Human Immunology 70 (2009) 645–650

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Emerging role of donor-specific anti–human leukocyte antigen antibody determination for clinical management after solid organ transplantation

Adriana Zeevi [a,b,d,*], John G. Lunz III [a,d], Ron Shapiro [b,d], Parmjeet Randhawa [a,d], George Mazariegos [b,c,e], Steven Webber [b,c,e], Alin Girnita [a,d,*]

[a] Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA
[b] Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA
[c] Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA
[d] University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA
[e] Children's Hospital of Pittsburgh of University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

ARTICLE INFO

Article history:
Received 26 February 2009
Accepted 9 June 2009
Available online 12 June 2009

Keywords:
HLA antibody
Antibody-mediated rejection
C4d
Donor-specific antibody
Solid organ transplantation

ABSTRACT

Preformed and *de novo* donor-specific HLA antibodies (DSA) have been associated with allograft dysfunction and failure. The application of solid-phase methods have increased the sensitivity and specificity of antibody detection; however the clinical significance of these DSA is under evaluation. In the present study, we summarize six cases (four renal transplant recipients, one multivisceral recipient, and one heart-and-lung transplant recipient) to illustrate the role of the histocompatibility laboratory in providing the most comprehensive workup to assess the risk of graft dysfunction associated with antibody-mediated rejection (AMR). These cases illustrate the potential risk assessment for AMR in various situations: (1) in patients exhibiting low levels of DSA pretransplantation; (2) protocol immunosuppression minimization during stepwise weaning; and (3) desensitization protocols. Furthermore, increased sensitivity of DSA determination is indicated for the interpretation of focal C4d and its clinical significance. The clinical relevance of monitoring for circulating DSA with solid-phase single-antigen assays is also discussed. These cases exemplify the rationale for all patients to be monitored for DSA post-transplantation, with the frequency adjusted based on the individual risk for AMR.

1. Introduction

The deleterious effects of anti–human leukocyte antigen (HLA) antibodies in solid organ transplantation have been reviewed extensively [1–4], with the consensus among clinicians that preformed donor-specific HLA antibodies (DSA) are associated with graft dysfunction and failure [5,6]. Furthermore, post-transplantation anti-HLA antibodies are also associated with allograft dysfunction [7,8]. As the methodologies of alloantibody detection have evolved from mainly cell-based to multiparameter solid-phase assays, including single-antigen or allele-specific determinations, the sensitivity and specificity of antibody determination have increased [9–11]. Furthermore, the use of HLAMatchmaker has enhanced our ability to identify specific epitopes shared by the immunizing donor [12–14]. However, the clinical relevance of DSA is still continuously being evaluated [15]. Depending on multiple factors including the patient's disease, history of sensitization, living or deceased donor, type of organ, center-specific protocol, and immunosuppressive strategy, the presence of DSA before transplantation may be a contraindication to transplantation [16,17]. Therefore, it is important to re-evaluate the role of the histocompatibility laboratory in providing the most comprehensive workup before and after transplantation to assist the clinician's decision-making process in managing transplant recipients. In the present study, we illustrate several cases to show how laboratory information is applied to assess the risk of graft dysfunction associated with antibody-mediated rejection (AMR) in various solid organ transplant recipients.

2. Subjects and methods

Donors and recipients were typed by rSSO and/or single-strand polymorphism (SSP) (Invitrogen, Carlsbad, CA) with serologic confirmation. Anti–HLA-antibody panel-reactive antibody (PRA) and titers were performed by commercially available enzyme-linked immunoabsorbent assay (ELISA) kits (LAT; OneLambda, Canoga Park, CA) [18]. Antibody specificity was assigned by Luminex single-antigen bead analysis (LabScreen, OneLambda) [19]. Cytotoxic crossmatch (CXM) was performed using donor T and B cells by anti–human globulin(AHG)–enhanced or extended-incubation/modified Amos technique. We used both the serum with the highest PRA within 1 year before transplantation ("peak" serum) and the current serum. Immunohistochemical staining for vascular C4d

* Corresponding authors.
E-mail address: zeevia@upmc.edu (A. Zeevi; cases 3, 4, and 5).
E-mail address: girnitaal@upmc.edu (A. Girnita, cases 1, 2, and 6).

0198-8859/09/$32.00 - see front matter © 2009 Published by Elsevier Inc. on behalf of American Society for Histocompatibility and Immunogenetics.
doi:10.1016/j.humimm.2009.06.009

deposition in kidney, heart, and lung allografts was performed as described previously [20,21].

3. Results

3.1. Patterns of anti–class II HLA antibodies associated with rejection: Anti–HLA-DP antibodies

HLA-specific antibodies, including anti-class II HLA-DR and -DQ, have been associated with worse outcomes in renal transplantation [7,8]. However, the impact of anti-HLA-DP antibodies has only recently emerged [22–25].

3.1.1. Case 1

We present an index case of a 29-year-old patient who received a nonrelated, living renal allograft with zero HLA-DR and zero HLA-DQB/DQA mismatches. The recipient HLA was A2,24; B7,51; Cw7,14; DR4,11; DQ7 (DQB1*0301DQA1*0501), DQ8; DR52,53, whereas the donor HLA was A2;B44,51;Cw5,14;DR11;DQ7; (DQB1*0301DQA1*0501);DR52. Nine months after transplantation we detected *de novo* anti–class II HLA antibodies (class II PRA, 45% by ELISA). The graft failed 2 years post-transplantation and the patient became an active candidate for retransplantation. Pathologic examination showed acute cellular rejection with grade v3 intimal arteritis (Banff type III) superimposed on severe chronic allograft nephropathy. C4d immunohistochemistry was not performed. Subsequently, CXM with five zero-antigen mismatched HLA-DR and HLA-DQ donors were B-cell strongly positive and AHG T-cell negative. Anti-HLA antibody specificity analysis demonstrated only an anti-HLA DP antibody pattern (DP1, 3, 5, 9, 10, 11, 13, 14, 17, and 19, which share the common 84DEAV epitope) without other class I or II specificities [26]. The beads representing recipient HLA (HLA-DP2 and HLA-DP4) provided negative reactions.

In this case, anti-HLA DP antibodies were associated with renal allograft loss and with decreased probability of retransplantation with a random donor even in the absence of other anti–class I and/or anti–HLA-DR, –HLA-DQB, or –HLA-DQA antibodies.

3.2. Patterns of anti–class II HLA antibodies associated with rejection: Anti–HLA DQA1 antibodies

Although anti–class II HLA antibodies have been extensively reported as risk factors for allograft outcomes [1,7,27], the role of the polymorphic HLA-DQA chain in humoral allosensitization is less well known [28,29].

3.2.1. Case 2

This is the case of 48-year-old patient who received a living-related kidney transplant. Screening for HLA antibody results before transplantation by ELISA and Luminex were negative. The T- and B-cell CXM were also negative. The recipient typing was HLA-A1,11; B7,8; Cw7; DR12,17; DQB1*0201,*0301; DR52, whereas the donor HLA was A1,–;B8,57; Cw6,7; DR15,17; DQ2,6; DR51,52.

Eight months post-transplantation, we detected *de novo* anti-class I and II HLA antibodies by ELISA and Luminex (PRA, 77%). The donor specificities were directed toward HLA-B57, -DR15, -DR51, and -DQ6. After another month, the class II antibody titer rose from 1:2 to 1:512, and the ELISA class II PRA was in the 80–100% range. Allograft needle biopsy showed mild acute cellular rejection, with intimal arteritis, Banff type IIA; however C4d immunohistochemistry was not performed. Seventeen months after transplantation, the renal allograft failed and was removed. The specificity analysis revealed antibodies toward HLA-DQ2, which were shared by the recipient and donor. The issue of self-reactivity toward HLA-DQ2 was solved by analysis of the HLA-DQA pattern (Table 1). The beads exhibiting the recipient's HLA-DQA were negative (the recipient was typed as HLA-DQA1*0501, *0505), whereas the reactions toward donor HLA-DQA were positive.

Table 1
Pattern of polymorphic amino-acid residues on HLA-DQA molecules that provided positive or negative reactivity by Luminex (case 2)

Reaction	Allele specificity	Polymorphic epitopes[a]	
Positive	DQA1*0102 (donor HLA)	107T	161D
Positive	DQA1*0301	107T	161D
Positive	DQA1*0201	107T	161D
Positive	DQA1*0301	107T	161D
Positive	DQA1*0201	107T	161D
Positive	DQA1*0601	107T	161D
Positive	DQA1*0303	107T	161D
Negative	DQA1*0501 (recipient HLA)	107I	161E
Negative	DQA1*0505 (recipient HLA)	107I	161E

[a]Polymorphic epitopes defined by HLAMatchmaker [12–14].

Because the immunizing haplotype was HLA-DR15, DR51, DQA1*0102, DQB1*0602, we sought to identify the mismatched DQA epitopes shared by the immunizing donor and the positive beads [26]. As Table 1 shows, all positive beads shared the same amino acid residues as the immunizing donor HLA-DQA1*0102. Furthermore the self HLA-DQA and the negative beads contained different polymorphic residues. The combination of high-definition assays for both antibody screening and molecular four-digit HLA typing enabled the determination of clinically relevant anti-DQ alpha antibodies. The clinical impact of anti–HLA-DQA antibodies cannot be documented in this case, because of the presence of multiple donor-specific antibodies. Nevertheless, the HLA-DQ alpha pattern was used in this case to exclude apparent self-reactivity toward HLA-DQB1*0201.

3.3. Correlation between focal C4d and DSA in renal transplant recipients

Renal allograft recipients have benefited from close post-transplantation monitoring with clinical indicators, such as serum creatinine, to detect graft dysfunction. However, there are myriad underlying causes of dysfunction that can elevate creatinine; often multiple tests need to be performed to accurately diagnose the problem, and this frequently includes a biopsy. Immunohistochemical staining for C4d was recommended in a 2007 update to the Banff classification for renal biopsies to assess the risk for humoral rejection [30]. The clinical significance of diffuse peritubular capillary (PTC) C4d staining in renal biopsies is well established [20]; however the significance of focal PTC C4d staining is not as clear. We therefore also began to determine the significance of focal PTC C4d staining in relation to the presence of DSA [31].

We observed that patients with focal PTC C4d biopsy staining may exhibit ELISA screens of less than 10%, and further antibody testing is not typically pursued, leaving the clinical significance of focal PTC C4d staining in question. We therefore examined 11 such kidney allograft recipients with focal PTC C4d staining and a concomitant ELISA screen of less than 10% to determine whether more sensitive screening by Luminex could detect DSA. Luminex screens detected in three of 11 patients (27%) either HLA class I or II antibodies (three patients). Specificity testing for HLA antibody by Luminex high definition revealed DSA in all three patients with positive HLA Luminex screens. These three patients had previously circulating DSA determined by ELISA, whereas the eight patients with negative Luminex screen results did not. Below we describe a case of a recipient with a history of post-transplantation DSA with a negative ELISA screen result at the time of biopsy with focal PTC C4d.

3.3.1. Case 3

A 53-year-old recipient HLA: A3,68; B13,60; Cw3,6; DR4,7; DQ2,3 DR53,– received a living donor kidney allograft from donor HLA: A24,32; B44,61; Cw1, –; DR1,103; DQ1,7. The recipient had