DEVELOPMENT OF AN ANTIBODY SPECIFIC TO MAJOR HISTOCOMPATIBILITY ANTIGENS DETECTABLE BY FLOW CYTOMETRY AFTER LUNG TRANSPLANT IS ASSOCIATED WITH BRONCHIOLITIS OBLITERANS SYNDROME

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Background. Chronic allograft rejection manifested as bronchiolitis obliterans syndrome (BOS) is the leading cause of late death after lung transplantation. Although increasing evidence suggests an association between anti-human leukocyte antigens (HLA) antibodies and chronic rejection of kidney or heart allografts, the clinical significance of anti-HLA antibodies in lung recipients is less clear, especially in previously unsensitized recipients. The use of flow cytometry based panel reactive antibody (flow-PRA) provides a highly sensitive means to identify the development of de novo anti-HLA antibodies in lung recipients.

Methods. Flow-PRA testing was used to analyze the pre- and posttransplant sera in stable BOS free lung recipients who survived at least 6 months. Patients without prior sensitization as defined by a negative pretransplant flow-PRA were analyzed posttransplant for the presence of anti-HLA antibodies by flow-PRA. A proportional hazards model was used to determine the impact of anti-HLA antibody on BOS risk.

Results. Sera from 90 recipients at Duke University with negative pretransplant flow-PRA were tested by flow-PRA at various time points after transplant. Sera from 11% (10/90) of recipients were found to contain anti-HLA antibodies detectable by flow-PRA. Nine patients (90%) developed anti-HLA antibodies specific for donor antigens, and one patient developed anti-HLA class II antibodies, not specific to donor antigens. Among the nine patients with donor antigen specific antibodies, flow-PRA specificity analysis demonstrated eight were specific for class II antigens and one for class I antigens. In a multivariate model that controls for other BOS risk factors, a positive posttransplant flow-PRA was significantly associated with BOS grades 1,2, or 3 (hazard ratios [HR] 3.19; 95% confidence interval [CI]: 1.41–7.12, P=0.005) and BOS grade 2 or 3 (HR 4.08; 95% CI: 1.66–10.04, P=0.002). Four patients with de novo anti-HLA antibodies died during follow-up; all four had BOS. Among BOS patients, the presence of anti-HLA antibodies was associated with a significantly worse survival (P=0.05, log-rank test).

Conclusions. Although uncommon, previously unsensitized lung transplant recipients can develop anti-HLA antibodies to donor class II antigens. The development of de novo anti-HLA antibodies significantly increases the risk for BOS, independent of other posttransplant events. Furthermore, de novo anti-HLA antibodies identify BOS patients with significantly worse survival. Additional studies are needed to determine if class II–directed anti-HLA antibodies contribute mechanistically to the chronic rejection process in lung recipients.

INTRODUCTION

Chronic rejection is the leading cause of allograft loss among solid organ transplant recipients. Each type of solid organ recipient has distinct pathological and clinical manifestations of chronic rejection (*1*). In the lung, chronic rejection is defined pathologically as obliterative bronchiolitis (OB), a dense fibrous scarring of the bronchioles accompanied in some cases by vascular sclerosis affecting pulmonary arteries and veins (*2*). Clinically, chronic graft dysfunction in the lung is defined as bronchiolitis obliterans syndrome (BOS), a significant decrease in the posttransplant forced expiratory volume in one second (FEV_1) graded according to the guidelines established by the International Society for Heart and Lung Transplantation (ISHLT) (*2*).

Antibodies to human leukocyte antigens (HLA) are associated with poor outcomes among all solid organ transplant recipients. Previous studies, including our own, have demonstrated that the presence of preexisting anti-HLA antibodies identifies lung allograft recipients at increased risk for early allograft failure and death (*3*). Similar studies in other organ transplant recipients suggest preexisting antibody increases the rate of early kidney allograft failure or heart allograft dysfunction (*4*). Additional evidence also suggests the development of posttransplant antibodies to HLA antigens, especially donor specific antigens, is significantly associated with acute, and particularly with chronic, allograft rejection in heart, kidney, and perhaps lung recipients (*3, 5*).

Despite reports that link the development of anti-HLA antibodies to chronic allograft rejection in lung recipients, most previous studies are limited by the use of the complement dependent cytotoxicity (CDC) anti-human globulin enhanced technique (*5*). Limitations of the CDC techniques include detection of non-HLA autoantibody and difficulty defining antibody specificity. Specifically, identification of class II antibodies in the presence of class I antibodies is difficult, and weak-binding class I antibodies may bind only to B cells, thereby confusing the interpretation. Furthermore, most previous studies in lung recipients have consid-

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ered the effect of anti-HLA antibody on BOS in a univariate manner that did not control for time or other covariates that influence the risk for BOS. Therefore, a precise determination of the frequency, specificity, and clinical significance of de novo anti-HLA antibodies in lung recipients has been difficult. We have recently implemented a panel reactive antibody (PRA) technique using solid phase matrix to which purified soluble HLA class I or class II antigens are attached, flow-PRA, because several studies have demonstrated the superiority of this method to the antihuman globin (AHG)-CDC technique (*6, 7*). The aims of the current study were to use the flow-PRA technique to determine the rate of development of posttransplant anti-HLA antibody production in patients negative pretransplant by flow-PRA and determine the association between posttransplant antibody development and BOS.

MATERIALS AND METHODS

Duke Transplant Population

282 lung transplant operations (140 bilateral, 140 single lung transplants, and 2 living-related lobar transplants) were performed at Duke University Medical Center between 4/24/1992 and 6/8/2000. These patients serve as the population for analysis. Standardized surgical techniques and posttransplant management protocols were employed as described elsewhere (*8*). Patients received cyclosporine (5–10 mg/kg per day) for primary immune suppression, generally in conjunction with azathioprine (1–2 mg/kg per day) and corticosteroids (methylprednisolone, 125 mg every 12 hr for the first 48 hr, followed by prednisone, 20 mg per day). Since January 1999, patients received a monoclonal interleukin (IL)-2 receptor antibody (daclizumab or basiliximab) as part of their induction immunosuppression (83 patients). Episodes of acute allograft rejection were treated with methylprednisolone, 500 mg per day for 3 days, followed by a 2-week oral prednisone taper. Bronchiolitis obliterans was treated generally with a change from cyclosporine to tacrolimus or a change from azathioprine to mycophenolate, or both.

Posttransplant Flow-PRA Screening

Flow-PRA testing is now routinely performed at Duke at least once per year in all posttransplant lung recipients. Patients included in this retrospective analysis were stable, without prior sensitization (i.e., negative pretransplant CDC-AHG PRA and flow-PRA) and survived at least 6 months posttransplant. Serial samples were analyzed for the presence of anti-HLA antibodies by flow-PRA. A total of 90 patients met entry criteria and had posttransplant sera available for testing to comprise the study population. All patients with BOS and anti-HLA antibodies were noted to have developed a positive flow-PRA assay before the diagnosis of BOS.

Definition of Acute Rejection and BOS

Acute rejection was defined and graded according to standard histological criteria (*2*). The acute rejection score (ARS) was calculated by adding the sum of the grades of each rejection episode. BOS was defined according to the ISHLT guidelines (*2*). Briefly, a BOS score of 0 corresponds to a current FEV_1 of 80% or greater of the posttransplant baseline, a BOS score of 1 corresponds to an FEV_1 of 66 to 80% of baseline, a BOS score of 2 corresponds to an FEV_1 of 51 to 65% of baseline, and a BOS score of 3 corresponds to an FEV_1 of less than 50% of baseline.

Flow-PRA Assay

Flow-PRA analysis was performed as previously described (*6*). The flow-PRA assay (One Lambda, Inc, Canoga Park, CA) uses a pool of beads that are microparticles 2 to 4 μm in diameter coated with purified HLA antigens. Distinct beads are coated with class I or class

II molecules isolated from Epstein-Barr virus (EBV) transformed cells lines from 30 different donors of known HLA specificities. Briefly, the flow-PRA assay was performed by mixing 20 μL serum and 5 μL each of class I and class II coated microparticles and incubating for 30 minutes in the dark. After washing three times with phosphate buffered saline, the microparticles were stained with 25 μL of pretitered fluorescein isothiocyanate (FITC)-conjugated anti-human immunoglobulin (Ig)G and incubated for 30 minutes in the dark with gentle shaking. After two additional washes, 0.5 mL fixing solution was added and flow cytometry analysis performed. The class I microparticles are nonfluorescent while the class II microparticles fluoresce when excited at 488 nm, generating an emission of approximately 580 nm, similar to phycoerythrin. Thus, the class I and class II microparticles can be separated and anti-class I versus anti-class II antibodies can be identified independently. The percent PRA is determined by the percent of microparticles that are bound by the antibodies in the serum.

The specificity of the antibody is determined by using a flow-PRA specificity detection system. For this assay, four pools containing class I or class II coated microparticles from eight different EBV lines are used. Eight different colored microparticles are used with different fluorescent properties that generate an emission similar to phycoerythrin. Microparticles that have antibody bound to them produce a clear peak of fluorescence that is distinct from microparticles not bound by antibodies. The volumes, incubation times, etc., are the same as described above. The specificity of the antibody can be determined by identifying which of the 32 microparticles are bound by antibodies in the serum.

Statistics

Patient demographic characteristics are reported by using descriptive statistics. For between-group comparisons of proportions, Chi-square testing or the Fisher exact test was used, as appropriate. For BOS risk analysis, a Cox proportional hazard model was used with flow-PRA status as a predictor variable in a multivariable model and time to BOS as the outcome variable. Results are presented with hazard ratios (HR) and 95% confidence intervals (CI). The Kaplan-Meier method and log-rank test were used for survival analysis among posttransplant BOS patients. *P* values of <0.05 were considered significant. SAS statistical software was used for all the analyses (version 8.0, Cary, NC).

RESULTS

Development of de Novo Anti-HLA Antibodies Posttransplant in Flow-PRA Negative Pretransplant Recipients

Sera from 90 recipients at Duke University with negative pretransplant flow-PRA were tested by flow-PRA at various time points after transplant. Recipients underwent a median of two posttransplant flow-PRA tests per patient (range, 1–4). The initial posttransplant flow-PRA testing was performed on patients a median of 789 (range, 192–2627) days after lung transplant. Sera from 11% (10/90) of recipients were found to contain anti-HLA antibodies detectable by flow-PRA.

Demographic Characteristics of Patients with and without Posttransplant Anti-HLA Antibodies

The demographic characteristics of patients with and without positive posttransplant flow-PRA are illustrated in Table 1. There were no significant differences between the two groups of patients. Patients in both groups were of similar age and gender. The number of bilateral transplants in the positive flow-PRA group was greater than that in the PRA

TABLE 1. Characteristics of lung transplant recipients with or without anti-HLA antibodies as detected by Flow-PRA

Characteristic	Flow-PRA positive (n=10)	Flow-PRA negative (n=80)
Recipient age (mean±SD)	38±15	45±13
Donor age (mean±SD)	37±14	32±15
Gender (%)		
Male	6 (60)	45 (56)
Female	4 (40)	35 (44)
Type of transplant (%)		
Single	2 (20)	41 (51)
Bilateral	8 (80)	39 (49)
Native lung disease (%)		
COPD	2 (20)	41 (51)
CF	5 (50)	18 (23)
Others	3 (30)	21 (26)
HLA mismatches (%)	5.0±0.67	4.6±1.3
Second transplant (%)	1 (10)	4 (5)

Flow-PRA, flow cytometry based panel reactive antibody; COPD, chronic obstructive pulmonary disease; CF, cystic fibrosis; HLA, human leukocyte antigen.

negative group, consistent with the observed differences in native disease among the two groups. The native diseases for patients with positive posttransplant flow-PRA are as follows: cystic fibrosis, n=5; idiopathic pulmonary fibrosis, n=2; chronic obstructive pulmonary disease, n=2; and OB, n=1. The single patient with OB initially underwent a transplant for IPF and was negative by flow-PRA at the time of retransplant. Patients with negative flow-PRA measurements underwent a mean of 1.89 measurements per patient while patients with positive measurements underwent a mean of 1.96 measurements per patient. The time to initial posttransplant flow-PRA testing and the total postoperative follow-up days were similar among patients with and without development of de novo anti-HLA antibody.

Posttransplant Anti-HLA Antibody and BOS

When the current BOS scores were analyzed, 80% (8/10) of recipients with de novo posttransplant anti-HLA antibodies were found to have a BOS score of 1, 2, or 3, while only 38% (30/80) of flow-PRA negative patients developed BOS ($P=0.016$). Furthermore, high grade BOS (grade>1) was present in 70% (7/10) recipients with positive flow-PRA but was present in only 24% (19/80) of those without a positive flow-PRA ($P=0.005$). Nine patients (90%) developed anti-HLA antibodies specific for donor antigens, and one patient developed anti-HLA class II antibodies, not specific to donor antigens. Among the nine patients with donor antigen specific antibodies, flow-PRA specificity analysis demonstrated eight were specific for class II antigens and one for class I antigens, as shown in Table 2. Two patients with donor specific antibodies have remained BOS free over 119 or 340

days of additional follow-up. The development of a positive flow-PRA preceded the development of BOS by a median of 30 days (range, 7–183). The relationship between the positive flow-PRA, posttransplant time, and FEV_1 in a single patient is illustrated in Figure 1. In those patients with multiple measurements, flow-PRA results generally became negative over time (for example, Figure 1).

Effect of Flow-PRA Status on BOS in a Multivariate Model

In this cohort of 90 patients, BOS (grade 1 or higher) was present at the time of analysis in 38 patients, while 52 were free from BOS at follow-up. To determine if flow-PRA represented a significant risk independent of other known risk factors for BOS, univariate and multivariate analyses were performed. To control for the effects of posttransplant time on BOS, a Cox proportional hazards model was used with time to BOS as the outcome variable. The following risk factors were considered in univariate analysis: flow-PRA, age, gender, transplant type, transplant number, donor age, ARS, HLA mismatches, and cytomegalovirus (CMV) pneumonitis. The ARS was calculated by adding the grade of each rejection episode. Factors in univariate analysis with a $P<0.10$ were included in the multivariate model.

The four factors significant in the univariate analysis (ARS, flow-PRA, bilateral transplant, and CMV pneumonitis) were entered into a multivariate Cox proportional hazards model. All four factors remained significant in the multivariate model, with a positive posttransplant flow-PRA significantly associated with BOS (HR 3.19; 95% CI: 1.41–7.12, $P=0.005$), independent of the other variables.

A similar analysis was performed using a Cox proportional hazards model for patients with BOS grades 2 or 3 only. In univariate analysis, ARS, flow-PRA status, and CMV pneumonitis were significant predictors of time to BOS 2 or 3. In contrast with the previous analysis, type of transplant was no longer significant, but donor age was significant. When all four factors were entered into a multivariate model, only ARS (HR 1.19; 95% CI: 1.06–1.34, $P=0.004$) and flow-PRA status (HR 4.08; 95% CI: 1.66–10.04, $P=0.002$) remained significant predictors, as shown in Table 3.

Impact of Anti-HLA Antibodies on Survival

Four patients with de novo anti-HLA antibodies died during follow-up; all four had BOS. Deaths occurred 25, 69, 87, and 274 days from the time of detection of anti-HLA antibodies. Survival analysis was performed among the 38 patients with BOS using the Kaplan-Meier method to determine the impact of a positive flow-PRA on outcomes (Fig. 2). Despite the small numbers, BOS patients with de novo anti-HLA antibodies have a significantly worse survival as compared with those without antibodies ($P=0.05$, log-rank test).

DISCUSSION

In the current study, we found anti-HLA antibodies developed in 11% of a population of stable lung transplant recipients who survived the initial 6 months after transplantation. Most of the antibodies were specific for donor class II HLA, and the development of antibodies was highly associated with the development of BOS in a multivariate model independent of other known risk factors for this condition,

TABLE 2. Posttransplant antibody specificity and BOS

BOS grade	No.	Post-transplant antibody	Donor antigen specific	Class I	Class II
BOS 1–3	38	8	7	1	6
BOS 0	52	2	2	0	2

BOS, bronchiolitis obliterans syndrome.
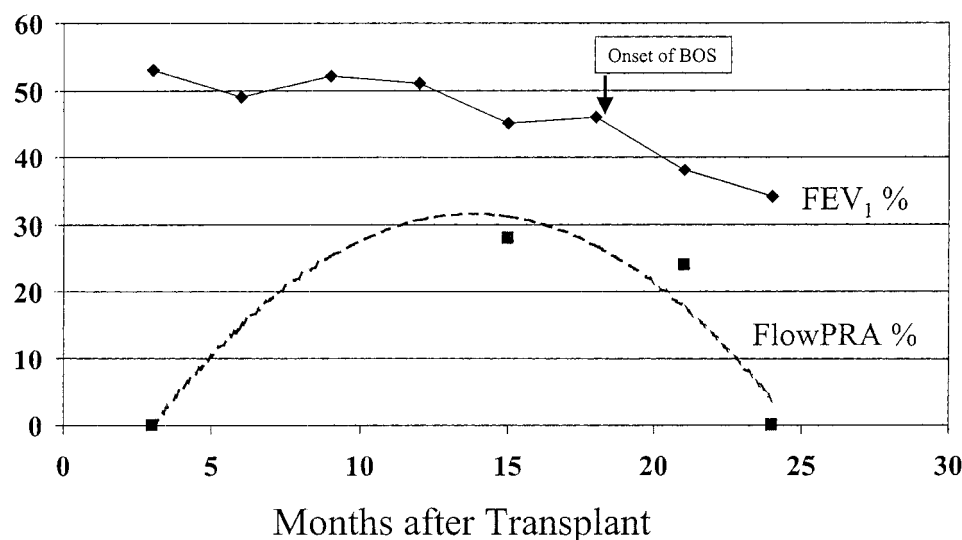
FIGURE 1. Relationship between flow cytometry based panel reactive antibody (flow-PRA) and forced expiratory volume in one second (FEV_1) in a lung transplant recipient. BOS, bronchiolitis obliterans syndrome.

TABLE 3. Risks for time to BOS grade 2 and 3 in multivariate Cox model

Variable	Hazard ratio (95% CI)	P value
Flow-PRA	4.09 (1.66–10.05)	0.002
Acute rejection score	1.19 (1.06–1.34)	0.004
Donor age	1.023 (.99–1.05)	0.11
CMV pneumonitis	2.34 (0.84–6.48)	0.10

BOS, bronchiolitis obliterans syndrome; Flow-PRA, flow cytometry based panel reactive antibody; CMV, cytomegalovirus.

including acute rejection. In addition, survival was significantly worse among BOS patients with anti-HLA antibodies as compared with those BOS patients without antibody.

Our results provide several insights into the importance of anti-HLA antibodies in the serum of lung recipients. First, our results demonstrate that although infrequent, de novo anti-HLA antibodies develop in some previously unsensitized recipients. Second, consistent with the donor allograft as the inciting antigen, all but one of our patients developed antibody specific to donor HLA antigens. The single patient with nonspecific HLA reactivity had received blood transfusions after the initial transplant operation that may have served to sensitize that recipient.

The relative paucity of de novo antibody production posttransplant, however, contrasts with some previous studies of anti-HLA antibody development in lung recipients. Although only 11% of recipients developed de novo anti-HLA antibody posttransplant at Duke, previous studies have generally found higher rates of antibody production (9). One explanation for the difference is our use of flow-PRA method to screen pretransplant sera and specifically exclude patients with pre-existing antibodies from the analysis. Studies that have found higher rates of posttransplant antibody production have used less sensitive techniques to screen pretransplant sera (e.g., enzyme-linked immunosorbent assay [ELISA] or CDC methods) (9, 10). Antibodies that become detectable by ELISA or CDC methods posttransplant may have actually been present pretransplant but not detectable by these less sensitive methods. In a larger series from another group,

Gammie and colleagues (11) found a similar rate (8.5%) of de novo antibody production to our results.

Also in contrast with our results, some previous studies have found that the anti-HLA antibody was directed to donor and unrelated HLA class I antigens but not to class II antigens (9). One explanation for these different results is that the ELISA method used by Jaramillo and colleagues (9) was not designed to detect class II molecules optimally. The flow-PRA method we used, employing microparticles coated with either class I or class II purified antigens, allows for the separate and optimal detection of both class I and class II antibodies. Furthermore, our finding of predominately anti-HLA antibody to donor class II antigens is consistent with recent observations in renal transplant recipients that found antibody to class II much more frequent than antibody to class I HLA (6% vs. 1.4%) and demonstrated a significant association between anti-class II antibody and acute or chronic rejection (12).

Although it is possible that antibody production occurs as an epiphenomenon as a result of activation of other elements of the chronic rejection response, we suspect antibody production contributes directly to airway damage. A plausible role for anti-class II antibody in OB may be offered by the observation that many tissues in the lung, including vascular endothelium and bronchial epithelium, can be induced to express class II antigens by a variety of stimuli, such as interferon gamma or IL-2 (13). Indeed, inducible expression of class II antigens has been documented in tracheal and bronchial tissues in an animal model of OB (14). We hypothesize that antibodies bind to class II antigens induced in such tissues by a variety of stimuli (e.g., infections, mechanical trauma, and cell-mediated rejection). Direct antibody binding to airway epithelium could produce a variety of deleterious effects, including complement-mediated cytotoxicity. Recently, anti-HLA antibody binding to class I MHC in a lung epithelium line in vitro has been shown to induce fibroblast proliferation through a tyrosine phosphorylation dependent mechanism (15).

Our survival analysis demonstrates that once BOS occurs, the prognosis is much worse in patients with anti-HLA antibodies than those BOS patients without humoral sensitiza-

FIGURE 2. Impact of anti-human leukocyte antigen (HLA) antibody on survival in lung recipients with BOS.
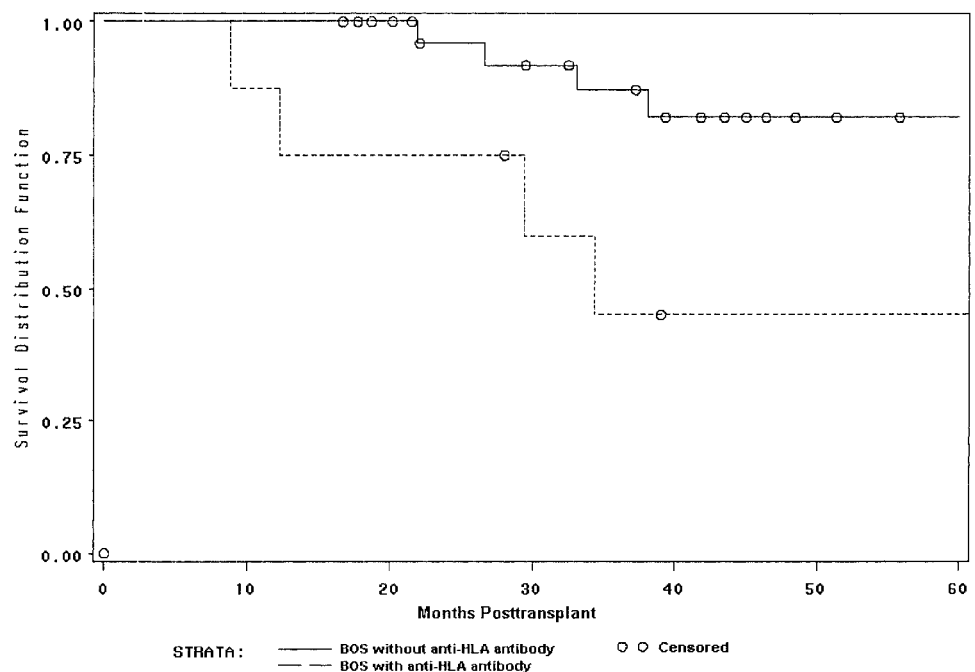
tion. Although survival among BOS patients has not been previously stratified by immune parameters, our results are consistent with previous reports on the effect of anti-HLA antibodies in lung recipients. Smith and colleagues (*10*) found that among lung recipients who survived at least 3 months posttransplant, early antibody development (within the first 2 years posttransplant) was associated with a significantly worse overall survival (*P*=0.04). Although the number of patients in our BOS analysis is small, our results suggest that heterogeneity may exist among BOS patients both in terms of pathogenesis and prognosis. This heterogeneity is consistent with the variable survival times reported by Valentine et al. (*16*) in a large analysis of lung recipients with BOS.

Our study suffers from several limitations. First, while our sample size (n=90) is relatively large for a lung study, a larger multicenter study would be useful to confirm our observations. Second, we chose to sample a cohort of stable lung transplant recipients at selected time points posttransplant. A prospective study with serial monitoring for anti-HLA antibody would be needed to determine the true incidence and natural history of de novo antibody production. Third, only a limited number of factors were considered in the univariate and multivariate BOS model because of the relatively small sample size. However, the factors employed were significant in previous studies examining BOS risk (e.g., ARS) (*17*). Furthermore, we did carefully control for posttransplant follow-up time using the Cox proportional hazard method.

In conclusion, although uncommon, previously unsensitized lung transplant recipients can develop anti-HLA antibodies to donor class II antigens. The development of de novo anti-HLA antibodies significantly increases the risk for BOS, independent of other posttransplant events. Furthermore, de novo anti-HLA antibodies identify BOS patients with significantly worse survival. Therefore, it may be useful to measure posttransplant flow-PRA in all lung recipients early posttransplant to identify those patients at increased risk for

BOS. In addition, it may be useful to measure flow-PRA in those patients with BOS because of the worse prognosis associated with anti-HLA antibodies as detected by the positive flow-PRA. Additional prospective studies are needed before we would recommend the widespread application of serial posttransplant flow-PRA measurements in all lung transplant recipients. Such studies are needed to confirm the associations between positive flow-PRA and BOS, to determine the optimal timing of monitoring, and to determine what impact, if any, immunotherapy directed at reducing or preventing anti-HLA antibody production has upon BOS.

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INCIDENCE, THERAPY, AND CONSEQUENCES OF LYMPHOCELE AFTER SIROLIMUS-CYCLOSPORINE-PREDNISONE IMMUNOSUPPRESSION IN RENAL TRANSPLANT RECIPIENTS[1]

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Background. In a retrospective study we sought to dissect the factors associated with an increased occurrence of clinically significant perinephric fluid collections and lymphoceles among sirolimus-treated renal transplant recipients.

Methods. We compared the incidence, predisposing factors, and consequences of these fluid collections among patients treated with sirolimus-cyclosporine (CsA)-prednisone (Pred) (n=354, group I) versus CsA-Pred with or without azathioprine (n=136, group II).

Results. More group I patients (135/354; 38.1%) displayed perinephric fluid collections (denoted as group III) then group II patients (24/136; 17.6%) (denoted as group IV) (*P*<0.001). In both subgroups the serum creatinine levels were elevated at the time of diagnosis from a nadir of 2.04±1.61 to 4.09±2.95 mg/dL (group III) and from 2.53±2.34 to 4.36±2.90 mg/dL (group IV). A significantly greater number of patients required treatment for lymphoceles among group I (56/354; 15.8%) versus group II recipients (6/136; 4.4%; *P*<0.001). Single or repeated percutaneous drainage procedures successfully treated 35 group I patients versus all 6 group IV patients (*P*=0.033). No patients in group II versus 21 patients in group I underwent surgical procedures (*P*<0.001). A significantly higher rate and higher histologic grade of acute rejection epi-

sodes, particularly proximate to the onset of the lymphocele, occurred among group IV patients, namely 54.2% (13/24) versus 21.4% (29/135) group III patients (*P*<0.001).

Conclusions. Addition of sirolimus to a CsA-Pred regimen resulted in both a higher incidence and a requirement for more aggressive treatment of perinephric fluid collections and lymphoceles.

Lymphocele, a well-known complication after kidney transplantation, occurs among 0.6% to 22% of patients treated with cyclosporine (CsA)-based immunosuppression (*1–7*). The term lymphocele (lymphatic collections lacking epithelial lining) originated in the Japanese literature (*8*), after an initial description of the condition as a complication of pelvic lymphadenectomy by Kobayashi (*9*). As demonstrated by lymphangiography (*10, 11*), two pathophysiologic mechanisms may result in lymphocele formation: drainage from open lymphatics divided during surgery to dissect the host iliac vessels (*5, 12*) and injured lymph channels in the donor kidney hilum vessels (*7, 13*). In his report of a case of lymphocele after kidney transplantation in 1968, Hume (*14*) recommended ligation of all major lymphatic channels at the time of skeletonizing the iliac vessels to minimize their occurrence. Other factors predisposing to lymphocele formation include an acute allograft rejection episode (*2, 11, 15*), acute tubular necrosis (*2*), a transplant biopsy (*6*), retransplantation (*16*), and, interestingly, adult polycystic kidney disease as the original renal disease (*17*).

The literature reports a wide range of incidences of lymphoceles, reflecting the use of different detection methods and various degrees of clinical significance in their interpretation. Lymphoceles have been detected more sensitively by routine ultrasonography (*18*) than by intravenous pyelography, lymphangiography (*19*), or computed axial tomography, which shows bladder compression (*19, 20*). Ultrasonography

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