations were done with the HLAMatchmaker. HLA-DQ similarities were compared further by dissimilarity scoring in HistoCheck. We observed that Luminex beads with donor-type HLA-DQα and HLA-DQβ bound less antibody than beads with donor-type HLA-DQα combined with recipient HLA-DQβ. In HLAMatchmaker, we identified all eplet mismatches between donor and recipient HLA-DQ. Next, we counted how many of these eplets were represented on the various Luminex beads. We found that antibody binding to the bead increased with the number of such mismatches for HLA-DQα. Surprisingly, antibody binding decreased as the number of eplet mismatches for HLA-DQβ increased, from a mean fluorescence intensity (MFI) value of 18,800 for no mismatched eplets to approximately 10,000 for 12 mismatched eplets. These findings were confirmed by comparing antibody binding with the structural dissimilarity score between the recipient HLA-DQ type and the HLA-DQ bound to the Luminex beads. In this patient, clinically relevant antibodies bound strongly to donor-like HLA-DQα chains when combined with recipient-like HLA-DQβ. HLA-DQβ chains more similar to those of the donor reduced the binding of donor- specific HLA-DQα antibody.

IN KIDNEY transplantation, the degree of matching is often determined by counting the number of mismatched human leukocyte antigens (HLA)-A, -B, and -DR of the donor. Zero-antigen mismatched transplantations are generally considered most likely to succeed. Conversely, antibodies against mismatched HLA antigens increase the risk of transplant failure. HLA antigens have multiple motifs that can be recognized by specific antibodies.[1] HLA-DQ is an $\alpha\beta$ heterodimer of the HLA class II type. The α and the β chains are encoded by HLA-DQA1 and HLA-DQB1, respectively. The serological nomenclature of HLA-DQ molecules is generally equivalent to the typing of the β chain (DQB1).

Historically, HLA-DQ antibody specificities have been difficult to identify because of the strong association between HLA-DR and HLA-DQ. Now, antibodies against HLA-DQ are easier to identify with the Luminex single antigen bead assay (One Lambda Inc., Canoga Park, USA). HLA-DQ–specific antibodies are common in organ transplant recipients. The α chain is less polymorphic than the β chain. Consequently, the immunization is usually β-chain specific and HLA-DQα antibodies are rarely demontrated.[2]

From the Department of Clinical Immunology, Aarhus University Hospital, Aarhus, Denmark.

Address reprint requests to Susan Mikkelsen, Department of Clinical Immunology, Aarhus University Hospital, Brendstrupgaardsvej 100, 8200 Aarhus N, Denmark. E-mail: susanmikkelsen@live.dk

0041-1345/–see front matter
http://dx.doi.org/10.1016/j.transproceed.2012.10.006

This study is a case report of a patient with clinically relevant anti-HLA- DQα antibodies.

Other studies report DQα immunizations in patients waiting for kidney graft transplantation, but in these cases, the patients did not undergo transplantation. Barabanova et al reported 2 cases with "anti-self-antibodies": both patients have strong reactivity against DQ7-coated beads, but only when the self-DQβ chain is combined with nonself DQα chains.[3] Tambur et al studied sera from 104 patients awaiting kidney transplantation known to have HLA-DQ antibodies. A total of 34% of these patients have antibodies against self-DQβ chain combined with nonself DQα chains.[2]

To identify potential organ donors with acceptable mismatches, computational techniques that describe antibody reactivity at the structural level can be used.

HLAMatchmaker[4] is a theoretical computer algorithm that determines HLA compatibility at the structural level. In HLAMatchmaker, each HLA antigen is modelled as a sequence of so-called "eplets." These eplets are akin to epitopes, but are defined as patches of polymorphic residues within a radius of 3.0–3.5 Å. Usually, eplets consist of 3 contiguous amino acid residues; however, the residues need not be sequential to form an eplet. Residues from different positions in the protein primary sequence may form an eplet if they are sufficiently close in the tertiary structure. HLAMatchmaker thus assigns donor-recipient histocompatibility by comparing eplets. The class II eplet version of HLAMatchmaker is based on 33 DQB and 29 DQA polymorphic positions that contribute a repertoire of 74 DQB and 58 DQA eplets.[5]

HLAMatchmaker makes it possible to determine which eplets on mismatched HLA molecules are different or shared between donor and patient. Immunogenic eplets can induce cross-sensitization between antigens encoded by different DQB loci.[6] Apparently, sensitization by an epitope of an HLA-mismatched antigen may cause other HLA antigens to become unacceptably mismatched due to sharing of that epitope, even when the patient has never been exposed to such antigens.[7]

HistoCheck[8,9] is an online internet based tool to visualize the amino acid differences between the HLA molecules of the donor and recipient and calculate a "dissimilarity score" (DSS). The allogenicity of mismatches is estimated using a sequence-similarity concept. Amino acid differences between HLA alleles are evaluated and rated according to position within the molecule (peptide binding, contact with the T-cell receptor) and functional similarity of amino acids. This procedure leads to a DSS, with high values representing a high degree of dissimilarity.[9]

CASE DESCRIPTION

A 34-year-old woman received her first renal allograft from a blood type–identical deceased donor in 2010. The patient type was HLA-A2,30(19); B18,44(12); DRB1*03; DQB1*02 and the donor type was HLA- A3,32(19); B44(12),57(17); DR07, 12; DQB1*03:01,03:03.

In pretransplantation serum of the patient, no HLA class I or class II antibodies were detected using complement-dependent cytotoxic (CDC) technique. Pretransplantation CDC crossmatch was negative against both T cells and B cells.

During pregnancy, the patient was noncompliant to immunosuppresive therapy and had kidney graft failure with biopsy-proven humoral and cellular rejection 23 months posttransplantation. The 23 month posttransplantation cytotoxic crossmatch (CDC) was positive against B cells and negative against T cells.

METHODS

HLA antibodies in posttransplantation serum of the patient were detected with the Luminex single-antigen bead assay LABScreen. HLA-coated beads enabled identification of Immunoglobulin G (IgG) alloantibody specificities against HLA-A,-B,-C,-DRB1,-DQA1,-DQB1 antigens. The threshold for a positive response was set at a mean fluorescence intensity (MFI) of 1000.

High-resolution DQA1/DQB1 typing was done with single specific primer-polymerase chain reaction technique. The patient type was HLA- DQA1*05:01; DQB1*02:01, and the donor type was HLA-DQA1*02:01,05:05; DQB1*03:01, 03:03. We used the HLAMatchmaker and the HistoCheck programs for a structurally based HLA matching. Calculation of eplet mismatches in HLA-DQ was done using the eplet pair–based HLAMatchmaker antibody analysis program (eplet matching v2.1).[4] First, we identified whether eplets on mismatched HLA-DQ molecules were different or shared between patient and donor. We then determined the number of these eplets present on the various Luminex beads. Only patient/donor–relevant eplets present on the various beads were considered. The Luminex reactivity was then compared with the degree of eplet mismatching between patient's own HLA-DQ chains and the HLA-DQ chains present on the Luminex beads.

DSSs were calculated using the HistoCheck website.[8] Patient DQα was compared with the DQα chains on the Luminex beads to find DSS. The same was done for DQβ. The DSS of both DQα and DQβ were categorized into groups of null, low, intermediate, and high dissimilarity and then compared with the Luminex reactivity.

RESULTS

The Luminex assay on the 23-month posttransplantation serum of the patient revealed anti-DQ2 autoreactive antibodies (MFI 12,000–18,000) when using beads with the serological equivalent of HLA-DQβ. Considering both DQβ and DQα chains, the strongly reactive anti-DQ2 antibodies bound only to beads with recipient-type DQβ chains in combination with nonself DQα chains. The patient had no anti-DQ2 antibodies against the beads with self-type DQα/DQβ.

Examining DQβ chains and their serological equivalents, we found 2 donor-specific antibodies (DSA), namely, anti-DQ7 and anti-DQ9, but with low to moderate reactivity (MFI 1000–7800). The patient had antibodies against all beads with donor-type DQα chains regardless of the DQβ association; so when taking the DQβ and the DQα chains into account, the patient had broadly reactive DSA.

Luminex beads with donor-type DQα/DQβ bound less antibody than beads with donor-type HLA-DQα combined with patient HLA-DQβ. Indeed, HLA-DQβ chains more

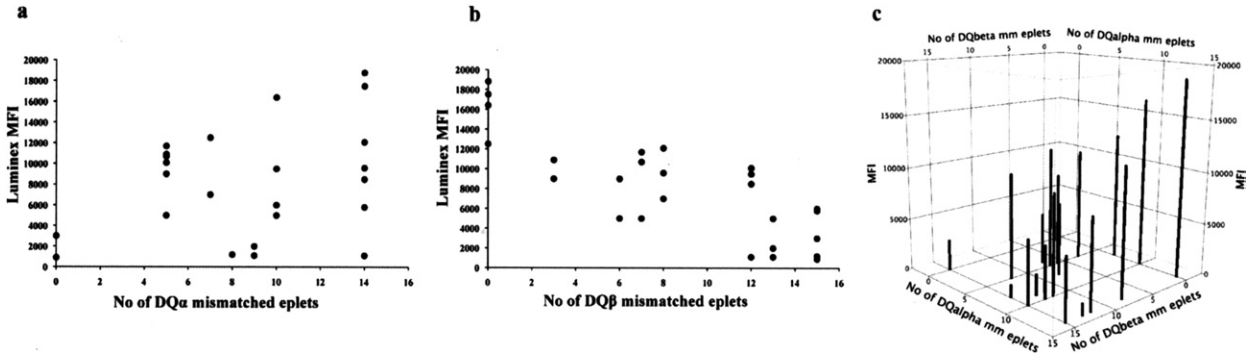

Fig 1. Comparison of Luminex reactivity with the degree of eplet mismatching on DQα (**a**) and DQβ (**b**). Only mismatched eplets represented in the donor HLA-DQ types are depicted. (**c**) The combined effect of mm eplets in DQα and DQβ chains, where the height of the bar represents MFI and the position of the bar represents the number of mm eplets.

similar to those of the donor reduced the binding of donor-specific HLA-DQα antibody.

When we compared Luminex reactivity with the degree of eplet mismatching (calculated in the HLAMatchmaker), antibody binding to the bead increased with the number of eplet mismatches for HLA-DQα (Fig 1a). Conversely, antibody binding decreased with increasing eplet mismatching for HLA-DQβ (Fig 1b). The combined effect of DQα and DQβ mismatched eplets is demonstrated in Figure 1c.

HLA-DQ similarities were compared by DSS in HistoCheck,[8] and the amino acid differences between the HLA molecules of the donor and patient were visualized.

Results for HLA-DQB1: patient DQB1*02:01 versus donor DQB1*03:01, DSS = 19.2. Patient DQB1*02:01 versus donor DQB1*03:03, DSS = 15.0. Results for HLA-DQA1: patient DQB1*05:01 versus donor DQB1*05:05, DSS = 0.0 (similar). Patient DQB1*05:01 versus donor DQB1*02:01, DSS = 2.97.

Multivariate cluster analysis identified 4 bead clusters based on DSS (Fig 2). We found the highest Luminex reactivity (MFI 17,500) in the cluster with donor type HLA-DQα chains of intermediate dissimilarity to "self" type in heterodimeric combination with an HLA-DQβ chain of low dissimilarity. Beads with higher dissimilarity had lower Luminex reactivity (MFI 9000), as did beads with HLA-DQα chains of intermediate dissimilarity in heterodimeric combination with HLA-DQβ chains of high dissimilarity (MFI 6000).

DISCUSSION

Herein, we have demonstrated a surprising binding of an anti-HLA DQ antibody dependent on similarity to donor DQα chain and to recipient DQβ chain.

The Luminex analysis revealed anti-DQ7 and anti-DQ9 reactivity examining only the DQβ chains and their serological equivalents. The patient had antibodies against all

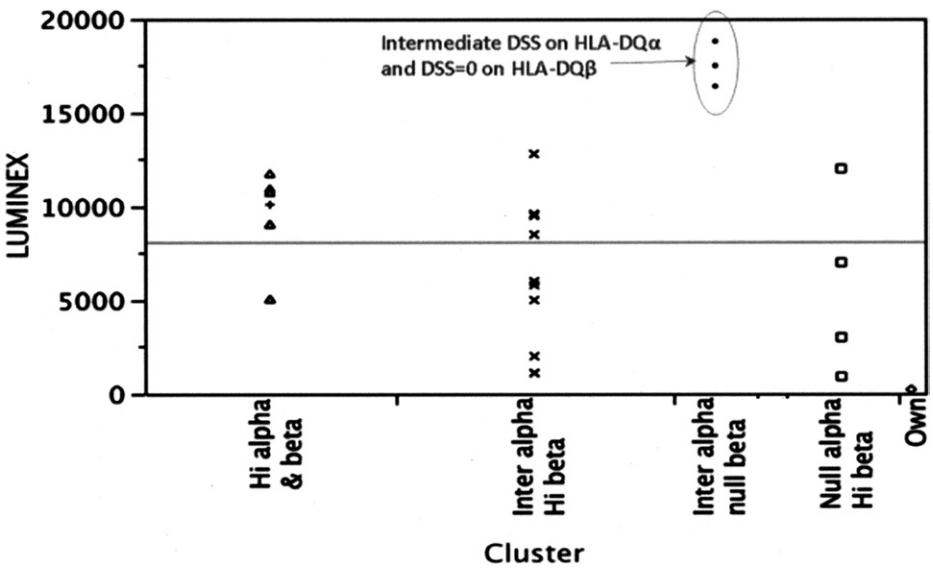

Fig 2. One-way analysis of Luminex by clusters of DSS. The DSS of both DQα and DQβ are categorized into groups of null, low, intermediate, and high dissimilarity and then compared with the Luminex reactivity.

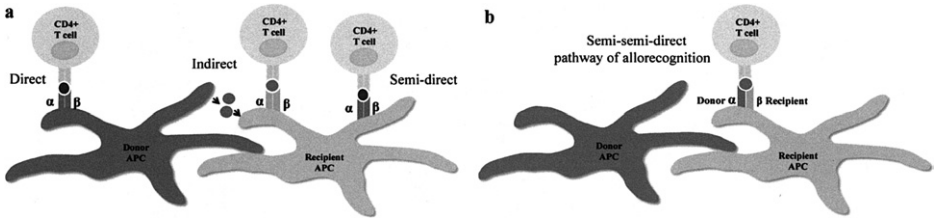

Fig 3. Pathways of allorecognition. (**a**) Direct, indirect, and semi-direct pathway of allorecognition. (**b**) Semi-semi-direct pathway of allorecognition.

beads coated with donor-identical DQα chains, so examining the DQβ as well as the DQα chains, the patient had broadly reactive DSAs. This indicates that the serological nomenclature is insufficient. Indeed, the structural analysis of antibody-specificity suggests that the DQβ chain modifies the antibody reactivity of the DQα chain.

Transplantation between genetically disparate individuals leads to immunologic reactivity directed against the graft. The consequence of allorecognition is the initiation of an adaptive immune response (the alloresponse) with recruitment of allospecific T cells. There are 3 pathways of allorecognition (Fig 3a). By the direct pathway, recipient T cells recognize intact HLA and peptide complexes expressed by the donor's antigen- presenting cells (APCs). By the indirect pathway, recipient T cells recognize peptides derived from donor HLA proteins presented by the recipient's own APCs. By the semi-direct pathway, intact donor HLA is transferred to recipient APCs and expressed intact on the surface of recipient APCs and presented to recipient T cells.[10,11] The α and the β chain of HLA-DQ may rearrange.[12] To explain the strong reactivity of antibodies against beads coated with donor-type HLA-DQα chain combined with patient HLA-DQβ chain, we speculate that donor HLA molecules are transferred to the patient's APCs through direct cell-cell contact or via exsomes. In the patient's APCs, HLA-DQ may then be rearranged and expressed as a combination of recipient-type DQβ chain and donor-type DQα chain on the surface of APCs, where it is ultimately presented to alloreactive T cells. We propose the term "semi-semi-direct" to describe this tentative pathway of allorecognition (Fig 3b).

We cannot exclude the possibility that the recipient's pregnancy is a confounder. The HLA types of the expected child and the father are unknown. Nevertheless, we have recently found similar cases of HLA-DQα immunizations that remain to be analyzed at the structural level.

In summary, allo-DQα in combination with auto-DQβ may induce clinically significant HLA antibodies in kidney transplant recipients. HLA-DQα immunization is of clinical importance and may necessitate an extension of the match requirements for highly immunized patients with high-resolution DQA1/DQB1 typing of potential transplant donors.

ACKNOWLEDGMENTS

We thank the staff at the Tissue Typing Laboratory for their technical assistance.

REFERENCES

1. Duquesnoy RJ. Clinical usefulness of HLAMatchmaker in HLA epitope matching for organ transplantation. *Curr Opin Immunol*. 2008;20:594.

2. Tambur AR, Leventhal JR, Friedewald JJ, et al. The complexity of human leukocyte antigen (HLA)-DQ antibodies and its effect on virtual crossmatching. *Transplantation*. 2010;90:1117.

3. Barabanova Y, Ramon DS, Tambur AR: Antibodies against HLA-DQ α-chain and their role in organ transplantation. *Human Immunol*. 2009;70:410.

4. HLAMatchmaker: Available at: http://www.HLAMatchmaker.net. Accessed February 15, 2012.

5. Duquesnoy RJ, Askar M. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. V. Eplet matching for HLA-DR, HLA-DQ, and HLA-DP. *Human Immunol*. 2007;68:12.

6. Duquesnoy RJ, Marrari M. HLAMatchmaker-based definition of structural human leukocyte antigen epitopes detected by alloantibodies. *Curr Opin Organ Transplant*. 2009;14:403.

7. Duquesnoy RJ. Antibody-reactive epitope determination with HLAMatchmaker and its clinical applications. *Tissue Antigens*. 2011;77:525.

8. HistoCheck: Available at: http://www.mh-hannover.de/institute/transfusion/histocheck/. Accessed.

9. Elsner H-A, DeLuca D, Strub J, et al. HistoCheck: rating of HLA class I and II mismatches by an internet-based software tool. *Bone Marrow Transplant*. 2003;33:165.

10. Safinia N, Afzali B, Atalar K, et al. T-cell alloimmunity and chronic allograft dysfunction. *Kidney Int*. 2010;78:S2.

11. Afzali B, Lombardi G, Lechler RI. Pathways of major histocompatibility complex allorecognition. *Curr Opin Organ Transplant*. 2008;13:438.

12. Tollefsen S, Hotta K, Chen X, et al. Structural and functional studies of trans-encoded HLA-DQ2.3 (DQA1*03:01/DQB1*02:01) protein molecule. *J Biol Chem*. 2012;287:13611.