CARDIOTHORACIC TRANSPLANTATION

EFFECT OF DEVELOPMENT OF ANTIBODIES TO HLA AND CYTOMEGALOVIRUS MISMATCH ON LUNG TRANSPLANTATION SURVIVAL AND DEVELOPMENT OF BRONCHIOLITIS OBLITERANS SYNDROME

Michael A. Smith, MD[a]
Sudhir Sundaresan, MD[a]
T. Mohanakumar, PhD[b]
Elbert P. Trulock, MD[c]
John P. Lynch, MD[c]
Donna L. Phelan, BA, CHS[d]
Joel D. Cooper, MD[a]
G. Alexander Patterson, MD[a]

Objective: **A retrospective analysis was performed to examine the role of HLA antibodies and cytomegalovirus mismatch on the development of bronchiolitis obliterans syndrome and survival after lung transplantation.** ***Methods:*** **Of 339 consecutive lung transplantations performed over a 102-month interval, 301 patients survived at least 3 months. There was a minimum follow-up period of 13 months. Bronchiolitis obliterans syndrome was defined as a decline in forced expiratory volume in 1 second less than 80% of posttransplantation baseline and/or histologic presence of obliterative bronchiolitis and was defined as occurring "early" if documented within 3 years of transplantation. Variables analyzed included preoperative donor and recipient cytomegalovirus status and the development of antibodies to human leukocyte antigens after transplantation. Microcytotoxicity was used to determine the presence of antibodies to human leukocyte antigens. Variables were subjected to Kaplan-Meier analysis to determine their impact on freedom from bronchiolitis obliterans syndrome and survival.** ***Results:*** **The development of antibodies to human leukocyte antigens after transplantation correlated significantly with bronchiolitis obliterans syndrome (*P* = .02). The development of antibodies to human leukocyte antigens did not affect survival (*P* = .33) unless they were detected within 2 years of transplantation (*P* = .04). There was greater frequency of early bronchiolitis obliterans syndrome in cytomegalovirus seronegative patients who received allografts from seropositive donors compared with all other combinations (*P* = .02). There was also a trend toward worse survival of cytomegalovirus seronegative patients who received allografts from seropositive donors (*P* = .13).** ***Conclusion:*** **These data suggest that bronchiolitis obliterans syndrome is the result of an immune-mediated process in which HLA antibodies and cytomegalovirus may play a significant role. (J Thorac Cardiovasc Surg 1998;116:812-20)**

The most common cause of late morbidity and death after lung transplantation is bronchiolitis obliterans syndrome (BOS).[1,2] The cause of this chronic lung allograft dysfunction is uncertain. It is presumed that the pathogenesis of BOS has an immunologic basis and that BOS is a manifestation of chronic rejection.

From the Divisions of Cardiothoracic Surgery[a] and Transplantation,[b] Department of Surgery; Division of Pulmonary and Critical Care Medicine, Department of Medicine[c]; and the HLA Laboratory[d]; Barnes-Jewish Hospital, Washington University School of Medicine, St Louis.

This work supported in part by NIH grant HL56643.

M.A.S. is a US Public Health Service National Research Service Award recipient.

Read at the Seventy-eighth Annual Meeting of The American Association for Thoracic Surgery, Boston, Mass, May 3-6, 1998.

Received for publication May 8, 1998; revisions requested June 12, 1998; revisions received July 3, 1998; accepted for publication July 13, 1998.

Address for reprints: Sudhir Sundaresan, MD, Washington University School of Medicine, Division of Cardiothoracic Surgery, Suite 3108, Queeny Tower, One Barnes Hospital Plaza, St Louis, MO 63110.

0022-5223/98 $5.00 + 0 **12/6/93089**

Table I. *Demographic characteristics of lung allograft recipients (n = 301)*

Age (yr)	45 ± 12
Gender (n, %)	
Male	146 (49)
Female	155 (51)
Primary disease	
Chronic obstructive pulmonary disease	98 (33)
α_1-Antitrypsin deficiency	54 (18)
Cystic fibrosis	47 (15)
Idiopathic pulmonary fibrosis	42 (14)
Primary pulmonary hypertension	35 (12)
Others	25 (8)
Transplant type (n, %)	
Single lung	121 (40)
Bilateral single lung	178 (59)
Double lung	2 (1)

Data presented as mean ± standard deviation wherever possible

Previous investigators have identified several potential risk factors for the development of BOS that include frequent or severe early acute rejection,[3-5] cytomegalovirus infection,[6-10] class I human leukocyte antigen (HLA) mismatches,[10-13] and development of antibodies to class I HLA.[11] The prevalence of BOS has been shown to exceed 50%.[2] However, long-term follow-up data regarding freedom from BOS are limited, so the notion that this process is inevitable in all long-term survivors of lung transplantation is controversial.

BOS frequently does not respond to medical therapy, and the fibrosis resulting from obliterative bronchiolitis is irreversible once established. A more detailed understanding of the pathogenesis of BOS may lead to more effective prevention and treatment. This study used Kaplan-Meier methods to analyze overall survival, the incidence of BOS, the effect of BOS on survival, and to further evaluate specific risk factors (cytomegalovirus mismatch and development of antibodies to HLA) for the development of BOS.

Methods

Patient population. We retrospectively analyzed 339 consecutive lung transplantations performed at Barnes-Jewish Hospital from August 1988 to December 1996. Three hundred one patients survived at least 3 months after transplantation and form the basis of this analysis. The minimum follow-up period was 13 months (median, 41 months). Operative techniques[14-16] and immunosuppression protocols[17] have been reported. Cytomegalovirus prophylaxis was used only in recipients who were cytomegalovirus seronegative and who received allografts from seropositive donors. From July 1989 to August 1991 (14 patients), prophylaxis was given in the form of high-dose oral acyclovir or a 2- to 3-week course of intravenous ganciclovir. After August 1991, cytomegalovirus prophylaxis was given as a 16-week course of intravenous ganciclovir.

Table II. *Cytomegalovirus (CMV) status and HLA antibody development after transplantation and development of BOS*

Variable (n, %)	*Group I (free of BOS; n = 121)*	*Group 2 (with BOS; n = 180)*	*Significance*
CMVD+ to CMVR–	28 (23)	46 (26)	$P = .63$
PRA– to PRA+	14 (14)*	30 (19)†	$P = .12$
PRA– to early PRA+	9 (9)	20 (13)	$P = .32$

Direct comparisons made by Fisher's exact test.
*PRA data available in 100 patients.
†PRA data available in 158 patients.

Definition of BOS. The diagnosis of BOS was based on pulmonary function data and histologic evidence from lung biopsy results after transplantation with criteria established by the 1993 International Society for Heart and Lung Transplantation report.[1] (1) Recipients were considered at risk for BOS only if they survived 90 days after transplantation. (2) Recipients were determined to be free of BOS (group 1) if their forced expiratory volume in 1 second remained at 80% or more of their posttransplantation baseline value and if there was no histologic evidence of obliterative bronchiolitis. (3) Patients were defined as having BOS (group 2) if their forced expiratory volume in 1 second fell persistently below 80% of their posttransplantation baseline value and/or if obliterative bronchiolitis was documented histologically. (4) Patients were defined as having "early" BOS (group 3) if the preceding BOS criteria were met within 3 years after transplantation.

Before the diagnosis of BOS was applied, other specific causes for declining pulmonary function (such as graft infection and bronchial anastomotic stricture) were ruled out.

Variables analyzed. The following variables were analyzed. (1) The effect of pre-operative cytomegalovirus mismatch (patients who were cytomegalovirus seronegative and who received allografts from donors who were cytomegalovirus seropositive were compared with all other combinations collectively [ie, cytomegalovirus seronegative recipient and donor, cytomegalovirus seropositive recipient and seronegative donor, cytomegalovirus seropositive recipient and donor]). (2) The presence of lymphocytotoxic HLA antibodies was determined in the HLA laboratory with the use of a panel of T and B lymphocytes from 36 random individuals of known–HLA type (panel reactive antibody test [PRA]). The presence of HLA antibody was determined by using the Amos-modified and antiglobulin-augmented microcytotoxicity assays. The existence of HLA antibodies was sought before transplantation and after transplantation. PRA was considered positive before transplantation when there was cytotoxicity to any cells in the panel. PRA was considered positive after transplantation when there was cytotoxicity

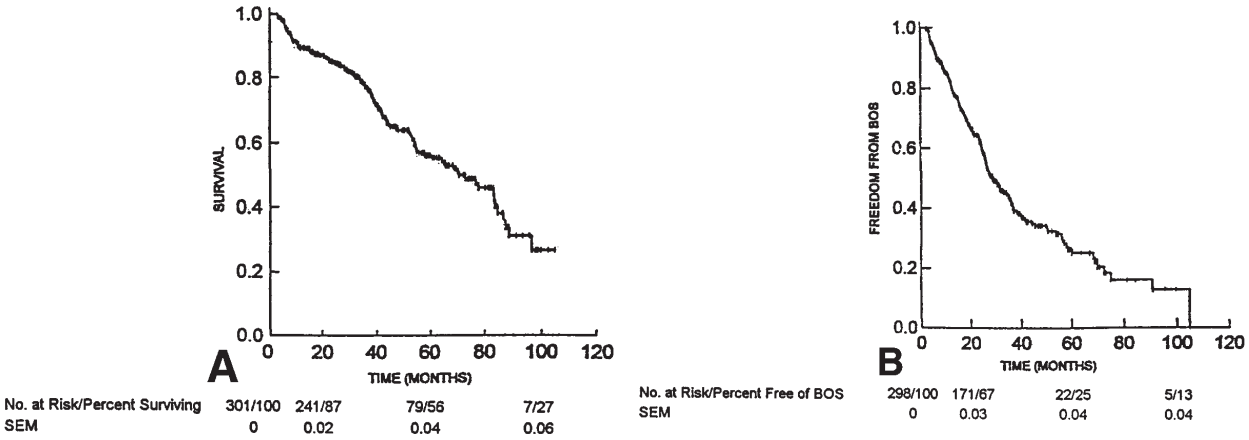


Fig 1. A, Actuarial survival scale for the 301 evaluable lung transplant recipients in this study. **B**, Actuarial freedom from BOS for the 298 evaluable lung transplant recipients in this study.

Table III. *Cytomegalovirus (CMV) status and HLA antibody development after transplantation and development of BOS*

Variable (n, %)	*Group 1 (free of BOS) (n = 121)**	*Group 3 (with early BOS) (n = 146)†*	*Significance*
CMVD+ to CMVR–	28 (23)	44 (30)	*P* = .08
PRA– to PRA+	14 (14)*	26 (20)†	*P* = .09
PRA– to early PRA+	9 (9)	19 (15)	*P* = .12

Direct comparisons made by Fisher's exact test.
*PRA data available in 100 patients.
†PRA data available in 130 patients.

to 10% or more of the cells in the panel. Recipients in whom cytotoxicity developed to 10% or more of the cells in the panel within 2 years of transplantation were defined as having early development of HLA antibodies.

Data analysis. The available data were first subjected to direct comparisons between groups 1 and 2 and groups 1 and 3 with the Fisher's exact test. Actuarial freedom from BOS and actuarial survival were determined by the Kaplan-Meier method[18] and comparisons made with the Mantel-Haenszel test.

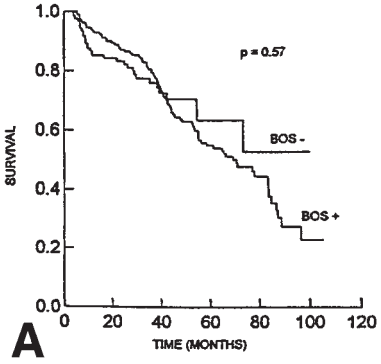
Results

Patient population and survival. Of 339 lung transplantations, the 301 patients (89%) who survived at least 3 months after the transplantation form the basis of this study. The demographic and clinical data are shown in Table I. The duration of survival was as follows: range, 3.9 to 104.8 months; mean, 43.6 months; and median, 40.7 months.

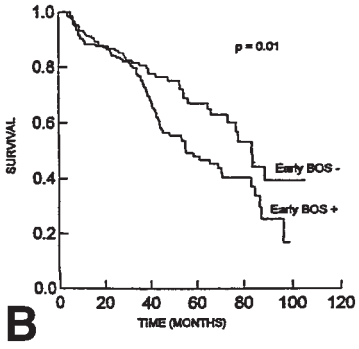
Comparison of variables between groups. Sera from both before and after transplantation were available (for determination of HLA antibody status) on 258 of the 301 patients evaluated (86%). The number of posttransplantation serum samples varied from 1 to 34 per patient. These serum samples were obtained over intervals ranging from 1 to 73 months after transplantation. Direct comparisons were made between cytomegalovirus status and development of HLA antibodies overall and within 2 years of transplantation between each of the patient groups (Tables II and III). There were trends toward greater proportions of patients with cytomegalovirus-D+ to cytomegalovirus-R– and the development of HLA antibodies in patients with early BOS (group 3) compared with those patients free of BOS (group 1), which did not reach significance.

Kaplan-Meier analysis. Overall survival of the study population was 78%, 55%, and 38% at 3, 5, and 7 years after lung transplantation, respectively (Fig 1, *A*). Actuarial freedom from BOS was 42%, 25%, and 16% over the same time intervals (Fig 1, *B*). Freedom from BOS steadily declined over time so that no patient was free of BOS at the latest follow-up interval (105 months; Fig 1, *B*). There was a trend toward worse late survival for patients with BOS compared with those patients without (Fig 2, *A*; *P* = .57). Because there appeared to be a divergence in survival curves within the first 3 years, we further stratified patients with BOS into a subgroup in which BOS developed less than 3 years after transplantation (group 3). On the basis of this stratification, we identified significantly worse survivals in patients who experienced early BOS onset compared with those patients who did not (Fig 2, *B*; *P* = .01).

The development of HLA antibodies after transplan-



BOS-: No. at Risk/Percent Surviving	121/99	94/84	19/63	6/53
SEM	0.01	0.03	0.07	0.11
BOS+: No. at Risk/Percent Surviving	180/99	156/89	62/55	6/23
SEM	0.01	0.02	0.04	0.06



Early BOS-: No. at Risk/Percent Surviving	153/99	126/88	40/67	9/39
SEM	0.01	0.03	0.05	0.09
Early BOS+: No. at Risk/Percent Surviving	148/99	124/86	40/48	3/17
SEM	0.01	0.03	0.05	0.08

Fig 2. A, Actuarial survival scale based on the presence (*BOS+*) or absence *(BOS-*) of BOS. **B**, Actuarial survival scale based on the presence (*Early BOS+*) or absence (*Early BOS–*) of BOS onset within 3 years of the transplantation.

tation correlated with the earlier development of BOS (Fig 3, *A*; *P* = .02). The development of HLA antibodies overall was not associated with significantly worse survivals (Fig 3, *B*). Because the greatest impact of antibody development on BOS appeared to be in the first 24 months, we further stratified antibody producers into those patients in whom antibodies developed within 2 years of transplantation and those patients in whom development did not occur within 2 years of transplantation. On this basis, there was a significant association between the early development of HLA antibodies and worse survivals (Fig 3, *C*; *P* = .04).

Recipients who were cytomegalovirus seronegative and who received allografts from seropositive donors showed a trend toward greater development of BOS overall (Fig 4, *A*; *P* = .16) and also early BOS (Fig 4, *B*; *P* = .02). Cytomegalovirus mismatched patients also showed a trend toward poorer survivals, which was not significant (Fig 4, *C*; *P* = .13).

Discussion

BOS remains the most significant long-term complication after lung transplantation.[1,2] This chronic allograft dysfunction is poorly understood, inadequately treated, and, in most cases, fatal. In an attempt to confirm previous observations that development of BOS correlates with poor survival,[2] we looked at actuarial survivals of lung transplant recipients who had BOS compared with those who did not. Surprisingly, there was no significant difference in long-term survival between the 2 groups. In fact, at close examination, it appears that the patients free of BOS actually fared worse in terms of survival at follow-up periods of less than 36 months (Fig 2, *A*). Examination of the cause of death in these subjects with short follow-up periods revealed many early deaths secondary to infection and malignancy (data not shown), likely resulting from immunosuppression. We therefore analyzed the effect of development of BOS within 36 months of transplantation on survivals and found that early BOS correlates with decreased survival. This concurs with the findings of Kroshus and associates[13] who observed that greater declines in cumulative survival were found in patients in whom BOS developed early compared with those in whom BOS developed later. This observation by this laboratory and others[13] that early development of BOS correlates with earlier death after transplantation supports previous observations that development of BOS is the most important limiting factor in the long-term success of lung transplantation. In addition, this finding implies that there may be certain markers that may herald the onset of BOS and that their detection can allow closer vigilance, new and earlier treatment methods, and potential avoidance of BOS.

A previous retrospective analysis has shown that the prevalence of BOS approaches and possibly exceeds 50% in long-term survivors.[2] In the current report, with a larger number of patients and longer follow-up time, we observed a steady decline to zero in the proportion of patients who were free of BOS. These discouraging data suggest that the development of BOS is virtually inevitable in all lung-transplant recipients who survive long enough.

Previous investigators have identified and studied several potential risk factors for the development of BOS, including frequent or severe early acute rejection,[3-5] cytomegalovirus infection,[6-9] class I HLA mis-

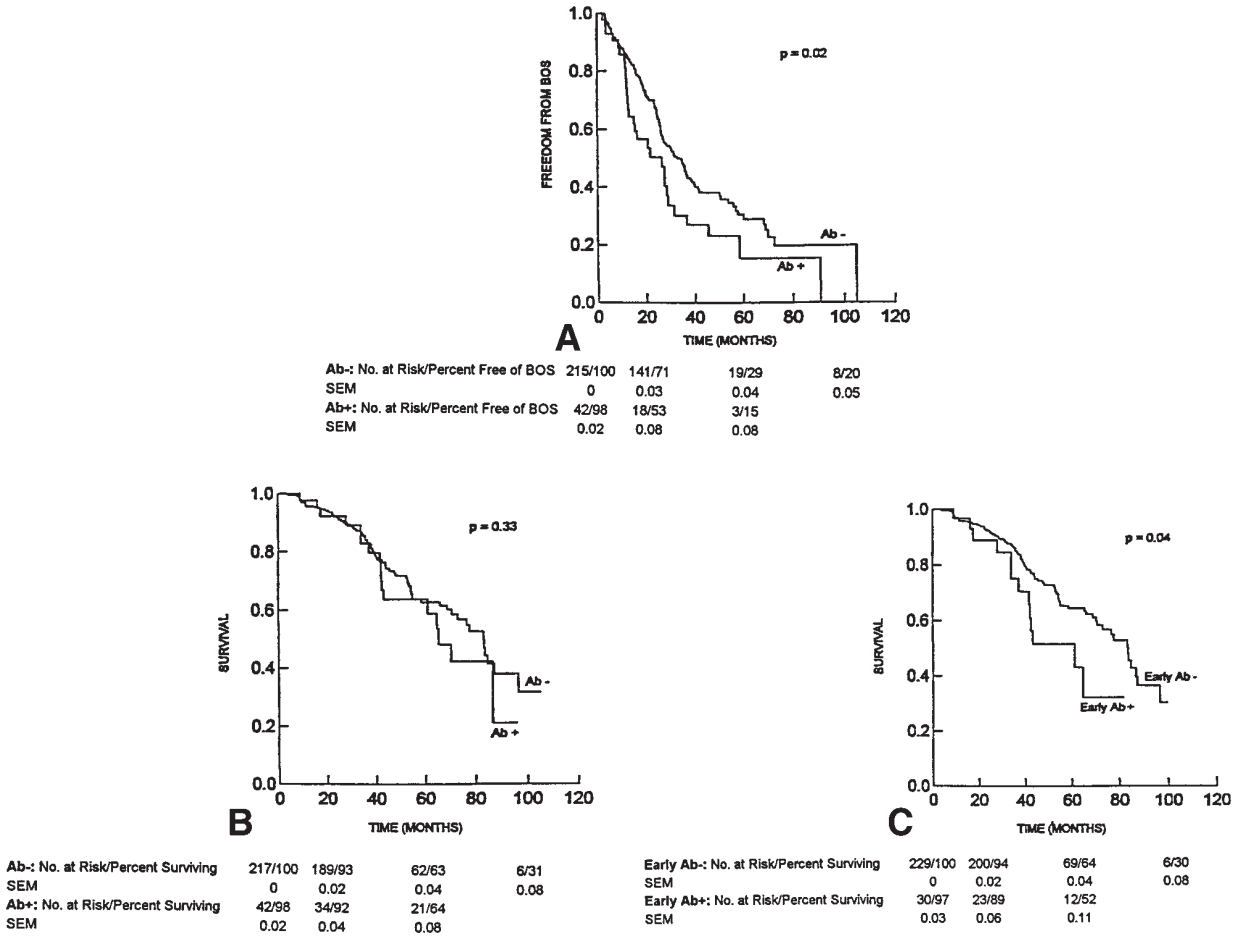


Fig 3. A, Actuarial freedom from BOS based on the presence (*Ab+*) or absence (*Ab–*) of HLA antibodies developed after transplantation. **B**, Actuarial survival scale based on the presence (*Ab+*) or absence (*Ab–*) of HLA antibodies developed after transplantation. **C**, Actuarial survival scale based on the presence (*Early Ab+*) or absence (*Early Ab–*) of HLA antibodies developed within 2 years of the transplantation.

matches,[10-13] and development of antibodies to class I HLA.[11]

Cytomegalovirus has long been implicated as a risk factor for the development of BOS and poor survival.[6-10] Many investigators believe that the greatest risk for cytomegalovirus-related complications after lung transplantation lies with patients who are cytomegalovirus seronegative and who receive allografts from donors who are cytomegalovirus seropositive.[9] In this study, we found that this group of patients was more likely to experience the development of BOS within 3 years of transplantation. In addition, there was a trend toward less favorable long-term survival in these patients, which was not significant. The current data are somewhat discrepant from our previous report that showed no correlation between cytomegalovirus infection and the development of BOS.[19] The apparent discrepancy may be explained by the larger sample size, longer follow-up period, and examination of patients with early development of BOS as a separate group in this study. Further study is needed to identify the true risk of cytomegalovirus on the development of BOS. The mechanism by which cytomegalovirus may play a role in the development of BOS is speculative. Associations between cytomegalovirus infection, increased donor-specific alloreactivity, and the development of BOS have been demonstrated.[7] It has also been shown that bronchoalveolar lavage, but not peripheral blood lymphocytes, are capable of specific proliferation to cytomegalovirus with an accumulation of cytomegalovirus-primed lymphocytes within the graft for months.[20] One potential mechanism for the association

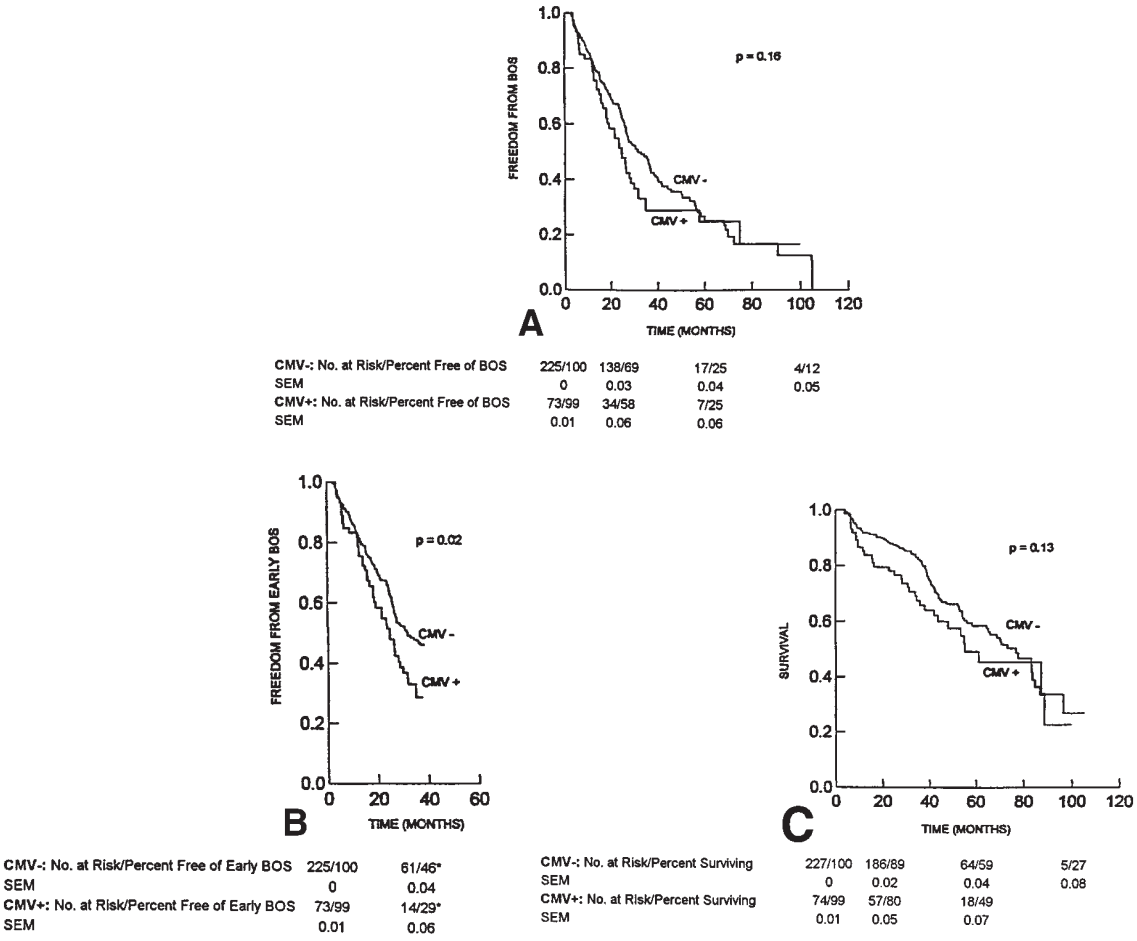


Fig 4. A, Actuarial freedom from BOS based on the presence (*CMV+*) or absence (*CMV–*) of a patient who is seronegative and who is receiving an allograft from a seropositive donor. **B**, Freedom from BOS within 3 years of transplantation (*Early BOS*) based on the presence (*CMV+*) or absence (*CMV–*) of a patient who is seronegative and who is receiving an allograft from a seropositive donor. **C**, Actuarial survival scale based on the presence (*CMV+*) or absence (*CMV–*) of a patient who is seronegative and who is receiving an allograft from a seropositive donor. *Representative data at 36 months.

of cytomegalovirus infection with BOS is immunomodulation of the graft milieu by inducing increased production of inflammatory cytokines. This idea is supported by findings that cytomegalovirus can increase expression of IFN-γ, TNF-α, IL-4, and IL-6.[21] It has also been demonstrated that cytomegalovirus can increase HLA class I and intercellular adhesion molecule I expression on airway epithelial cells,[22] thereby making them more immunogenic.

In the present study we show that the development of HLA antibodies correlates with earlier development of BOS. Further, we determined that development of HLA antibodies within 2 years of transplantation correlated with less favorable survivals. The association between chronic allograft rejection and the posttransplantation development of HLA antibodies has been made by this laboratory in lung transplantation[11] and by other investigators in heart[23] and kidney[24] transplantation. Whether these antibodies are simply a marker of the immune response or whether they play a direct role in the development of chronic allograft rejection is unclear. The finding that HLA-A locus mismatches have also been shown to correlate with the development of BOS in lung transplant recipients[11] makes it feasible that these antibodies are against donor HLA and that they do play a direct role in the development of chronic allograft rejection. Although acute cellular rejection may be a TH1 response resulting in T-lymphocyte activation, there may also be a TH2 response with subsequent B-lymphocyte activation and

alloantibody production. This laboratory has previously shown that class I soluble HLA levels in bronchoalveolar lavage fluid from lung transplant recipients increase during acute rejection.[25] In addition, other investigators have found elevated levels of soluble HLA in the periphery of heart, liver, and kidney allograft recipients.[26] Therefore the source of antigen for this alloantibody production may be soluble HLA in the peripheral circulation and the allograft itself. Recipients who share at least 1 class II HLA antigen can participate in indirect antigen presentation by donor epithelial cells. Although this seems feasible, to date there is no evidence that epithelial cells with upregulated HLA class II expression can act as antigen-presenting cells. It is more conceivable that indirect alloantigen presentation by recipient macrophages within the lung allograft may occur, leading to TH2 stimulation and subsequent alloantibody production.

The specific cascade of events that follow alloantibody production outside of hyperacute rejection is speculative. Whether the antibodies remain in the circulation in the form of immune complexes or bind to the allograft leading to cytokine release and/or proliferation of the bronchial epithelium and subepithelial components of the airway walls is unknown. Saint Martin and associates[27] reported on 2 patients with failing pulmonary function early after transplantation that responded to plasma exchange; however, subsequent study failed to demonstrate antibody binding to allograft tissue in a lung transplant population that included patients with evidence of acute and chronic rejection. Failure to show antibody binding to any of the lung allografts in this study could have resulted from sampling error, lack of sensitivity of the test used to detect the antibodies, and inadequate sample size of patients with chronic rejection. Further investigation is needed to determine whether alloantibodies bind to lung allografts and what role they may play in the development of BOS.

In summary, this study has demonstrated (1) the development of BOS appears to be an inevitable long-term complication of lung transplantation, (2) there is a significant correlation between the development of HLA antibodies on the development of BOS and poor survival, and (3) there is a significant correlation between unfavorable cytomegalovirus matching and the development of early BOS.

We thank Mary S. Pohl, RN, BSN, MHS, and Tracey Guthrie, RN, BSN, for their assistance with data collection and Richard B. Schuessler, PhD, for his help with the statistical analysis.

REFERENCES

1. Cooper JD, Billingham M, Egan T, Hertz MI, Higenbottam T, Lynch J, et al. A working formulation for the standardization of nomenclature and for clinical staging of chronic dysfunction in lung allografts. International Society for Heart and Lung Transplantation. J Heart Lung Transplant 1993;12:713-6.
2. Sundaresan S, Trulock EP, Mohanakumar T, Cooper JD, Patterson GA. Prevalence and outcome of bronchiolitis obliterans syndrome after lung transplantation. Washington University Lung Transplant Group. Ann Thorac Surg 1995;60:1341-6.
3. Rabinowich H, Zeevi A, Paradis IL, Yousem SA, Dauber JH, Kormos R, et al. Proliferative responses of bronchoalveolar lavage lymphocytes from heart-lung transplant patients. Transplantation 1990;49:115-21.
4. Scott JP, Higenbottam TW, Sharples L, Clelland CA, Smyth RL, Stewart S, et al. Risk factors for obliterative bronchiolitis in heart-lung transplant recipients. Transplantation 1991;51:813-7.
5. Yousem SA, Dauber JA, Keenan R, Paradis IL, Zeevi A, Griffith BP. Does histologic acute rejection in lung allografts predict the development of bronchiolitis obliterans? Transplantation 1991; 52:306-9.
6. Burke CM, Glanville AR, Macoviak JA, O'Connell BM, Tazelaar HD, Baldwin JC, et al. The spectrum of cytomegalovirus infection following human heart-lung transplantation. J Heart Transplant 1986;5:267-72.
7. Keenan RJ, Lega ME, Dummer JS, Paradis IL, Dauber JH, Rabinowich H, et al. Cytomegalovirus serologic status and postoperative infection correlated with risk of developing chronic rejection after pulmonary transplantation. Transplantation 1991; 51:433-8.
8. Duncan SR, Paradis IL, Yousem SA, Similo SL, Grgurich WF, Williams PA, et al. Sequelae of cytomegalovirus pulmonary infections in lung allograft recipients. Am Rev Respir Dis 1992; 146:1419-25.
9. Duncan AJ, Dummer JS, Paradis IL, Dauber JH, Yousem SA, Zenati MA, et al. Cytomegalovirus infection and survival in lung transplant recipients. Heart Lung Transplant 1991;10:638-44.
10. Harjula AL, Baldwin JC, Glanville AR, Tazelaar H, Oyer PE, Stinson EB, Shumway N. Human leukocyte antigen compatibility in heart-lung transplantation. J Heart Transplant 1987;6:162-6.
11. Sundaresan S, Mohanakumar T, Smith MA, Trulock EP, Lynch J, Phelan D, et al. HLA-A locus mismatches and development of antibodies to HLA after lung transplantation correlate with the development of bronchiolitis obliterans syndrome. Transplantation 1998;65:648-53.
12. Wisser W, Wekerle T, Zlabinger G, Senbaclavaci O, Zuckermann A, Klepetko W, et al. Influence of human leukocyte antigen matching on long-term outcome after lung transplantation. J Heart Lung Transplant 1996;15:1209-16.
13. Kroshus TJ, Kshettry VR, Savik K, John R, Hertz MI, Bolman RM III. Risk factors for the development of bronchiolitis obliterans syndrome after lung transplantation. J Thorac Cardiovasc Surg 1997;114:195-202.
14. Sundaresan S, Trachiotis GD, Aoe M, Patterson GA, Cooper JD. Donor lung procurement: assessment and operative technique. Ann Thorac Surg 1993;56:1409-13.
15. Patterson GA, Cooper JD, Goldman B, Weisel RD, Pearson FG, Waters PF, et al. Technique of successful clinical double-lung transplantation. Ann Thorac Surg 1988;45:626-33.
16. Cooper JD, Pearson FG, Patterson GA, Todd TR, Ginsberg RJ,

Goldberg M, et al. Technique of successful lung transplantation in humans. J Thorac Cardiovasc Surg 1987;93:173-81.
17. Trulock EP. Management of lung transplant rejection. Chest 1993;103:1566-76.
18. Kaplan EL, Meier EP. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457-81.
19. Ettinger NA, Bailey TC, Trulock EP, Storch GA, Anderson D, Raab S, et al. Cytomegalovirus infection and pneumonitis: impact after isolated lung transplantation. Washington University Lung Transplant Group. Am Rev Respir Dis 1993;147:1017-23.
20. Zeevi A, Uknis ME, Spichty KJ, Tector M, Keenan RJ, Rinaldo C, et al. Proliferation of cytomegalovirus-primed lymphocytes in bronchoalveolar lavages from lung transplant patients. Transplantation 1992;54:635-9.
21. Geist LJ, Dai LY. Cytomegalovirus modulates interleukin-6 gene expression. Transplantation 1996;62:653-8.
22. Ibrahim L, Dominguez M, Yacoub M. Primary human adult lung epithelial cells in vitro: response to interferon-gamma and cytomegalovirus. Immunology 1993;79:119-24.
23. Suciu-Foca N, Reed E, Marboe C, Harris P, Yu PX, Sun YK, et al. The role of anti-HLA antibodies in heart transplantation. Transplantation 1991;51:716-24.
24. Barr ML, Cohen DJ, Benvenisty AI, Hardy M, Reemtsma K, Rose EA, et al. Effect of anti-HLA antibodies on the long-term survival of heart and kidney allografts. Transplant Proc 1993; 25:262-4.
25. Rizzo M, Sundaresan S, Lynch J, Trulock EP, Cooper J, Patterson GA, et al. Increased concentration of soluble human leukocyte antigen class I levels in the bronchoalveolar lavage of human pulmonary allografts. J Heart Lung Transplant 1997;16:1135-40.
26. Puppo F, Scudeletti M, Indiveri F, Ferrone S. Serum HLA class I antigens: markers and modulators of an immune response? Immunol Today 1995;16:124-7.
27. Saint Martin GA, Reddy VB, Garrity ER, Simpson K, Robinson JA, Adent JK, et al. Humoral (antibody-mediated) rejection in lung transplantation. J Heart Lung Transplant 1996;15:1217-22.

Discussion

Dr R. Morton Bolman III *(Minneapolis, Minn).* The Washington University group has made many contributions to our understanding of lung transplantation and continue to be leaders in our field. This report is a landmark study of the risk factors and the prevalence of BOS after lung transplantation. Specifically, the investigation examines 2 risk factors: cytomegalovirus donor-recipient mismatch and the development of posttransplantation HLA antibodies and the subsequent development of BOS.

Several interesting findings emerge. First, and most discouraging, if followed long enough, BOS is likely to develop in all recipients of lung allografts. Second, cytomegalovirus-negative recipients of cytomegalovirus-positive donors experience a higher incidence of early BOS, occurring within 3 years of transplantation as defined by the authors. Third, the development of posttransplant HLA antibodies portends earlier development of BOS and, if it occurs in the first 2 years, it is associated with decreased survival. All this confirms what others have shown, namely, that BOS represents an immunologic process consisting of some combination of injury related to antigen-antibody deposition, cytokine release, cell-mediated injury, and ultimately, fibrosis.

We have also been interested in delineating the factors for development of BOS after lung transplantation. Tim Kroshus of our group published our data in *The Journal of Thoracic and Cardiovascular Surgery* in August of 1997 (1997;114: 195-202). Somewhat in contrast to the study presented today, we found cytomegalovirus pneumonitis, late acute rejection, and HLA mismatch at the A locus to be statistical predictors of BOS by multivariate analysis. Interestingly, asymptomatic cytomegalovirus had no association with increased BOS in our data.

Furthermore, in our cohort, BOS was a statistical predictor of worse survival regardless of when it appeared after transplantation. Again, this is somewhat in contrast to your data.

I have 3 questions. First, in the article you did not mention the incidence of cytomegalovirus disease in the recipients. Is there any correlation between cytomegalovirus disease, rather than merely recipient-donor mismatch, and the subsequent development of BOS?

Second, did you study the relationship of the incidence of acute graft rejection to the development of BOS? Several groups, including ours, have found a correlation between these entities.

Third, and I think most provocative, the incidence of chronic rejection after lung transplantation is by far the highest of any solid-organ recipients. The notion of immunologic markers for the development of BOS has been very intriguing to us, and Nancy Reinsmoen, formerly of our center, developed the concept of donor antigen-specific hyporeactivity, which is a long phrase, that really tests for a specific recipient's reaction to his or her specific donor. Nancy developed a test for this and, in a small subpopulation of our transplant recipients, found that those who at 1 year or later after transplantation had no reaction to their donor did not experience obliterative bronchiolitis, at least up to 2 years.

These data prompted us to consider modifying the immunosuppression of our patients based on these markers. Have you considered any modification of immunosuppression based on the data you showed, demonstrating that patients who experience the development of HLA antibodies, especially early after transplantation, not only experience the development of BOS earlier but also have a decreased survival?

Dr Smith. Let me start with the third question. At this time we are investigating the mechanism by which HLA antibody and/or cytomegalovirus leads to the development of BOS. We have not made definitive steps toward using this information clinically. At some point the data regarding development of HLA antibodies may prove useful in treating patients prophylactically for the development of BOS. However, currently patients are treated preemptively with increased immunosuppression only when pulmonary function begins to decline significantly. There are no other laboratory tests that are being used at this time to identify these patients and make decisions regarding the management of their conditions.

To answer the questions regarding cytomegalovirus dis-

ease, we only looked at mismatch status in these patients. The Washington University Lung Transplant Group has previously published an analysis of the relationship between cytomegalovirus pneumonitis and BOS, which showed that there was no statistically significant difference in the development of BOS between patients in whom cytomegalovirus pneumonitis developed and those in whom it did not. However, that study analyzed a much smaller patient population with a shorter follow-up period. At this point we have not looked at cytomegalovirus disease in the current study population. It is assumed, based on data from previous analyses, that patients who are cytomegalovirus negative who receive allografts from donors who are cytomegalovirus positive have a higher incidence of cytomegalovirus-related complications.

This study also did not analyze the incidence of acute rejection and its effect on the development of BOS.

Dr Douglas J. Mathisen *(Boston, Mass).* In your analysis of these patients, were there any patients who underwent retransplantation, and if any, did you factor that into your survival analysis and did it have any impact on that?

Dr Smith. There were only 6 patients who underwent retransplantation of the 339 patients in the study. The survival data in this study refer to survival of the allograft. Both deaths and retransplantations were considered failures when the Kaplan-Meier estimations were computed.

Dr Axel Haverich *(Hannover, Germany).* This is an important yet not very optimistic study, because, like in all other single-center series, the incidence of obliterative bronchiolitis is still very, very high.

Maybe I missed it in your method section when you described the way you assessed the HLA antibodies. Were these antibodies always donor specific? I am asking this question because there is a growing body of evidence that blood transfusions at the time of transplantation in other solid organs may have a significant impact on long-term graft survivals in kidney transplantation, for instance; this is data from Germany. So were those donor-specific HLA antibodies or not?

Do you have a way to assess obliterative bronchiolitis during the entire course of this study? Was that a constant way, did you perform biopsies all the time, or were the functional parameters the constant factor over time?

Dr Smith. The way that these antibodies were determined in the sera was by panel-reactive antibody testing, which is a microcytotoxicity analysis in which the sera is incubated with a panel of T and B cells from normal individuals with known HLA types in the presence of complement. The proportion of lymphocytes within the panel that are lysed determines the percent of the HLA types represented in the panel for which the recipient has cytotoxic antibodies. This test does not determine whether these antibodies are donor specific. However, we do have a smaller group of patients that we have analyzed for HLA antibodies determined by ELISA. Using homozygous typing cells, we are seeing that these antibodies are reactive to donor HLA. In addition, there is some cross-reactivity with other HLA alleles.

To answer your second question, these patients are followed with pulmonary function testing on a routine basis throughout the posttransplantation period. In addition, fiberoptic bronchoscopy is performed liberally in the immediate postoperative period. Subsequently, surveillance bronchoscopy with transbronchial biopsy is performed at 3 to 4 weeks, at around 3 months, at 1 year, and then annually thereafter. Patients will also undergo bronchoscopy whenever there are clinical indications. There were few patients in whom histologic obliterative bronchiolitis developed without any evidence of a decline in pulmonary function.