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Transfer of donor anti-HLA antibody expression to multiple transplant recipients- a potential variant of the Passenger Lymphocyte Syndrome?

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ABBREVIATIONS:

AMR	Antibody-mediated rejection
CMV	Cytomegalovirus
CT	Computed Tomography
DSA	Donor specific antibody
EBV	Epstein Barr virus
HLA	Human leukocyte antigen
MFI	Mean fluorescence intensity
PLS	Passenger Lymphocyte Syndrome
TMAT	Transplantation-mediated allo-immune thrombocytopenia

ABSTRACT

Antibody mediated rejection (AMR), whereby transplant recipient B cells and/or plasma cells produce allo-reactive anti-human leukocyte antigen (HLA) antibodies, negatively influences transplant outcomes and is a major contributor to graft loss. An early humoral immune response is suggested by the production of anti-HLA donor-specific antibodies (DSA) that can be measured using solid phase assays. We report the early post-transplant co-existence of a shared anti-HLA antibody profile in five solid organ transplant recipients who received organs from the same donor. Retrospective analysis of the donor's serum confirmed the presence of the same anti-HLA profile, suggesting the transfer of donor-derived anti-HLA antibodies, or the cells that produce them, to multiple solid organ transplant recipients. The time frame and extent of transfer suggest a novel variant of the Passenger Lymphocyte Syndrome. These findings have important implications for the consideration of all post-transplant antibody measurements, particularly the interpretation of non-DSAs in the sera of transplant recipients.

INTRODUCTION

The immune response to the allograft is the major barrier to enduring survival following transplantation. Alloreactivity is largely an adaptive immune response, targeted against major transplanted organ antigens in the form of highly polymorphic HLA molecules. The humoral immune response involves the production of *de novo* anti-HLA antibodies that target and damage epithelial and/or endothelial cells through complement activation, natural killer cell recruitment and direct cytotoxicity. Solid phase assays, such as the Luminex platform (One Lambda Inc, Canoga Park, CA, USA), allow a quantitative and qualitative assessment of the humoral antibody response (1).

However, the production early post-transplant of *de novo* DSA does not always equate to graft dysfunction or mandate intensification of immunosuppression (1). Additionally, anti-HLA antibodies that are not specific to the HLA expressed by the donated allograft can also be detected early post-transplant and are assumed to reflect a non-specific non-alloreactive response by the recipient's immune system to transplantation (2, 3). We describe a case series of 5 solid organ transplant recipients who received organs from the same donor, and early post-transplant displayed donor-derived third party anti-HLA antibodies representing the transfer of donor antibodies, or cells from the donor that produce antibodies, along with the allograft.

Case Series

The organ donor was a 37 year old blood group B female, who had a witnessed collapse whilst exercising. There was no relevant past medical history and she was on no medications. A computed tomography (CT) brain scan subsequently demonstrated a left internal carotid artery dissection and infarction of the left hemisphere. Following a further clinical deterioration brain death was confirmed and organ donation proceeded. HLA donor typing was obtained by real-time PCR (Linkseq HLA-ABCDRDQDP rapid typing kit, One Lambda Inc) and is shown in Table 1. Molecular typing was subsequently confirmed by Luminex oligonucleotides (SSO, One Lambda Inc) as also shown in Table 1. Organ procurement resulted in five solid organ transplants; kidney, kidney and pancreas, lung, liver and heart.

The index case, the recipient of the donated lungs, was a 57 year old male with end-stage alpha-1-anti-trypsin deficiency and bronchiectasis. Past medical history also included ankylosing spondylitis and an IgA monoclonal gammopathy of unknown significance. Prospective T and B cell crossmatches were negative and anti-HLA antibodies (mixed bead and assay, One Lambda Inc) were not present pre-transplant. HLA mismatches with the donor are shown in Table 1. Related to a previous pleurodesis, the case was performed on cardiopulmonary bypass and was complicated by massive blood transfusion requirements. Protocol immunosuppression included tacrolimus (target level 9-11 ng/ml), azathioprine (1 mg/kg) and prednisolone (weaning from 1mg/kg to 0.3mg/kg). Surveillance

bronchoscopies with transbronchial biopsies at 2 and 6 weeks post-transplant showed no evidence of acute cellular rejection; however the initial biopsy showed faint capillary C4d staining raising the possibility of antibody-mediated rejection. Spirometry revealed a mild restrictive pattern. Chest radiography showed pleural thickening but clear lung fields.

Reflecting the C4d staining, Luminex screening for anti-HLA antibodies was performed 2 months post-transplant and showed considerable antibody production against a large number of HLA alleles, most of which were not donor-specific (Table 2). The extent of the antibodies and the persistent mild lung allograft dysfunction prompted a review of the results of other transplant recipients from the same donor (Table 2). The donor's anti-HLA antibody profile accounts for virtually all the class I antibodies from all recipients. Strikingly, the 4 highest mean fluorescent intensity (MFI) anti-HLA antibodies present in the donor were observed in all 5 recipients. The exceptions are the lung recipient's true DSA to the donor's A*24 and the absence of the B64 antibody which is likely bound to the recipient's B*64 antigen. The donor had no significant anti-HLA class II profile, while the lung recipient had DSA to DQ7, DQ5, DR4, DR53, DQA1*03, DPA1, DR1 and the kidney-pancreas recipient DSA to DQ7, DP5, DP1, DR53 and DP20. Assessment of eplet mismatching (HLAMatchmaker, version 2.0, www.epitopes.net) did not explain the extent or similarities in the HLA class I antibody profile.

Patient consent and Alfred Hospital Ethics Committee approval were obtained.

We have further encountered 2 additional cases whereby HLA antibody specificities in the non-sensitized lung recipients which could not be explained by the donors' HLA typing.

Discussion

This clinical series clearly shows that the anti-HLA antibody profile demonstrable from the peripheral blood of an organ donor can be reproduced subsequently in the blood of the various solid organ recipients. This observation has the potential to explain the previously poorly understood issue of anti-HLA non-donor specific antibody (non-DSA) in the sera of transplant recipients (4).

The donor's medical history was unremarkable, but past allo-sensitizing events (eg pregnancy or blood transfusion) are not necessarily known by the next-of-kin or routinely sought, and are theoretical but plausible explanations of the donor's profile. Samples taken from the lung recipient between 2 and 4 months after transplantation are unlikely to represent the passive transfer of pre-formed pre-donation graft antibodies, noting the half-life of immunoglobulins is less than 1 month (5). With significant MFIs and consistent antibodies in the sera of all of the organ recipients, it is likely

donor-derived B or plasma cells within the transplanted allografts were continuing to produce anti-HLA antibodies that were not directed against the allograft.

Several case reports describe a similar situation and timeline, and combined together offer a potential insight regarding the immune cell type responsible (6, 7. 9). Maxfield et al described 2 non-sensitized renal transplant recipients who developed third party anti-HLA antibodies at 7 days that matched the sensitized donor profile and lasted beyond 3 months (6). B cells or plasma cells were considered the likely culprits. Arnold et al (7) described the non-sensitized recipient of a maternal allogeneic hematopoietic (naïve T cell depleted) cell transplant with third party anti-HLA antibodies evident at day 7, matching the mothers anti-HLA antibody profile. Antibody levels increased over subsequent weeks – despite the protocolized use of rituximab (anti-CD20) and an absence of measureable plasma B cells. Again it was concluded that plasma cells or a subset of memory B cells (8) without surface - CD20 were producing the antibodies. Recently Koshizuka et al (9) detected donor derived anti-cytomegalovirus (CMV) antibody beyond 6 months post-lung transplantation in 2 recipients and subsequently characterized the presence of donor plasma cells (via their flow cytometry $CD19^-$ /$CD138^+$ profile) in the recipients serum.

We have additionally recently described a series of 5 hepatitis C naive lung transplant recipients who received antibody positive (but PCR negative) lungs with subsequent evidence of hepatitis C antibody positivity beyond one year post-transplant (without PCR positivity). As in our current case study, other solid organs transplanted from the same donor (kidneys and a heart) showed the same antibody positive/PCR negative pattern (10). Refractory transplantation-mediated allo-immune thrombocytopenia (TMAT) due to intra-graft production of donor antibody against recipient platelets, has also been described (11).

All these cases are likely variations of the well-recognized donor Passenger Lymphocyte Syndrome (PLS) where allografts act as reservoirs of donor B cells that manufacture red blood cell alloantibodies and cause hemolytic anemia. This syndrome has also been described across multiple organ recipients from one sensitized donor (12, 13). Lymphoid micro-chimerism was notable in one early PLS series, but the origin of these cells is not clear (12, 13).

Potentially the extent of both TMAT and PLS can be linked to the actual mass of co-transplanted lymphoid tissue (11, 12). Supporting this theory, in the current case series the lung recipient had the highest number of antibodies and the highest MFI of all the recipients. The variability in number and type of HLA antibodies evident in different recipients may represent a degree of randomness with regard to the transfer process of individual B cell or plasma cell clones. Additionally, there are differences in the timing of testing post-transplant, Luminex batching and technical considerations, and even recipient immunological responses.

Combined with our own report, these various case series (6, 7, 9) suggest that donor lymphoid tissue originating memory cells [likely plasma cells (9)] enter and remain in the recipient circulation long term. The case report utilizing rituximab, with no circulating B cells (7) and the 2 case reports observing antibodies within a short 7 days from transplant (6, 7), argue against the theory that B cells are the bridging cell that subsequently differentiates into plasma cells (8, 14). Additionally, tissue-resident B cells are associated with local antigens (14), so should not be outside the allograft in significant numbers. Conversely, while plasma cells typically live in the bone marrow, they are able to persist in secondary lymphoid tissue and have been shown to survive and secrete antibodies for a year in the circulation (15).

The current case series results are not explained by the existing theories regarding polyreactive antibodies. Polyreactive antibodies are present from birth and produced without evidence of immunization (2, 16). They function in tissue injury repair and host-defence and can recognize self-antigens as part of these processes. They have been implicated in contributing to primary graft dysfunction (2, 16) and non-DSA related AMR (17). However, noting the current study results, the novel concept of donor B or plasma cells contributing a component of polyreactive antibodies via an allograft, may warrant consideration.

The clinical relevance of demonstrating the donor's specific profile of anti-HLA antibodies in recipient sera include:

1) It confuses and drowns out the actual recipient DSA result, potentially convincing the clinician not to act, 'as it's all non-specific'. There may be less confusion if donors with sensitizing events are screened for anti-HLA antibodies up front;

2) They are potentially a source of allo-reactive antibodies, as there are studies showing poorer outcomes compared to the presence of no anti-HLA antibodies at all (2, 16-19). This may be particularly relevant to pediatric recipients were the aim is to achieve a very long lasting allograft, and re-transplantation may be necessary at some point.

3) Recognizing that donor B cells can be present in the recipients making a range of antibodies [against HLA, cytomegalovirus, hepatitis C, platelets etc (8,9)], could this extend to the interpretation of other serological tests- including those of CMV, Epstein Barr Virus (EBV) or other antigens?

In conclusion, this case series strongly suggests that an organ donors' anti-HLA antibody profile can be seen in the sera of multiple transplant organ recipients, months after transplant. It is likely a variant of the PLS with plasma cells as the culprit. These findings have important implications for the consideration of all post-transplant antibody measurements, particularly the interpretation of non-DSAs in the sera of the transplant recipients.

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation.*

DATA AVAILABILITY STATEMENT

The data are not publicly available due to patient privacy concerns.

REFERENCES

1. Roux A, Levine DJ, Zeevi A, Hachem R, Halloran K, Halloran PF, et al. Banff Lung Report: Current knowledge and future research perspectives for diagnosis and treatment of pulmonary antibody-mediated rejection (AMR). Am J Transplant. 2018.

2. Zorn E, See SB. Polyreactive natural antibodies in transplantation. Curr Opin Organ Transplant. 2017;22(1):8-13.

3. Lee PC, Chen YL, Wang WM, Tu WC, Chen HY. Clinical relevance of pre- and post-transplant HLA antibodies, donor-specific, and nondonor-specific HLA antibodies detected by ELISA in renal transplantation. Clin Transpl. 2013:385-91.

4. Malheiro J, Tafulo S, Dias L, Martins S, Fonseca I, Beirao I, et al. Impact on mid-term kidney graft outcomes of pretransplant anti-HLA antibodies detected by solid-phase assays: Do donor-specific antibodies tell the whole story? Hum Immunol. 2017;78(9):526-33.

5. Mankarious S, Lee M, Fischer S, Pyun KH, Ochs HD, Oxelius VA, et al. The half-lives of IgG subclasses and specific antibodies in patients with primary immunodeficiency who are receiving intravenously administered immunoglobulin. J Lab Clin Med. 1988;112(5):634-40.

6. Maxfield SJ, Taylor CJ, Kosmoliaptsis V, Broecker V, Watson CJ, Bradley JA, et al. Transfer of HLA-Specific Allosensitization From a Highly Sensitized Deceased Organ Donor to the Recipients of Each Kidney. Am J Transplant. 2015;15(9):2501-6.

7. Arnold PC, S; Triplett; B. Donor-derived anti-HLA antibodies in haploidentical hematopoietic cell transplant recipient shortly after transplant. Hum Immunol. 2018;79:S51.

8. Luque S, Lucia M, Bestard O. Refinement of humoral immune monitoring in kidney transplantation: the role of "hidden" alloreactive memory B cells. Transpl Int. 2017;30(10):955-68.

9. Koshizuka T, Matsuda Y, Suzuki H, Kanno R, Ikuta K, Kobayashi T, et al. Detection of engraftment of donor-derived antibody producing cells in a lung transplant recipient by anti-cytomegalovirus IgG avidity test. Transpl Immunol. 2018.

10. Smibert OC, Doyle JS, Pilcher D, Paraskeva MA, Snell GI. Unexplained PersistentHepatitis C Seropositivity (Hepc Ab+Ve) in the Absence of Viremia in LungTransplant (Ltx) Recipients from Hepc Ab+Ve/Nucleic Acid Test Negative (NAT-Ve)Donors Associated with Excellent Clinical Outcomes and Implications for FuturePractice. The Journal of Heart and Lung Transplantation. 2018;37(4):S77.

11. Trotter PB, Robb M, Summers D, Watson CJ, Clatworthy M, Bradley JA, et al. Donors With Immune Thrombocytopenia: Do They Pose a Risk to Transplant Recipients? Am J Transplant. 2017;17(3):796-802.

12. Seltsam A, Hell A, Heymann G, Salama A. Donor-derived alloantibodies and passenger lymphocyte syndrome in two of four patients who received different organs from the same donor. Transfusion. 2001;41(3):365-70.

13. Shortt J, Westall GP, Roxby D, Chen JW, Snell GI, Polizzotto MN, et al. A 'dangerous' group O donor: severe hemolysis in all recipients of organs from a donor with multiple red cell alloantibodies. Am J Transplant. 2008;8(3):711-4.

14. Allie SR, Bradley JE, Mudunuru U, Schultz MD, Graf BA, Lund FE, et al. The establishment of resident memory B cells in the lung requires local antigen encounter. Nat Immunol. 2019;20(1):97-108.

15. Radbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dorner T, et al. Competence and competition: the challenge of becoming a long-lived plasma cell. Nat Rev Immunol. 2006;6(10):741-50.

16. Dieude M, West LJ, Muruve DA, Gunaratman L, Mohanakumar T, Zorn E, et al. New Answers to Old Conundrums: What Antibodies, Exosomes and Inflammasomes Bring to the Conversation. Canadian National Transplant Research Program International Summit Report. Transplantation. 2018;102(2):209-14.

17. Gao B, Rong C, Porcheray F, Moore C, Girouard TC, Saidman SL, et al. Evidence to Support a Contribution of Polyreactive Antibodies to HLA Serum Reactivity. Transplantation. 2016;100(1):217-26.

18. Gao B, Moore C, Porcheray F, Rong C, Abidoglu C, DeVito J, et al. Pretransplant IgG reactivity to apoptotic cells correlates with late kidney allograft loss. Am J Transplant. 2014;14(7):1581-91.

19. See SB, Aubert O, Loupy A, Veras Y, Lebreton X, Gao B, et al. Post-Transplant Natural Antibodies Associate with Kidney Allograft Injury and Reduced Long-Term Survival. J Am Soc Nephrol. 2018;29(6):1761-70.

Table 1. Molecular HLA typing

Organ Donor	HLA A*03:88,*24:02; B*07:02,*-; C*07,*-; DRB1*01:01,*04:01; DRB4*01:01/03; DQA1*01:01/04/05,*03:01/02/03; DQB1*03:01,*05:01; DPA1*01,*02; DPB1*04:01,*09:01
Transplant Recipients	
Lung	HLA A*02:01/189,*-; B*14:01,*27:05; C*01:02,*08:02; DRB1*07:01,*08:02; DQA1*02:01,*04:01; DQB1*03:03,*04:02; DPA1*01:03,*-; DPB1*04:01,*-
Liver	HLA A*02:01,*11:01; B*27:05,*40:01; C*01:02, *03:04; DRB1*01:01,*07:01
Kidney #1	HLA-A*03:01,*24:02; B*07:02,*56:01; C*01:02,*07:02; DRB1*01:01,*04:01; DRB4*01:03; DQA1*01:01,*03:03; DQB1*03:01,*05:01; DPA1*01:03,*02:01; DPB1*04:01,*17:01
Kidney #2 (+Pancreas)	HLA-A*01,*11; B*48,*55; C*03:03,*08:22; DRB1*03,*16; DQA1*01:02,*05:01; DQB1*02:01,*05:02; DPB1*04:01,*-
Heart	HLA-A*02,*11; B*51,*52; C*07,*14; DRB1*08,*11; DQA1*05:05/09,*06:01; DQB1*03:01,*-; DPA1*01:03,*01:04; DPB1*02:01,*15:01

Notes:

-All molecular HLA typing was performed for donor and recipients using Labtype SSO (One Lambda Inc).

-Recipient serum samples were screened pre-transplant, and post-transplant at various time points for anti-HLA antibodies by either mixed bead or single antigen beads (One Lambda Inc.) as per standard protocol and assessed for the presence of de-novo DSA with a mean fluorescence intensity (MFI) cut off >1000. According to local laboratory practices, serum samples were pre-treated using heat inactivation, hypotonic dialysis and/or Absorb Out (One Lambda Inc) where required, to remove non-specific binding and/or inhibitory factors.

Table 2. Anti-HLA Antibody Profile

Case	Days post-Tx	HLA Class I HLA antibodies (MFI)	HLA Class II antibodies (MFI)
Donor	-	***B45 (18152), B44 (17677), B76 (16532), B82 (15003),*** *B50 (12646),*	DQ2 (2078)

		B49 (11738), B41 (10428), B60 (9553), B62 (9144), B61 (8764), B72 (8259), B75 (8142), B13 (7848), B71 (7803), B47 (6300), B35 (6252), B78 (6018), *B52 (5479), B48 (5186), B53 (5032), B51 (4932),* B18 (4786), *B56 (4392), B77 (4331),* B38 (4233), Cw15 (4211), B39 (4205), Cw6 (4192), *B46 (3910),* A29 (3850), Cw18 (3689), B64 (3310), *B63 (3277),* Cw5 (3112), *A1 (3048),* Cw2 (2744), B37 (2703), B59 (2007), B65 (1904), B57 (1826), *B54 (1805), Cw17 (1659), B58 (1513),* B55 (1459), Cw4 (1404), A34 (1366), *B8 (1233),* A33 (1183), A68 (1128), B67 (1092), A30 (1031)	
Lung	65	***B44 (20017), B45 (19698), B76 (18107), B82 (17485),*** *B49 (16060), B50 (15832), B72 (12641), B62 (12453), B71 (11672), B41 (10325), B52 (9586), B75 (9053), B51 (8299), B56 (7852), B46 (7489),* B60 (7247), *B35 (6799), B53 (6392), B13 (6101), B61 (5268), B47 (4898), B63 (4752), B77 (4618), B78 (4176), B54 (2113),* A24 (1958), B55 (1915), *A1 (1912), B58 (1555),* A23 (1517), *B48 (1113), B8 (1048),* B59 (1030)	DQ7 (8341), DQA1*06:01 (8341), DQ5 (7295), DR4 (6792), DQ6 (6191), DR53 (5862), DQA1*05:05 (4492), DQA1*03:01 (4420), DP5 (3534), DPA1*02:02 (3534), DR1 (3335), DP3 (2699), DP17 (2448), DR103 (2299), DP9 (2293), DP14 (2123), DP10 (1929), DR51 (1675), DP20 (1479), DR10 (1043)
Liver	91	***B45 (13913), B44 (13045), B76 (11447), B82 (6287),*** *B50 (2444), B49 (2419)*	Non-specific binding
Kidney #1	92	***B45 (12605), B44 (10022), B76 (4240), B82 (3467)***	-
Kidney #2 (+Pancreas)	120	*B13 (2213), Cw17 (1913),* ***B44 (1879), B45 (1731)****, B52 (1520),* Cw1 (1313), ***B76 (1307), B82 (1192),*** *Cw15 (1146), B75 (1099), B46 (1081),* Cw14 (1069)	DQ7 (2231), DQ9 (2108), DP5 (1796), DP1 (1598), DQ8 (1525), DR53 (1302), DP20 (1270), DP23 (1084)
Heart	85	***B45 (5226), B44 (3792), B76 (2587),*** B80 (1984), ***B82 (1886),*** Cw1 (1452)	DQB1*06 (3372)

Tx: Transplant

Notes:

- ***Bold + italic*** = shared across donor and all 5 recipients
- *Italic* = shared between donor and at least 1 recipient
- Underlined = True Donor Specific Antibodies

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