Cytolytic Therapy for the Bronchiolitis Obliterans Syndrome Complicating Lung Transplantation*

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The bronchiolitis obliterans syndrome (BOS) is the major cause of late morbidity and mortality after lung transplantation (LTx). Previous studies suggest cytolytic therapy may be effective for the BOS but this therapy has not been proved effective or safe.

**Method:** A retrospective study of a predetermined treatment regimen to determine if the rate of fall in FEV₁ can be reduced by corticosteroids and cytolytic therapy. Since August 1992, 10 of 65 long-term survivors of LTx (5 men, 5 women; mean age 36±10 years) developed BOS. All had previously had lymphocyte immune globulin, antithymocyte globulin (equine) (ATGAM sterile solution; Upjohn Pty Ltd; Sydney, Australia) induction therapy and corticosteroid avoidance for the first 7 to 10 days post-LTx. Therapy for the BOS was initiated with pulse methylprednisolone and ATGAM (aiming for an absolute CD3 count of ≤100 cells per microliter for 5 days). ATGAM therapy was initiated at a mean 657±323 days post-LTx. Subsequent follow-up has been for 310±110 days (range, 163 to 530 days).

**Results:** Nine of ten patients had a response with tolerable side effects. Preintervention, there was a linear fall in FEV₁ of 0.22±0.15% predicted FEV₁ per day (mean±SD) (range, 0.06 to 0.56%) compared with a postintervention linear fall of 0.036±0.019% predicted per day (range, 0 to 0.13%) (paired t test; p<0.005). This effect is sustained over the follow-up period.

**Conclusion:** The fall off in FEV₁ that characterizes the BOS may be altered usefully by augmented immunotherapy. This effect can be rapid and sustained although it is neither completely arrested nor ever reversed. These data are preliminary but encourage a randomized control trial in the BOS.

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**BOS=bronchiolitis obliterans syndrome; CMV=cytomegalovirus; LTx=lung transplantation**

**Key words:** bronchiolitis obliterans; cytolytic therapy; lung transplantation

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Lung transplantation (LTx) has become a successful therapeutic alternative for patients with end-stage pulmonary parenchymal or vascular disease. Survival to hospital discharge is expected for between 70% and 90% of lung transplant recipients.¹ The primary factor determining outcome in long-term survivors is the presence or absence of the bronchiolitis obliterans syndrome (BOS).²³ This syndrome is one of progressive airflow obstruction usually in the presence of histologic evidence of bronchiolitis obliterans. A diagnosis of the BOS requires the absence of other causes of airflow obstruction such as anastomotic stricture, bronchomalacia, infection, or acute rejection. The bulk of evidence suggests that the BOS is an immunologically mediated airway injury.²⁴ However, other processes, including cytomegalovirus (CMV) pneumonitis,⁵⁶ and airway ischemia² are risk factors for the development of the BOS, suggesting other possible mechanisms of airway damage. This process seems analogous to clinical and pathologic forms of chronic rejection that have been recognized in other solid organ transplants.³⁷

The rate of deterioration in lung function with the BOS is variable.² The conditions of some patients steadily decline while others eventually stabilize at a lower level of function. It is not known what factors can influence the course a recipient will follow, nor is it clear whether any intervention converts one curve to the other.

Strategies to avoid the development of the BOS are not of proved benefit, although it is generally agreed that attempts should be made to minimize recurrent bouts of acute rejection, CMV pneumonitis, and airway ischemia.²⁴ Successful therapy for progressive airflow obstruction due to the BOS is not well documented.²⁹ Suggested treatments include azathioprine,¹⁰ bolus corticosteroids,¹¹ corticosteroids and cytolytics,¹² and total lymphoid irradiation.² Retransplantation is the final surgical response to the problem.¹²¹³ Although

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there is some information on mortality, the morbidity and practical difficulties of such therapies are poorly documented.

There is an almost universal corticosteroid response in acute lung allograft rejection. Conversely, corticosteroid resistance is a common clinical problem in patients with the BOS.9 There is some experimental evidence to reinforce these observations. Yousem et al14 noted an increased percentage of lymphocytes with C494 antigens in transbronchial biopsy specimens of the BOS compared with biopsy specimens of acute allograft rejection. The C494 antigen has been linked previously to corticosteroid resistance in other organ transplant settings. Reinsmoen and coworkers,15 showed that lymphocytes from recipients with acute rejection will express different donor antigen-specific reactivity compared to those with the BOS, suggesting treatment for each of these conditions may need to target different lymphocyte subpopulations.

To overcome corticosteroid resistance in the BOS, a therapeutic combination of corticosteroids and cytolytics has been suggested.1,2 Corticosteroids have anti-inflammatory properties and suppress activated macrophages. Cytolytics are lymphocyte-selective immunosuppressants. One example of a cytolytic agent is a T-cell-specific immunosuppressant IgG derived from hyperimmune serum of horses immunized with human thymus lymphocytes (ATGAM sterile solution; Upjohn Pty Ltd; Sydney, Australia). A combination regimen of pulsed corticosteroids and ATGAM may be an effective therapy for the BOS because it may simultaneously target the broad inflammatory pathways and multiple T-cell subpopulations.

This article details the retrospective analysis of a predetermined and consistent approach to the clinical problem of the BOS. The aim was to determine if corticosteroid and cytolytic therapy could reduce the rate of fall in FEV1 seen in the BOS.

MATERIALS AND METHODS

Patient Population

Between March 1990 and March 1994, the Alfred Hospital Heart and Lung Transplant Service performed 82 heart-lung and lung transplants with an overall actuarial survival at 1 year of 77% and at 3 years of 62%. This includes 35 heart-lung, 24 single lung, and 23 double lung transplants. Since August 1992, 10 of 65 long-term survivors of lung transplantation (survived ≥30 days postoperatively) have developed the BOS and required treatment for this problem. Three other single lung transplant recipients developed the BOS prior to this date but did not receive specific immunosuppressive augmentation and are not included. One of these patients had recurrent pulmonary sepsis and ultimately died of this; another died of metabolic complications of a perforated ecum prior to BOS therapy. One recipient survives with significant bronchiolitis obliterans in the allograft but improved function in the native lung (the initial abnormality being severe usual interstitial pneumonia), thus aggressive further immunotherapy was not deemed indicated. Thus, from our transplant population, no control group is available.

Routine Immunosuppression and Surveillance

Immunosuppressive protocols were similar to those reported by other centers.1,16 Briefly, all recipients reported received cytolytic therapy with ATGAM for 7 to 10 days from the time of transplantation. Cyclosporine and azathioprine therapy was started at the same time and corticosteroids were then added from day 7. Maintenance triple therapy with cyclosporine (to achieve a blood level of 600 to 1,200 µg/L via TDX, polyclonal assay; Abbott Pty Ltd; Sydney, Australia), azathioprine (0.5 to 2 mg/kg/d), and prednisolone (maintenance, 0.15 mg/kg/d) continued thereafter. Patients diagnosed as having the BOS were maintained on a regimen of normal baseline levels of immunosuppression apart from a slightly increased oral corticosteroid dose (0.3 to 0.4 mg/kg/d prednisolone). Surveillance bronchoscopies were performed at 0.5, 1, 2, 3, 6, 9, 12, 18, and 24 months as well as yearly thereafter. Nonroutine bronchoscopies were performed for appropriate clinical indications.

Infection Prophylaxis

All patients received long-term prophylaxis for Pneumocystis carinii with low-dose oral trimethoprim-sulfamethoxazole or equivalent. CMV prophylaxis was undertaken with IV ganciclovir, 10 mg/kg/d, for 2 weeks followed by 5 mg/kg/d three times a week for 10 weeks in CMV serologically positive donors and/or recipients. Persistent colonization by Aspergillus species on routine BAL specimens was treated with oral itraconazole. No specific infection prophylaxis was undertaken at the time of enhanced steroid or ATGAM treatments other than IV ganciclovir, 10 mg/kg/d, in those with positive donor and/or recipient CMV serology (IgG).

Diagnosis and Therapy of the BOS

Since August 1992, treatment for the BOS has been undertaken in patients with a 20% or greater drop in predicted FEV1 in the absence of any other explanation. Fiberoptic bronchoscopy and transbronchial biopsy were performed in all patients to exclude acute rejection, infection, or airway stenosis. Cated cardiac scans at this time showed no evidence of left ventricular dysfunction. Therapy was initiated with pulse IV methylprednisolone (day 1, 1,000 mg; day 2, 500 mg; day 3, 500 mg) and an oral tapering dose of prednisolone (1 mg/kg/d tapering to baseline over 4 weeks). If this treatment failed to stabilize declining spirometry, ATGAM was administered. The exact timing of therapy depended on the clinical urgency and the coexistence of any other medical confounding problems, ie, an intercurrent chest infection. Appropriate informed consent was obtained from the subjects.

ATGAM dosages were maintained an absolute CD3 count below 100 cells per microliter for 5 days. The average starting dose was 500 mg. The average daily dose was 8.2 mg/kg/d for an average of 5.3 days. Promethazine was given routinely to cover any possible allergic side effects.

Gradients of the change in FEV1 were calculated using a computerized line of best fit of the graph of FEV1 vs time (Harvard Graphics version 3.0). The pre-ATGAM slope was derived from the best most recently stable FEV1 and the FEV1 at the time of ATGAM. The post-ATGAM slope was derived from the FEV1 at the time of ATGAM and the most recent FEV1. Statistical significance was accepted at p less than 0.05 level using the Wilcoxon rank sum test. Lung function was measured using a pneumotachograph (Jaeger) according to the guidelines of the American Thoracic Society.

RESULTS

Ten patients have been treated for the BOS (Table 1). Nine patients had a response (Fig 1 and 2; Table 2).
Table 1—Characteristics of Patients With the BOS*

<table>
<thead>
<tr>
<th>Patient/Age, yr/Sex</th>
<th>Underlying Disease</th>
<th>Type of Transplant</th>
<th>BOS Category at Time of ATGAM</th>
<th>Days Post-TX to ATGAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/51/F O</td>
<td>OB</td>
<td>HLTx</td>
<td>3a</td>
<td>1,152</td>
</tr>
<tr>
<td>2/22/M CF</td>
<td>HLTx</td>
<td>2a</td>
<td>1,002</td>
<td></td>
</tr>
<tr>
<td>3/22/M CF</td>
<td>HLTx</td>
<td>1a</td>
<td>942</td>
<td></td>
</tr>
<tr>
<td>4/44/F PPH</td>
<td>HLTx</td>
<td>2b</td>
<td>803</td>
<td></td>
</tr>
<tr>
<td>5/45/F EMPHYs</td>
<td>HLTx</td>
<td>2b</td>
<td>785</td>
<td></td>
</tr>
<tr>
<td>6/44/F EMPHYs</td>
<td>SLTx</td>
<td>2a</td>
<td>634</td>
<td></td>
</tr>
<tr>
<td>7/23/F PPH</td>
<td>HLTx</td>
<td>3b</td>
<td>477</td>
<td></td>
</tr>
<tr>
<td>8/40/M PPH</td>
<td>HLTx</td>
<td>2b</td>
<td>412</td>
<td></td>
</tr>
<tr>
<td>9/30/M CF</td>
<td>HLTx</td>
<td>2a</td>
<td>288</td>
<td></td>
</tr>
<tr>
<td>10/37/M EMPHYs</td>
<td>HLTx</td>
<td>2b</td>
<td>76</td>
<td></td>
</tr>
</tbody>
</table>

*OB=obstructive bronchiolitis; CF=cystic fibrosis; EMPHYs=emphysema; PPH=primary pulmonary hypertension; HLTx=heart-lung transplant; SLTx=single lung transplant; Tx=transplant.

1BOS category as per reference 3.

was an inverse association between the rate of the decline in lung function prior to ATGAM therapy and the time from transplant at which the decline occurred (p<0.05, R=0.7). The slope of the decline in lung function prior to ATGAM did not predict the slope after treatment. Subsequent follow-up has been for 310±110 days (range, 163 to 550 days) without significant alteration of the post-ATGAM slope, which continues to show a slow fall in lung function over time. At no time was significant acute rejection coincidentally evident on transbronchial biopsy specimen in this patient group.

Possible or definite viral or bacterial infections delayed a clear diagnosis of the BOS in four patients and treatment with ATGAM in three patients. A requirement to make a certain diagnosis of the BOS before committing to cytolytic therapy, plus a large degree of variability in the rate of fall off in FEV\textsubscript{1}, meant some recipients received therapy with a more advanced BOS category. Absolute levels of FEV\textsubscript{1} and the patient's subjective dyspnea influenced timing to a lesser degree.

Clinical outcomes and complications of therapy are listed in Table 3. No serious allergic or infective events directly followed the ATGAM. Patient 4 did not achieve the desired CD3 count with ATGAM and was

![Chart](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21730/ on 06/27/2017)
changed to a regimen of muromonab-CD3 (Ortho
clon OKT3; Janssen-Cilag Pty Ltd; Sydney, Australia).
Five months after treatment, patient 1 developed
mouth ulceration with adjacent lymphadenopathy.
Histologic features of the ulcer margin showed a lyn
phoproliferative disorder. This responded satisfac
torily to decreasing the baseline immunosuppression
and the patient remains well 12 months later with stable lung
function.

**DISCUSSION**

These results suggest the decline in lung function
associated with the BOS is able to be attenuated with
augmented immunotherapy with acceptable side ef
effects.

The time course of the change in slope of the FEV
with therapy suggests the ATGAM, rather than the corticosteroid, is probably the main contributor to a
response. There is only limited support for use of cor
ticosteroids alone in the literature. Paradis et al.12,17 and
Allen et al.11 report a response to corticosteroids alone
in the BOS, but the description is in only six patients
and one patient, respectively. Paradis et al.17 had an
initial favorable response in four of the six treated, but
three of these ultimately suffered relapses. Several
other commentators suggest advantageous effects, but
no details are supplied.2,18

Paradis et al.12 have reported the use of cytol
tic therapy for the BOS. Five different therapies (three
different cytolytics, corticosteroids, and total lymphoid
irradiation) were used for a total of 44 courses of
therapy to treat 16 patients with the BOS. The efficacy
of individual agents is impossible to discern from this.
This article reports that 12 patients ultimately achieved
long-lasting remissions, with 2 deaths in the 4 nonre
sponders. Morbidity data are not detailed but would
appear to be far greater than we have seen in our se
ries. Cooper et al.11 report one case showing a substan
tial improvement in lung function for a patient with
BOS who received eight augmented steroid courses
and two courses of cytolytics; again no morbidity data
are shown. Nathan and coworkers.18 describe two of
three patients with the BOS in whom lung function
stabilized with ATGAM therapy.

A response by the BOS to augmented immuno
erapy is consistent with the belief that the disease is immu
nologically mediated.2,4 The observation that the rate of drop off in FEV
with the BOS was inversely related to the time posttransplant is supportive of the hypo
thesis that the etiology of the BOS is an immu
nologic process initiated around the time of transplant.

The effect seen was slowing of the rate of fall in
FEV rather than complete stabilization or improve
ment. This limited response may be expected given the
typical extent of fibrosis and established damage seen
histologically with the BOS.19 In this context, the dra

**Table 2—Changes in Absolute Lung Function With
ATGAM (in Liters)**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Previous Stable FEV1</th>
<th>FEV1 at Time of ATGAM</th>
<th>Post-ATGAM Final Follow-up FEV1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.50</td>
<td>1.70</td>
<td>1.20</td>
</tr>
<tr>
<td>2</td>
<td>5.10</td>
<td>3.36</td>
<td>2.80</td>
</tr>
<tr>
<td>3</td>
<td>2.76</td>
<td>1.92</td>
<td>1.91</td>
</tr>
<tr>
<td>4</td>
<td>3.00</td>
<td>1.84</td>
<td>1.60</td>
</tr>
<tr>
<td>5</td>
<td>3.10</td>
<td>1.84</td>
<td>1.22</td>
</tr>
<tr>
<td>6</td>
<td>1.60</td>
<td>1.15</td>
<td>0.70</td>
</tr>
<tr>
<td>7</td>
<td>3.68</td>
<td>1.82</td>
<td>0.86</td>
</tr>
<tr>
<td>8</td>
<td>1.90</td>
<td>1.44</td>
<td>0.90</td>
</tr>
<tr>
<td>9</td>
<td>2.10</td>
<td>1.30</td>
<td>0.70</td>
</tr>
<tr>
<td>10</td>
<td>2.70</td>
<td>2.00</td>
<td>1.10</td>
</tr>
<tr>
<td>Mean</td>
<td>2.94</td>
<td>1.86</td>
<td>1.30</td>
</tr>
<tr>
<td>SD</td>
<td>1.01</td>
<td>0.60</td>
<td>0.71</td>
</tr>
</tbody>
</table>

*The previous stable FEV1 values are the average of values over the
previous 90 days of stability. ATGAM and post-ATGAM FEV1 readings are the average of two separate readings taken at those times.

**Table 3—Results of Treatment**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Therapy Complications</th>
<th>NYHA Status at Time of ATGAM</th>
<th>Final NYHA Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lymphoproliferative disease</td>
<td>II</td>
<td>I</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>4</td>
<td>CD3 levels too high, finished with OKT3</td>
<td>II</td>
<td>II</td>
</tr>
<tr>
<td>5</td>
<td>Exacerbation of bronchiectasis</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>III</td>
<td>III</td>
</tr>
<tr>
<td>7</td>
<td>Exacerbation of bronchiectasis</td>
<td>II</td>
<td>IV</td>
</tr>
<tr>
<td>8</td>
<td>No effect on FEV1, patient dies</td>
<td>II</td>
<td>NA</td>
</tr>
<tr>
<td>9</td>
<td>—</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>10</td>
<td>Low FEV1, underwent retransplantation</td>
<td>III</td>
<td>NA</td>
</tr>
</tbody>
</table>

*NYHA=New York Heart Association grading of physical status; NA=not applicable as patient/transplanted organ no longer alive; Rate of FEV1 fall=percentage predicted FEV1 fall per day.
matic response to steroid and cytolytic therapy in the case of the BOS described by Cooper et al is surprising.

These data appear to associate heart-lung transplants, rather than single or double lung transplants, with the development of the BOS. This is likely to relate to the historic perspective of having performed heart-lung transplants first and thereby having considerably greater follow-up with this group. Generally the incidence of the BOS is not seen as different between heart-lung and isolated lung transplants.9

This report has several important limitations. First, it was retrospectively analyzed and second it is possible that the patients may have spontaneously plateaued out around the point that cytolytic therapy was administered. Figure 1 suggests the latter conclusion is unlikely as the inflection point on the curve is clearly centered around the point of initiation of ATGAM therapy.

Notwithstanding these comments, the results do suggest that augmented immunotherapy (especially cytolytics) may have a role in the BOS. Furthermore, as the response observed is a reduced rate of decline in FEV1, rather than a complete restoration of function, it would appear appropriate to augment immunotherapy earlier in the course of the BOS. There is clearly a need for a randomized controlled trial (preferably multicentered) to quickly address this important issue. It is probable that improved outcomes from LTx will require very early detection of the BOS, or better still, prevention of the BOS with more effectively targeted immunosuppressive strategies.

REFERENCES