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De novo donor-specific HLA antibodies are associated with early and high-grade bronchiolitis obliterans syndrome and death after lung transplantation

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BACKGROUND: The development of human leukocyte antigen (HLA) antibody responses has been associated with worse clinical outcomes, such as bronchiolitis obliterans syndrome (BOS) and death, in lung transplant recipients (LTRs). However, the role of donor-specific HLA antibody (DSA) responses as a risk factor for poor outcomes remains controversial.

METHODS: We prospectively screened 445 LTRs for DSA at our institution at the time of surveillance bronchoscopies for the first 2 years after transplantation between 2003 and 2008, and evaluated clinical outcomes. For this purpose, we used the combination of panel-reactive antibodies (PRA) by enzyme-linked immunosorbent assay (ELISA) and the Luminex single-antigen bead (SAB) assay (One Lambda, Canoga Park, CA).

RESULTS: We detected de novo DSA (dnDSA) in 58 of 445 (13%) LTRs in our cohort. Freedom from BOS was significantly reduced in LTRs with dnDSA versus those without dnDSA ($p < 0.001$). Using a Cox proportional hazards model, the development of dnDSA was associated with a significantly increased hazard ratio (HR = 6.59 [4.53 to 9.59]; $p < 0.001$) for BOS and high-grade BOS (Stage ≥ 2) (HR = 5.76 [3.48 to 9.52]; $p < 0.001$). Freedom from death was significantly reduced in LTRs with dnDSA ($p < 0.001$), including mortality attributable to BOS (HR = 9.86 [4.91 to 19.78]; $p < 0.001$).

CONCLUSIONS: Taken together, our findings provide evidence that dnDSA is associated with accelerated BOS kinetics and severity, as well as death due to BOS after lung transplantation. In addition, these data support regular monitoring for the development of dnDSA in LTRs and underscore the need for novel strategies to mitigate the increased risk of poor outcomes associated with dnDSA.
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Long-term survival after lung transplantation is poor compared with other solid-organ transplants, and is most limited by bronchiolitis obliterans syndrome (BOS), a progressive airflow obstruction in the absence of other

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etiologies.[1,2] In fact, 48% of patients surviving to 5 years and 76% of patients surviving to 10 years after lung transplantation will develop BOS. The prognosis after the diagnosis of BOS is dismal, with a median survival of approximately 2.5 years.[3] Strategies for earlier identification of lung transplant recipients (LTRs) at high risk for BOS are urgently needed to develop effective interventions to improve outcomes.

Several risk factors for the development of BOS have been elucidated and include both alloimmune-dependent and -independent factors. Acute cellular rejection, lymphocytic bronchitis, human leukocyte antigen (HLA) mismatch, community-acquired respiratory viral infections, primary graft dysfunction and gastroesophageal reflux have all been implicated in the development of BOS.[2,4–6] Early studies showed that the development of HLA antibodies in LTRs was associated with BOS, and preceded its development.[7,8] Subsequently, the development of HLA antibodies was shown to correlate with persistent high-grade acute cellular rejection and lymphocytic bronchitis, 2 major risk factors for BOS, in conjunction with allograft dysfunction.[9,10] In another recent study, HLA antibodies were associated with allograft dysfunction and mortality; however, donor-specific HLA antibodies (DSA) were not found to be a risk factor for the development of BOS, despite being a risk factor for overall survival.[11] Overall, the clinical impact of DSA remains controversial after lung transplantation.

To further elucidate the role of DSA in lung transplant outcomes, we established a prospective protocol to screen for the development of DSA in LTRs at our institution. We sought to define the incidence of and determine the association of HLA antibodies, specifically de novo DSA (dnDSA), on the development of allograft dysfunction and mortality.

Methods

The protocol for this study was approved by the University of Pittsburgh Quality Improvement Committee and by the institutional review board of the University of Pittsburgh.

Study design and patient management

We performed a cross-sectional, observational study of all patients who underwent lung transplantation at the University of Pittsburgh Medical Center between January 1, 2003 and December 31, 2008. Study follow-up was completed through December 31, 2010. Patients were excluded if they died within the first 3 months after lung transplantation, or if they did not have spirometry results during the follow-up period.

Before lung transplantation, patients were screened for preformed HLA antibodies using the LABScreen enzyme-linked immunosorbent assay (ELISA) and, if positive (>5%), the Luminex-IgG single antigen bead (SAB) assay (One Lambda, Canoga Park, CA) was performed to determine HLA antibody specificity (>1,000 MFI was considered positive). Donor lung allografts were accepted only if a "virtual" crossmatch between all previously identified recipient anti-HLA sensitization and donor antigens were negative. All patients had a T- and B-cell complement-dependent cytotoxicity crossmatch (CDC-XM) within 24 hours of transplantation using peak panel-reactive antibody (PRA) historic sera and sera from the time of transplant. The T-cell CDC-XM was performed using anti-human globulin and dithiothreitol treatment if the untreated sera CDC-XM were positive, to differentiate between IgG and IgM antibodies. The B-cell CDC-XM was done by an Amos modified/extended incubation method. T- and B-lymphocytes were isolated using magnetic beads from the donor peripheral blood, lymph nodes or spleen. After lung transplantation, LTRs were screened for the presence of dnDSA using ELISA and, when positive (>5%), Luminex-IgG SAB assays were performed to determine HLA antibody specificity. This screening procedure was performed with routine surveillance bronchoscopies every 2 to 3 months during the first 2 years after lung transplantation and also when clinically indicated. Patients were classified as having dnDSA at the time of the initial antibody identification and did not cross over between groups, irrespective of future HLA testing results.

All patients received alemtuzumab (humanized anti-CD52 monoclonal antibody) for induction and were maintained on a triple-drug immunosuppressive regimen consisting of a calcineurin inhibitor (tacrolimus or cyclosporine), a cell-cycle inhibitor (mycophenolate or azathioprine) and corticosteroids (prednisone). All patients received voriconazole for fungal prophylaxis and valganciclovir for cytomegalovirus (CMV) prophylaxis at the time of lung transplantation. All episodes of rejection were diagnosed histologically after bronchoscopy according to the most recent 2007 International Society for Heart and Lung Transplantation (ISHLT) guidelines on standardization of nomenclature in the diagnosis of lung rejection.[12] Episodes of rejection of Grade >A1 were treated with steroids in addition to modification of the daily immunosuppressive regimen.

Allograft function was measured according to the American Thoracic Society guidelines and was monitored serially after lung transplantation. Bronchiolitis obliterans syndrome (BOS) was diagnosed according to criteria from the International Society for Heart and Lung Transplantation (ISHLT) and was treated with azithromycin, augmentation of the immunosuppressive regimen and/or with rabbit anti-thymocyte globulin.[2]

Statistical analysis

Baseline demographics and characteristics were compared between the 2 groups using Student's *t*-test and chi-square analysis, when appropriate. Normally distributed data were compared using Student's *t*-test and non-normally distributed data were compared using Wilcoxon's rank-sum test and Mann-Whitney *U*-test, when appropriate. Freedom-from-event analyses were performed using the Kaplan-Meier method with the log-rank test. Cox proportional hazards modeling was used to determine the risk associated with events in a time-dependent fashion without assigning pre-identification risk to each variable. Time-to-event data are reported as mean value and standard deviation (SD) for parametric values and median and interquartile range (IQR) for non-parametric values. All analyses were performed using SPSS (version 19.0) software (SPSS, Inc., Chicago, IL). A $p < 0.05$ was considered statistically significant.

Results

Patients' characteristics

We prospectively instituted a screening protocol of all LTRs for the detection of dnDSA. Patients were followed from the