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Donor-specific antibodies to human leukocyte antigens are associated with and precede antibodies to major histocompatibility complex class I–related chain A in antibody-mediated rejection and cardiac allograft vasculopathy after human cardiac transplantation

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ABSTRACT

Humoral immune responses to mismatched donor human leukocyte antigen (HLA) and major histocompatibility complex (MHC) class I–related chain A (MICA) have been reported to contribute to immunopathogenesis of antibody-mediated rejection (AMR) in the early period and cardiac allograft vasculopathy (CAV) in the late period after cardiac transplantation (HTx). The goal of this study is to define the roles of donor-specific antibodies (DSA) and anti-MICA in AMR and CAV. A total of 95 post-HTx recipients were enrolled; 43 patients in the early period (≤12 months post-HTx) and 52 patients in the late period (>12 months post-HTx). Development of DSA and anti-MICA were serially monitored using Luminex. Development of DSA (AMR+: $n = 6/8.75\%$, AMR−: $n = 4/35.11\%$, $p = 0.009$) and anti-MICA (AMR+: $n = 5/8.63\%$, AMR−: $n = 4/35.11\%$, $p = 0.002$) was significantly associated with AMR. AMR+DSA+ patients demonstrated increased anti-MICA levels compared with AMR+DSA− patients ($p=0.01$). Serial monitoring revealed DSA (2.7 ± 1.4 months) preceded development of anti-MICA (6.5 ± 2.1 months) in recipients diagnosed with AMR at 8.3 ± 2.5 months post-HTx. Development of DSA (CAV+: $n = 8/12.67\%$, CAV−: $n = 5/40.13\%$, $p = 0.004$) and anti-MICA (CAV+: $n = 9/12.75\%$, CAV−: $n = 5/40.13\%$, $p = 0.001$) was significantly associated with CAV. CAV+DSA+ patients demonstrated increased anti-MICA levels compared with CAV+DSA− patients ($p = 0.01$). Antibodies to HLA are associated with and precede development of anti-MICA in AMR and CAV. Therefore, DSA and anti-MICA can be used as noninvasive markers for monitoring AMR and CAV.

1. Introduction

The immediate and long-term success of human cardiac transplantation (HTx) is impeded by the development of antibody-mediated rejection (AMR) and cardiac allograft vasculopathy (CAV), respectively. During the early postoperative period, it is estimated that 20–40% of HTx recipients develop AMR [1–5]. During the late post-HTX period, CAV is a pathognomic feature of allograft dysfunction and contributes to increased mortality [6–9]. Alloantibodies directed against mismatched donor major histocompatibility complex (MHC) molecules have been linked to increased allograft failure during both the early and late postoperative period [10–12]. However, a significant proportion of solid organ transplant recipients demonstrate allograft failure although no antibodies (Abs) against major histocompatibility antigens are detected [13–15]. Increasingly, studies have demonstrated that Abs against nonclassical MHC molecules, such as MHC class I polypeptide–related sequence A (MICA), can induce complement-dependent cytotoxicity, and have been implicated in acute and chronic solid organ allograft rejection [13,16,17]. This extends to post-HTx recipients, in whom a positive correlation between the development of circulating non-MHC Abs with allograft dysfunction has been demonstrated [18].

MICA is a highly polymorphic cell surface glycoprotein expressed on endothelial cells as well as fibroblasts and activated monocytes [19]. MICA is a ligand for NKG2D, which is an activating immunoreceptor found on natural killer (NK) cells and on $CD8^+$ T cells [20,21]. The NKG2D receptor acts as a co-stimulatory signal for $CD8^+$ T cells that complements T-cell receptor–mediated antigen

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recognition of target cells [22,23]. The increased surface expression of MICA on graft endothelial cells during episodes of rejection can induce allorecognition leading to an amplified humoral and cellularly mediated immune response, as substantiated by enhanced detection of anti-MICA in sera of allograft recipients with acute and chronic rejection [24,25].

The incidence of anti-MICA varies considerably, between 3% in healthy individuals to more than 30% after kidney and heart transplantation [26]. despite confounding factors, such as lack of sensitivity and/or specificity in detection systems and lack of test standardization which may contribute to the varying incidence of anti-MICA in different patient populations, many studies have suggested that MICA plays an important role in the alloimmune response following solid organ transplantation [17,27]. In the case of HTx recipients, studies have demonstrated that nearly 40% of patients develop Abs against MICA during the first year post-transplant and are at an increased risk for the development of severe acute rejection [24]. Similarly, a recent retrospective study found that more than 20% of patients with cardiac rejection episodes had Abs against MICA, with the resultant multivariate analysis identifying anti-MICA positivity as an independent risk factor for the development of CAV [28]. Further, studies have demonstrated increased titers of anti-MICA in serum accompanied by an enhanced MICA expression on allograft endomyocardial biopsies in patients with increased episodes of acute cardiac allograft rejection [29].

It is important to elucidate whether there is a causative role for Abs to MICA in inducing adverse early or late cardiac allograft events. There is some evidence for the role of MICA in AMR in renal transplant recipients [30,31]. However, there is a dearth of studies that have examined the role of MICA in AMR in HTx, which is a frequent cause of early adverse graft function. Given the evidence for the role of donor-specific antibodies (DSA) in recipients who were subsequently diagnosed with AMR and CAV in the early and late post-HTx periods, respectively, the objective of our present study was to evaluate the association between the development of DSA to mismatched human leukocyte antigens (HLA) and serum levels of Abs against 10 commonly found MICA antigens in post-HTx patients. Our results demonstrate that DSA is significantly associated with the detection of anti-MICA in patients with AMR and CAV. Importantly, serial monitoring of postoperative sera in the early period indicated that detection of DSA precedes the detection of Abs to MICA in patients who developed AMR, thereby suggesting a temporal relationship between DSA and development of Abs to MICA after HTx.

2. Subjects and methods

2.1. Study population

In a protocol approved by the Institutional Review Board, 95 patients who underwent adult HTx at Barnes-Jewish Hospital/Washington University were enrolled in the study. Patient sera was collected and stored at −70°C. For the 43 patients in the early period (EP, ≤12 months post-HTx), serum samples were obtained every month after enrollment in the study. This coincided with the performance of surveillance or clinically indicated endomyocardial biopsy. In many cases, we were able to obtain a serum sample before HTx and immediately after HTx as well. For the 52 patients in the late period (LP, >12 months post-HTx), serum samples were obtained annually following enrollment in the study and it coincided with the performance of surveillance or clinically indicated angiogram. Using guidelines recommended by the International Society of Heart and Lung Transplantation, a diagnosis of AMR was reached by examining clinical, histologic, and serologic criteria [7]. Clinical criteria included patient symptoms (fatigue, palpitations), objective evidence of cardiac dysfunction (decreased ejection fraction and/or restrictive physiology on echocardiography, intravenous inotrope administration). Histologic and immunopathologic criteria included capillary endothelial swelling as a marker of acute capillary injury, macrophages or neutrophils in capillaries and lack of features consistent with cellular rejection, positive C4d capillary staining, and CD68 positivity for capillary macrophages. Serologic criteria included detection of DSA to mismatched donor HLA. Once the attending transplant cardiologist assessed each of the above criteria and reached a diagnosis of AMR, initial treatment was initiated that included methylprednisone, plasmapheresis, and intravenous immunoglobulins (IVIG). A diagnosis of CAV was reached based on angiographic evidence of coronary artery stenosis. Angiographic evidence of coronary artery stenosis (>50% luminal diameter) of one vessel or less was designated as none/minimal CAV while coronary artery stenosis (>50% luminal diameter) of two vessels or more was designated as moderate/severe CAV.

2.2. Detection of DSA by Luminex

Luminex technology (Biosource International, Amarillo, CA), which uses a solid-phase assay, was used to identify DSA in patient sera. Primary Ab-coated beads and incubation buffer were initially placed into 96-well filter plates. On an oribital shaker at room temperature, samples and standards were incubated with the primary Ab beads. Subsequently, the wells were washed and biotinylated secondary Abs were added before a 30-minute incubation period. Strepatividin-R-phyocoerythrin solution was added (after the wells were washed) and incubated for 15 minutes. The wells were washed for a final time and binding was determined with a dual-laser flow analyzer, the Luminex-100 system version 1.7. Data analysis was performed using the MasterPlex QT 1.0 system (MiraiBio Group, San Francisco, CA) and detection was compared with standard curves using a five-parameter regression formula.

2.3. Detection of Abs against MICA by Luminex

As described in a previous study from our laboratory, Abs to MICA were determined using LABScreen assay (Luminex Technology, Biosource International, CA) in 96-well filter plates according to the manufacturer's specifications (One Lambda, CA) [27]. The assay filter plate was prewet with 300 μl of wash buffer (catalog no. LSPWABUF) and, on a platform plate shaker at low speed, it was incubated for 10 minutes. After aspirating the buffer with a Millipore vacuum manifold, 5 μl of LABScreen beads and 20 μl of serum were dispensed into test wells. A negative control serum sample (OLI catalog no. LS-NC) was used in conjunction with a healthy volunteer's serum to establish a cut-off for each test. With gentle shaking, the mixture was incubated for 30 minutes and washed with 275 μl of wash buffer. A 100-μl quantity 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 a LABScan 100 machine.

Serum samples were tested at 1:3 dilution for Abs against a panel of 10 commonly found MICA antigens (MICA *001, *002, *004, *007, *009, *012, *017, *018, *019, and *027). The fluorescent signal was measured using LABScan and analyzed by HLA-Visual software (One Lambda, CA). The raw mean fluorescence intensity (MFI) values were normalized with negative control serum (OLI catalog no. LS-NC) and usng the following formula: ([sample N beads − sample negative control beads] − [negative control N beads − negative control beads]). A reading was considered positive if the fluorescent signal of each bead was above the MFI of volunteer control sera and normal control sera provided by the company. Serum samples containing Abs against all 10 tested MICA antigens were used as positive controls and these samples were a kind gift from Miyuki Ozawa (One Lambda, CA).