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Brief communication

Rapid and strong *de novo* donor-specific antibody development in a lung transplant recipient: Short communication/case report☆

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ABSTRACT

A 66-yo female patient (typed B*39:01, 44:02) underwent first left single lung transplant (typed **B*81:01, 15:17**) on 02/07/2016 with negative for DSA in current and historical samples. On 02/17/2016 strong *de novo* DSA (MFI = 15,200, C1q+) to B81 were detected. The recipient has two children typed **B*07:02**, 44:02 **B*27:03**, 39:01, and had received multiple vaccinations. Twinrix, Zostavax and MMR vaccines contain viruses grown on live human lung fibroblasts (MRC-5, typed **B*07:02**, 44:02, and WI-38, typed **B*08:01, 58:01**). Each dose of vaccine used for injection is known to contain protein components of fibroblasts including HLA. Most likely rapid *de novo* DSA development is due to booster effect produced by five exposures to mismatched B locus alleles which share the following epitopes: **70IAQ, 65QIA, 65QIA** + 76esn, **69aa** + 80n, and **163ew** + 73te. The later three consist of paired non-self and self eplets. Although likelihood of bystander effect produced by multiple vaccinations is low its impact cannot be ruled out.

1. Introduction

In most cases, the development of alloantibodies against human leukocyte antigens (HLAs) is related to immunization *via* blood and/or blood product transfusions, pregnancy, and transplants. The detection of anti-HLA Class I and Class II antibodies is an important component of the initial work-up of a potential transplant candidate. The importance of the post-transplant monitoring of DSAs in solid organ transplants has been widely described [1,2]. The *de novo* development of DSAs strictly depends on the antigenicity and immunogenicity of mismatched HLAs [3–5]. It is generally accepted that *de novo* developed DSAs represent a risk factor for graft failure, even at low concentrations. The substantial influences on antibody production include other factors such as the HLA Class II type of the responder, immunosuppressive medications, cytokine and chemokine genomic polymorphisms, and the hormonal background of the recipient [6–8]. The early detection of DSAs considerably reduces the incidence of antibody-mediated rejection and transplant failure [9].

Here, we describe the case of a lung-transplanted patient who rapidly developed (within 10 days) strong Class I DSAs after a single lung transplantation. We hypothesized that this event could have been associated with multiple exposures to mismatched HLA-B antigens *via* pregnancy and vaccinations.

2. Case description and methods

2.1. Patient, lung donor and sensitizing events characteristics

A 66-year old female patient with idiopathic pulmonary fibrosis underwent left single lung transplant on 02/07/2016 with negative DSAs and flow cytometry cross match (FC XM). No anti-HLA antibodies were detected in two historical (12/17/2015 and 01/20/2016) and pre-transplant (02/06/2016) serum samples. The FC XM results were negative when these samples were tested. The schedule of potential sensitizing events of the recipient is presented in Fig. 1. These include two pregnancies (children 33 and 35 years old) and multiple vaccinations. The type of the vaccines, their characteristics, and the time of injection are presented in Table 1. Twinrix is a bivalent vaccine containing

Abbreviations: AVE, antibody verified epitope; DSA, donor specific antibodies; FC XM, flow cytometry cross match; HLA, human leukocyte antigens; MCS, median channel shift; MFI, mean fluorescence intensity; MM, mismatch; MMR, mumps, measles, rubella; PC, plasma cells; POD, post-operative day; SPSA, solid phase single antigen.

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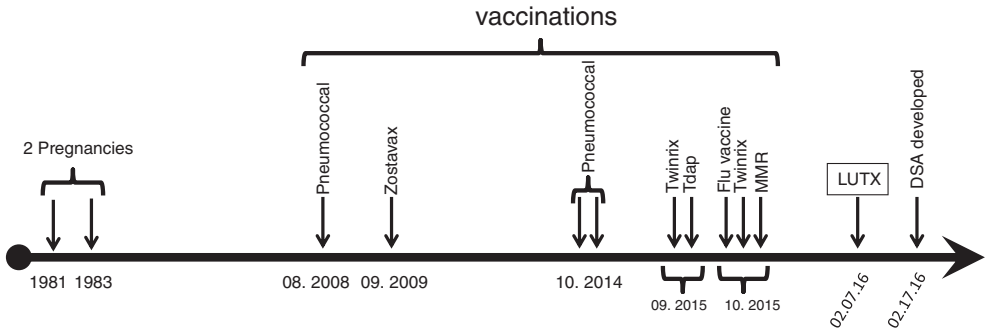

Fig. 1. Schedule of sensitizing events and vaccinations to which the lung transplant recipient was exposed. Arrows indicate the month and year of the event.

Hepatitis A inactivated and Hepatitis B recombinant vaccines. The Hepatitis A component consists of virus particles that are grown in human lung fibroblast (MRC-5) cell culture and then killed using heat or formaldehyde. Zostavax and MMR are live attenuated vaccines prepared by reducing the virulence of the viruses grown in MRC-5 and WI-38 human lung fibroblast. Each dose of vaccine used for injection is known to contain DNA and protein components of fibroblasts including HLA.

2.2. HLA typing

Typing of the HLA-A, -B, and -C loci of the donor, recipient, and human lung fibroblast cell lines was performed by DNA sequencing using a GenDx (Utrecht, Netherlands) reagent kit following the vendor's instructions. SBTengine software was used for the data analysis.

2.3. Immunosuppressive protocol

Immunosuppressive induction included methylprednisolone IV 500 mg × 1 prior to cross clamp removal followed by 4 doses of 125 mg IV Q12h of methylprednisolone and then prednisone 5 mg/day thereafter. Alemtuzumab (Campath®) 30 mg was given subcutaneously on post-operative day (POD) 0 within 12 h after cross clamp removal. Mycophenolate mofetil 500 mg every 12 h was started on POD 1 and adjusted post-transplant based on white blood cells counts. Tacrolimus was initiated at 48 h post-transplant and titrated to maintain levels in a goal range of 8–12 ng/mL. Pravastatin 20 mg daily was started during her hospitalization. At 3 months post-transplant, azithromycin 250 mg orally every Monday, Wednesday and Friday was started.

2.4. Anti-HLA antibody analysis

Anti-Class I IgG antibody analysis was performed using a solid phase single antigen (SPSA) assay (LAB-Screen® Single Antigen Class I, One Lambda Inc., Canoga Park, CA, USA). The tests were performed according to the vendor's instructions. Antibody specificity was analyzed manually using a baseline median fluorescence intensity (MFI) scale. Serum samples were treated with DDT to avoid prozone. The clinical significance (*i.e.*, antibody MFI values producing T cell positive FC CM) of the Class I DSAs was estimated based on an MFI cut-off value of 2600, as determined at our center. Anti-Class I antibody C1q test was performed according to the vendor's instructions (LAB-Screen® Single Antigen Class I, One Lambda Inc., Canoga Park, CA, USA). MFI value of 280–500 was used as a positive cut off.

2.4.1. Epitope analysis

Epitope analysis of mismatched and antibody-reactive antigens was conducted using the HLAMatchmaker computer algorithm [10,11].

2.5. FC XM assay

The details of the procedure for three-color FC XM have been described elsewhere [12]. The donor's T- and B-cells used for XM were treated with pronase [13]. The results of the assay were interpreted as median channel shift (MCS) to the right from the peak of normal human serum and donor cells, and T- and B-lymphocyte FC XM were accepted as positive at MCS values greater than 50 and 150, respectively.

3. Results

3.1. HLA typing

The recipient, donor, children and fibroblast cell lines Class I HLA typing results are presented in Table 2.

3.2. Antibody analysis in recipient serum samples

The first post-transplant serum sample was collected on 02/17/2016 (day +10), which tested strongly positive for *de novo* DSAs to B81 (MFI = 15.200). A C1q test was also positive (MFI = 4565), indicating that these antibodies bound complement. The remaining antibodies were against different HLA-B locus specificities. No anti-HLA-A, -C, or

Table 1
HLA Class I typing of the lung transplant recipient, deceased donor, two siblings, and lung fibroblasts cell lines.

Individual/cell line	HLA-A locus		HLA-B locus		HLA-C locus	
Recipient	02:01	32:01	39:01	44:02	05:01	02:02
Donor	02:01	**33:01**[a]	**81:01**	**15:17 (63)**[b]	**07:01**	**18:01**
Sib. 1 (33 yo)	**11:01**	32:01	**27:03**	39:01	**01:02**	02:02
Sib. 2 (35 yo)	02:01	02:01	**07:02**	44:02	05:01	**07:02**
MRC-5 (lung fibroblasts)	02:01	29:01	**07:02**	44:02	05:01	**07:02**
WI-38 (lung fibroblasts)	**02:05**	**68:01**	**08:01**	**58:01**	**07:01**	**07:01**

[a] – mismatched alleles are shown in bold font.
[b] – serological equivalent of B*15:17 allele.

Table 2
Vaccinations the recipient had received prior to lung transplantation.

Type of vaccine	Date	Description of vaccine	Human cell lines
Pneumococcal	08/29/2008	Polysaccharide	NA
Zostavax[a]	09/30/2009	Live (attenuated)	MRC-5, lung fibroblasts
Pneumococcal	10/14/2014	Polysaccharide	NA
Twinrix[b]	09/25/2015	Inactivated (killed)	MRC-5, lung fibroblasts
Twinrix	10/29/2015	Inactivated (killed)	MRC-5, lung fibroblasts
Tdap[c]	09/25/2015	Inactivated bacterial	NA
Influenza	10/29/2015	Chemical	NA
MMR[d]	10/29/2015	Live (attenuated)	WI-38, lung fibroblasts

[a] – Herpes Zoster Virus.
[b] – Hepatitis A and B viruses.
[c] – Diphtheria and tetanus toxoids and killed *Bordetella pertussis* bacteria.
[d] – MMR, Measles, Mumps, Rubella viruses.