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Development of antibodies to human leukocyte antigen precedes development of antibodies to major histocompatibility class I–related chain A and are significantly associated with development of chronic rejection after human lung transplantation

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ABSTRACT

The development of antibodies (Abs) to major histocompatibility (MHC) class I–related chain A (MICA) and human leukocyte antigen (HLA) and their role in the immunopathogenesis of chronic rejection (bronchiolitis obliterans syndrome [BOS]) after human lung transplantation (LTx) was analyzed. Sera from 80 LTx recipients were analyzed for anti-MICA and anti-HLA Abs using Luminex and flow PRA (panel reactive assay). Development of Abs either to MICA alone or MICA and HLA together significantly correlated ($p < 0.01$) with development of BOS. Kinetic analysis in the post-LTx period revealed that development of anti-HLA Abs (7.6 ± 4.7 months) preceded the development of anti-MICA Abs (10.0 ± 3.5 months). Abs to MICA alleles (*001 and *009) developed approximately 6 months after LTx and peak titers were present at the time of clinical diagnosis of BOS (16.3 ± 2.7 months). The development of Abs to both MICA and HLA was strongly associated with the development of BOS thereby suggesting a synergistic effect. Furthermore, immune response to mismatched HLA can lead to development of Abs to other MHC related antigens expressed on the airway epithelial cells. Cumulatively, these immune responses contribute to the pathogenesis of chronic rejection following human LTx.

1. Introduction

Long-term patient outcomes following lung transplantation (LTx) are limited by the development of chronic rejection (CR), clinically diagnosed as bronchiolitis obliterans syndrome (BOS). The incidence of BOS approaches 50% at 5 years and 90% after 9 years post-transplantation [1,2]. Obliterative bronchiolitis is the histologic correlate of BOS and is characterized by the cellular infiltration, fibrosis, collagen deposition and occlusion of small airways in the allograft. The diagnosis of BOS is clinically established based on a decline in pulmonary function in the absence of reversible causes of respiratory failure, such as infections and acute rejection (AR) [3,4].

Previous studies from our laboratory [5–7] and others have demonstrated that development of anti-human leukocyte antigen (HLA) antibodies (Abs) precedes the development of CR after LTx [8,9]. Studies have also shown that the development of anti-HLA Abs during the post-transplantation (Tx) period is a significant risk factor for the development of CR in kidney [10,11], and heart transplantation [12,13]. Recent studies suggest that Abs developed during the post-Tx period against the mismatched non-HLA antigens may also contribute to the pathogenesis of CR in solid-organ transplantation [14–16].

One target antigen that has received considerable attention recently is the major histocompatibility (MHC) class I–related chain A (MICA). MICA, a single chain glycoprotein expressed on cellular membrane, is determined by a genetic locus closely linked to the HLA-B. MICA is polymorphic, with 52 protein variants and more than 60 alleles [17,18]. MICA molecules function as signals of cellular stress and trigger a range of immune effecter mechanisms, including cellular cytotoxicity and cytokine secretion [19,20]. Although the MICA protein has not been implicated in antigen presentation, it plays an important role in initiating an immune response by binding to the immune-receptor NKG2D. Upon binding to MICA, NKG2D stimulates cell lysis pathways and hence MICA has

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an important role in providing a co-stimulatory signal for the activation of NK (natural killer) cells, $CD8^+$ T cells and $\gamma\delta$ T cells [21–23]. Recent reports suggest that post-Tx development of Abs to MICA correlate with renal allograft loss and is an independent risk factor for the development of CR [24–26]. Similarly a relationship between the development of anti-MICA Abs and CR has been reported in heart transplant recipients [27,28].

The increased expression of polymorphic MICA genes in the human lung airway epithelial cells during conditions of stress can lead to apoptosis of epithelial cells and pulmonary fibrosis [29]. During donor procurement and LTx, the airway epithelial cells undergo mechanical stress which causes damage to epithelial integrity. Similarly, anti-HLA Abs developed in the post-Tx period can also injure epithelial cells, leading to the expression of otherwise cryptic self-antigens and cell surface expression of MICA antigens. The objective of this study was to determine the significance of MICA in the development of CR after LTx, and to assess whether sensitization to MICA is an independent risk factor for the development of CR after human LTx.

2. Subjects and methods

2.1. Patients and samples

From May 1990 to December 2008, all patients undergoing LTx at Washington University/Barnes-Jewish Hospital were enrolled in the study in accordance with a protocol approved by the Institutional Review Board. Serum samples were collected serially in the post-LTx period and were stored at −80°C. Sera from 80 patients were used in this study. Of these, 50 recipients developed BOS (BOS+), and 30 did not develop BOS (BOS−−). The demographic profile of the two cohorts (BOS+ and BOS− patients), including age, gender, ethnicity, indication for transplantation, and type of Tx (unilateral or bilateral) were tabulated. A clinical diagnosis of BOS was made using the guidelines established by the International Society for Heart and Lung Transplant [30,31]. The maintenance immunotherapy protocol at our center consisted of cyclosporine, azathioprine, and prednisone. After BOS was diagnosed, the immunotherapy protocol was modified to FK-506 (tacrolimus), mycophenolate mofetil, and prednisone. Sera from 15 healthy adult volunteers were obtained and served as controls.

2.2. Luminex assay for detection of Abs against MICA

Abs to MICA alleles were determined using LABScreen assay by Luminex Technology, according to the manufacturer's specifications (One Lambda, , Canoga Park, CA) using 96-well filter plates. In brief, the assay filter plate was pre-wet by dispensing 300 μl of wash buffer (cat. no. LSPWABUF) and incubated for 10 minutes on a platform plate shaker at low speed. After aspirating the buffer using a Millipore vacuum manifold with pressure not exceeding 100 mm Hg, 5 μl of LABScreen beads with 20 μl of serum was dispensed into test wells. For each test, a negative control serum (One Lambda, Inc. cat. no. LS-NC) was used along with a healthy volunteer's serum to establish a cut-off. The above mixture was incubated for 30 minutes with gentle shaking and washed using 275 μl of wash buffer. Furthermore, 100 μl of 1X PE conjugated antihuman IgG was added to each well and incubated for 30 minutes. Finally, 80 μl of 1X PBS was added, and the samples were read using LABScan 100 machine (Austin, TX).

Serum samples of LTx subjects and healthy donors were tested at 1:3 dilution for Abs against a panel of 10 MICA alleles (MICA *001, *002, *004, *007, *009, *012, *017, *018, *019, and *027). The fluorescent signal was measured using LABScan and analyzed by HLA-VisualTM software (One Lambda). The raw MFI values were normalized using negative control serum (One Lambda, Inc. Cat no. LS-NC) by the following formula: ([sample # N (specific fluorescent value) beads − sample negative control beads] − [negative control # N beads − negative control beads]). Luminex reactions were considered positive when the fluorescent signal of each bead was above the normalized mean fluorescence intensity (MFI) of volunteer control sera and normal control sera provided by the company. In addition, the MICA-positive serum for each of the 10 alleles listed above (obtained from Miyuki Ozawa, One Lambda) were analyzed and served as positive controls.

2.3. Detection of HLA Abs by FlowPRA assay

FlowPRA assay was performed according to the manufacturer's directions (One Lambda). For classes I and II FlowPRA assays, positivity with a single bead produced a clear and distinct peak of

Table 1
Clinical and demographic profile of lung transplant patients

	All	BOS+	BOS−	*p* Value
Age (y)	48.8 ± 12.6	49.8 ± 11.7	47.7 ± 12.2	0.62
Sex:				
Female	42	28	14	0.41
Male	38	22	16	
Race:				
Caucasian	71	44	27	0.78
African American	9	6	3	
Pathology:				
COPD	40	25	15	1.00
A1AD	12	8	4	0.07
CF	11	7	4	0.07
IPF	8	4	4	0.42
PPH	6	4	2	0.06
Bronchiectasis	3	2	1	0.89
Type of transplant				
Bilateral	70	43	27	0.06
Single	10	7	3	

COPD, chronic obstructive pulmonary disease; A1AD, alpha-1 antitrypsin deficiency; CF, cystic fibrosis; IPF, idiopathic pulmonary fibrosis; PPH, primary pulmonary hypertension.

Table 2
Comparison of MICA antibodies in serum samples of patients before and after lung transplantation

Pretransplantation	Post-transplantation	
	BOS+	BOS−
00/20 (00.0%)	21/50 (42.0%)[a]	4/30 (13.3%)

[a] $p < 0.01$.

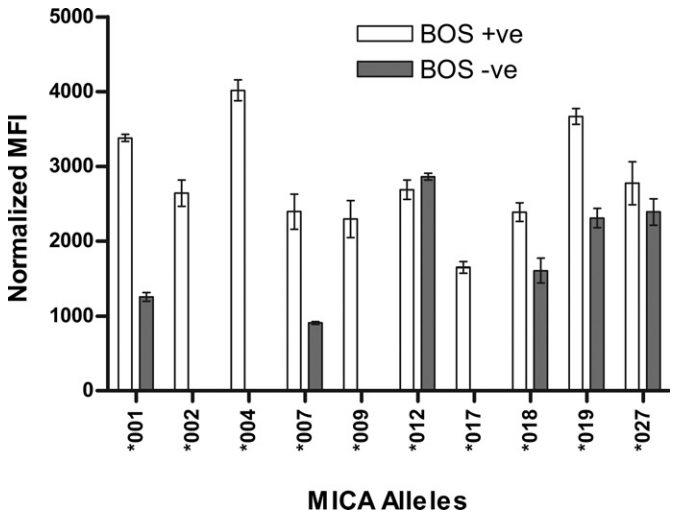

Fig. 1. Comparison of Abs to various MICA alleles in BOS+ and BOS− patient sera. Luminex reactions were carried in duplicates using 1:3 diluted sera. Data are representative of mean ± SD of all of the positive values obtained for each allele from various patients' sera of both the BOS+ and BOS− cohorts.