Immunologically Mediated Disease of the Airways after Pulmonary Transplantation

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Obliterative bronchiolitis has occurred in eleven of 30 recipients of cardiopulmonary allografts who survived at least 4 months after transplantation, has caused significant morbidity, and has been associated with four of eleven late deaths in this series. Although some improvement, or at least stability, of pulmonary function has followed augmented immune suppression, it appears that once the process is recognized clinically, much of the damage to the airways is irreversible. The histopathology, response to therapy, and, most important, the response of donor specific alloreactivity in the lymphocytes from the lung (bronchoalveolar lavage and peripheral blood) suggest immune- mediated basis for bronchiolitis obliterans. The presence of donor specific alloreactivity detected by primed lymphocyte testing predicted obliterative bronchiolitis in five of six recipients (83% sensitivity, 91% specificity) was absent in ten of eleven recipients who have not as yet developed the process (negative predicted value of 91%). Currently, the presence of a positive primed lymphocyte test in the bronchoalveolar lavage of the cardiopulmonary recipient is an indication for early treatment by augmented immune suppression.

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CARDIOPULMONARY TRANSPLANTATION has become a potentially useful therapy for patients with terminal cardiopulmonary disease.[1-3] Its emergence as a reliable treatment will depend on a lowered operative mortality and better understanding of the later-developing obliterative bronchiolitis, which causes a morbid obstruction of the airways.[4,5] Our experience with cardiopulmonary transplantation, which began in 1982 and now includes 64 procedures, suggests that the disease that can afflict the airways of the allograft is a form of chronic rejection, and is likely targeted against the respiratory epithelium of the donor. We believe this allogeneic response can be detected at a potentially reversible stage through analysis of lung and blood lymphocyte's phenotype and immunologic function. Clinical manifestations of obstructive disease of the airways occur when the process is in part irreversible, and effective treatment by augmented immune suppression will require the type of anticipatory signal provided by the functional analysis of lymphocytes within the allograft. This review describes our experience with obstruction disease of the airways after cardiopulmonary transplantation, and suggests early treatment based on lymphocyte functional and phenotypic predictors as a reasonable strategy for the future.

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Materials and Methods

Immunosuppressive

Generally, perioperative immunosuppression consisted of azathioprine (1–2 mg/kg/day), cyclosporine (5–7.5 mg/kg/day), and rabbit antithymocyte globulin. Unless we responded to suspicion of acute rejection of the lung with methylprednisolone (1 gm/day for 3 days), steroids were withheld until the fourteenth to twenty-first postoperative day. Before 1986, by the twenty-first postoperative day, prednisone (15–20 mg/day) was substituted for azathioprine as maintenance therapy with cyclosporine. Since 1986, we have become increasingly aware of the need to use less cyclosporine (400–500 ng/ml whole blood RIA, Sandoz) to avoid its nephrotoxicity, and also of the likelihood that obliterative bronchiolitis is immune-mediated and thus requires consideration for more, not less, immune suppression. For this reason, our chronic treatment since 1986 has included azathioprine. Obstructive disease of the airways detected

by progressive abnormalities in measured pulmonary function was treated with augmented oral and intravenous (I.V.) steroids and by depletion of lymphocytes by rabbit antithymocyte globulin and/or the monoclonal antibody OKT3™(Ortho Pharmaceutical, Raraztan, NJ).

Follow-up of Patients

Radiographs of the chest, bronchoalveolar lavage (BAL) and arterial blood gases, and pulmonary function studies that included spirometry, flow volume loops, lung volumes, and single breath diffusing capacity for carbon monoxide (DLCO) were performed at the end of the initial hospitalization and every 3 months thereafter, unless clinical circumstances indicated they be obtained more often.

Because isolated cardiac rejection has been rare and is detected within the first 3 postoperative weeks only, the endomyocardial biopsy that was initially used weekly early after operation and for 1 to 3 months thereafter is now used early less frequently and is discontinued after 3 months.[6] Initially, lung tissue was analyzed after open biopsy, when symptoms or pulmonary function abnormalities were noted. Recently, transbronchial biopsy of lung tissue has been used with success, and biopsies are now performed routinely every 3 to 4 months.

When our experience with cardiopulmonary transplantation first began in 1982, bronchoalveolar lavage was performed only when an abnormal chest radiograph indicated either infection or acute rejection, but is now performed regularly. The lavage fluid is cultured and stained, and the total number of cells counted and differentiated as lymphocytes, macrophages, or neutrophils. Immunostaining is performed on the BAL and peripheral blood (PBL) to identify lymphocyte phenotypes (CD_3, CD_4, CD_8, HNK-1, CD_{25}, and CD_{20}). Concentrated BAL fluid and cells are submitted for gram stain, direct fluorescent antibody for legionella, AFB smear, and cultures for cytomegalovirus (CMV), Epstein-Barr virus (EBV), adenovirus, Herpes simplex, influenza, varicella zoster, mycoplasma, bacteria, fungus, and Legionella. Methenamine silver and toluidene blue stained cytocentrifuge smears of BAL cells and lung tissue are examined for the presence of *Pneumocystis carinii* organisms. Recently, immunostaining for CMV has become routine.

Since 1984, we have studied the immunologic reactivity of lymphocytes in the BAL and PBL. A donor-specific allogeneic response is measured by the primed lymphocyte test (PLT). PBL lymphocytes are separated from whole blood by Ficoll-hypaque gradient centrifugation. BAL lymphocytes are separated by incubation of BAL cells with carbonyl iron followed by magnetic centrifugation. Lavage and blood lymphocytes are incubated in culture with irradiated lymphocytes obtained from the spleen of the donor at the time of cardiopulmonary transplantation. The proliferation of lung and blood lymphocytes is measured by their incorporation of tritiated thymidine, and this proliferation represented the degree of alloreactivity (PLT) present in a particular culture. The PLT is judged positive when the mean counts per minute of the recipient/donor cell cultures are four times those obtained from cultures of the recipient's blood or lung lymphocytes not stimulated by the donor's cells (spontaneous proliferation). Measurement of proliferation after a short 72-hour incubation period is chosen to detect a secondary or primed alloreactive response of the recipient lymphocytes against the donor cells.

Histologic specimens obtained from transbronchial and open biopsy and autopsy are serially sectioned and histochemically stained by hematoxylin and eosin, Grocott (fungi and protozoal), AFB (mycobacterial), Brown and Brenn (bacterial), trichrome (smooth muscle and collagen), and elastic van Gieson. Immunostaining is performed on freshly frozen tissue, using the avidin/biotin complex method for T and B lymphocytes and for Class II major histocompatibility antigens (DR, DQ, and DP) in the bronchiolar epithelium.

Results

Survival and Incidence of Obstructive Airway Disease

Between March 1982 and February 1988, 34 and 58 patients have been discharged from their operative hospitalization, and the overall survival rate between 1 and 65 months has been 41% (24 of 58). Infection within the thorax, usually of the lungs, has been the most common cause of death (19 of 37). Few infectious deaths, however, have occurred beyond the third postoperative month. Progressive pulmonary insufficiency that occurred in eleven of 30 recipients who survived at least 4 months has been responsible for 40% (4 of 10) of the late deaths. Obstructive disease of the airways was detected clinically between the 185th and 650th postoperative day (Table 1). Histologic diagnosis of obliterative bronchiolitis was made for eight of the eleven recipients with disease of the airways. Tissue was obtained through autopsy in two patients, open biopsy of the lung in four patients, transbronchial biopsy in two patients, and was clinically diagnosed through the use of radiographs and spirometry in three other recipients. Patients with obliterative bronchiolitis initially experienced a nonproductive cough and subsequent progressive shortness of breath. Although chest radiographs have generally been normal in this group, spirometric tests of pulmonary functions have confirmed a worsening restrictive and obstructive process.

TABLE 1.

Patient #	Initial PLT: Post-operative Day	Initial PLT: Location	Initial PLT: Immune Suppression (mg/kg/day)	First Clinical Symptoms: Post-operative Day	First Clinical Symptoms: Immune Suppression (mg/kg/day)	Abnormal PFT: Post-operative Day	Abnormal PFT: Immune Suppression (mg/kg/day)	Definite Diagnosis: Post-operative Day	Definite Diagnosis: Criteria	Definite Diagnosis: Immune Suppression (mg/kg/day)
2		N/A		176	CSA (7.8) Pred (0.22)	285	CSA (4.5) Pred (0.16)	300	clinical	CSA (4.5) Pred (0.16)
6		N/A		160	CSA (6.4) Pred (0.37)	274	CSA (5.6) Pred (0.37)	574	autopsy	CSA (4.6) Pred (0.74)
11		N/A			None		None	245	autopsy	—
14	135	BAL, PBL	CSA (4.4) Pred (0.22)	281	CSA (3.8) Pred (0.22) Aza (1.0)	281	CSA (3.8) Pred (0.22) Aza (1.0)	281	open biopsy	—
17	120	BAL, PBL	CSA (5.8) Pred (0.23)	551	CSA (3.3) Pred (0.18)	618	CSA (2.5) Pred (0.20) Aza (1.0)	618	open biopsy	—
30	359	BAL, PBL	CSA (14.2) Pred (0.23)	381	CSA (14.2) Pred (0.23)	381	CSA (14.2) Pred (0.23)	431	open biopsy	CSA (12.5) Pred (0.62)
31	367	BAL, PBL	CSA (7.6) Pred (0.23) Aza (1.15)	743	CSA (3.3) Pred (0.16) Aza (1.7)	367	CSA (7.6) Pred (0.23) Aza (1.2)	743	TTB	CSA (3.3) Pred (0.16) Aza (1.7)
32		N/A		486	CSA (11.0) Pred (0.33)	650	CSA (11.0) Pred (0.33)	650	clinical, bronchiectasis	CSA (11.0) Pred (0.33)
33		N/A		306	CSA (11.4) Pred (0.26)	306	CSA (11.4) Pred (0.26)	306	clinical, bronchiectasis	CSA (11.4) Pred (0.26)
46	335	BAL	CSA (3.3) Pred (0.43)		None		None	415	TBB	CSA (4.4) Pred (0.43)
49	95	PBL	CSA (12.2) Pred (0.22)	132	CSA (12.0) Pred (0.22)	185	CSA (10.0) Pred (0.22)	185	open biopsy	CSA (10.0) Pred (0.22)

PFT = Pulmonary function test.
TBB = Transbronchial biopsy.
CSA = Cyclosporine.
Pred = Prednisone.
Aza = Azathioprine.

Histopathology of Obstructive Airway Disease

The bronchiolar injury that results from obstructive disease of the airway is an obliterative bronchiolitis and presumably begins with epithelial injury induced by activated lymphocytes, resulting in epithelial necrosis with sloughing of cells into the lumen (Fig. 1). Fibroblasts and endothelial cells organize the intraluminal debris into polypoid masses of granulation tissue (Fig. 2) that may be reabsorbed, organized into eccentric or concentric plaques of dense collagen, or completely occlude and obliterate the lumen of the bronchiole. Injury to the small bronchi predisposes them to dilate, due to loss of smooth muscle support, and terminate in cylindrical bronchiectasis with mucopurulent secretions retained within the airways (Figs. 3, 4). This problem is compounded by superimposed infection.

Pulmonary Function Studies

In patients without apparent rejection or infection, pulmonary function studies reveal a moderate restrictive defect (decreased FVC and FRC) that nearly resolves by the first 12–18 months after transplantation. This was accompanied by improvements in diffusion (DLCO) and arterial blood oxygenation (Table 2). Importantly, in the absence of infection or rejection airway

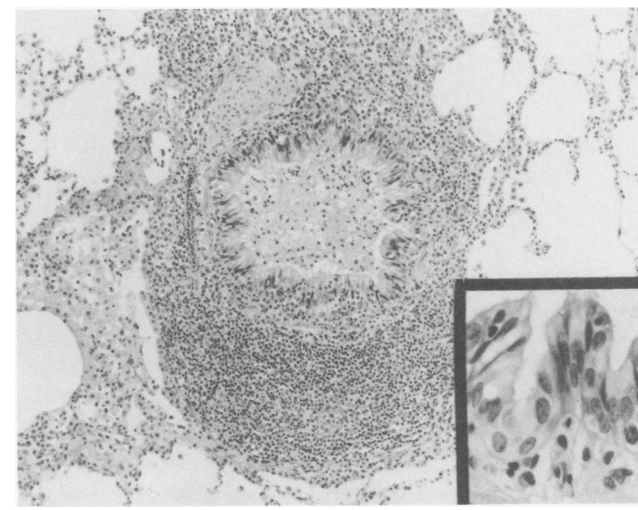

FIG. 1. Early injury in bronchiolitis obliterans consists of lymphocytes infiltrating the peribronchiolar regions and submucosa. Intraepithelial lymphocytes are prominent (insert) (H&E, 125; insert, H&E, ×500).

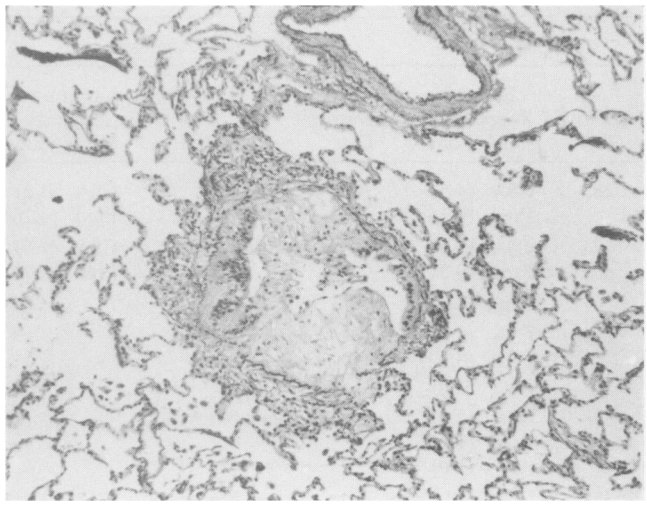

FIG. 2. With continued injury, plugs of granulation tissue occlude the lumen of bronchioles. This feature is associated with foamy macrophages, reflecting the obstruction of airways. (EVG_1, ×125).

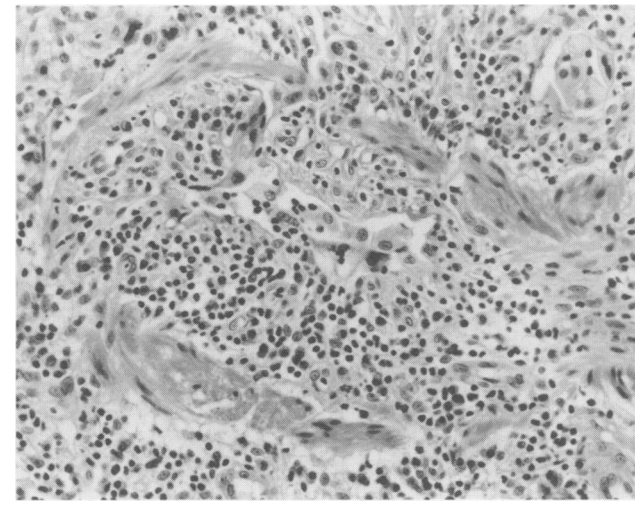

FIG. 4. Some bronchioles may have a small residual compression lumen (H&E, ×315).

function, as assessed by the FEV_1%, feF_{25-75}% and FEV_1/FVC, is normal as early as 3 months after transplantation and does not change for up to 6 years. In eleven recipients, a restrictive and obstructive defect was first noted at an average of 3 months after previously normal values on the 258 ± 140 postoperative day (Table 3). The abnormalities were associated with an elevated functional residual capacity, reduced diffusing capacity, and oxygenation. By the time definitive diagnoses of obliterative bronchiolitis were established, further deterioration had occurred (Table 3).

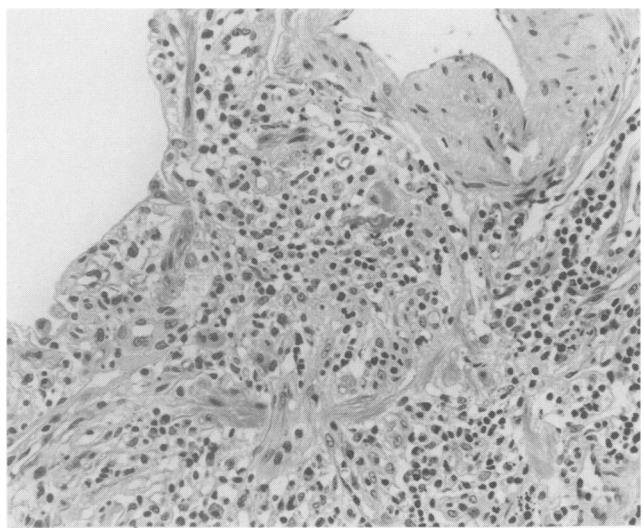

FIG. 3. At the end stage, bronchioles may be completely occluded by granulation tissue, with residual smooth muscle hinting at the original scarred bronchiole (H&E, ×315).

Immunopathology of Clinical Rejection

Evidence for donor-specific alloreactivity in the form of a positive PLT from lymphocytes separated from the BAL and PBL was present in five of six recipients who developed chronic rejection (83% sensitivity, 91% specificity), and was absent in ten of eleven recipients who have not developed disease airways (negative predictive value of 91%). In those recipients with chronic rejection, the correlation between PLT in the BAL or PBL was significant ($p \leq 0.05$) (Fischer's exact test). Clinical symptoms of cough and mild dyspnea (358 ± 205 days) and measurable decrease in pulmonary function (372 ± 159 days) followed detection of PLT (161 ± 192 days) (Table 3). Three months after clinical symptoms (and thus nearly 6 months after detection of the presence of PLT), a definitive diagnosis of chronic rejection and initiation of augmented suppression was achieved. In four of five recipients, both BAL and PBL expressed donor-specific alloreactivity, yet the magnitude of response was always greater for BAL lymphocytes. In the remaining recipient, the PLT was positive in the BAL and negative in the PBL. Before PLT monitoring was established, two patients exhibited obliterative tronchiolitis at the time of autopsy. BAL and, consequently, PLT have not been performed in two surviving patients with established and advanced obstructive disease associated with mucopurulent secretions from bronchiectasis, because specimens are likely to be contaminated with bacteria and neutrophils and because, in this group, BAL has been associated with significant morbidity.

Lymphocyte phenotypes from BAL and PBL revealed a relationship between an increased proportion of CD_4

TABLE 2. *Sequential Estimates of Pulmonary Lung Function in Recipients Without Infection or Rejection*

	Postoperative Months						
	3	6	12	18	24	30	48
FVC (%)†	58 ± 15	70 ± 17	74 ± 19*	74 ± 19*	83 ± 15	84 ± 13	77 ± 14
No.	(39)	26	23	14	4	6	4
FEV_1 (%)	67 ± 16	78 ± 19	84 ± 22*	84 ± 24*	95 ± 20	92 ± 14	87 ± 17
No.	(39)	26	23	14	4	6	4
FEV_1/FVC	90 ± 8	90 ± 8	90 ± 6	88 ± 7	89 ± 5	88 ± 4	87 ± 3
No.	(39)	26	23	14	4	6	4
FEF_{25-75} (%)	82 ± 32	85 ± 31	88 ± 28	87 ± 31	90 ± 29	86 ± 23	85 ± 24
No.	(38)	26	23	14	4	6	4
FRC (%)	72 ± 19	74 ± 21	77 ± 18	72 ± 25	84 ± 7	72	73 ± 5
No.	(27)	16	13	6	4	1	4
DLCO (%)	72 ± 20	81 ± 23	89 ± 23*	87 ± 29*	89 ± 25	92	103 ± 24
No.	(29)	17	15	7	4	1	2
PaO_2 (RA)	89 ± 12	87 ± 16	95 ± 6*	90 ± 16*	92 ± 2	83 ± 6	97
No.	(21)	13	14	7	3	3	1

* Significantly different as compared with 3 months after transplantation by the Wilcoxon signed rank test.

† Percent predicted. Data expressed as mean ± 150.

lymphocytes and an increased CD_4/CD_8 ratio and chronic rejection only (Table 4). In the absence of infection or rejection, the CD_4/CD_8 ratio in the pulmonary allograft was 0.8 ± 0.5, and in the PBL was 1.3 ± 1.1. A CD_4/CD_8 ratio greater than 1.8 in BAL and 2.5 in PBL was considered elevated. In both PBL and BAL, an elevated CD_4/CD_8 ratio occurred in four of the seven recipients who eventually developed chronic rejection (sensitivity 57%). An increased CD_4/CD_8 ratio was also detected in the PBL of two additional at-risk patients. A "normal" CD_4/CD_8 ratio was present in the BAL of 14 of 17 recipients (negative predictive value of 82%) and in the PBL of twelve of 15 recipients (negative predictive value of 80%) who exhibited no evidence of chronic rejection. Thus, a significant correlation ($p \leq 0.05$) (Fischer's Exact Test) exists between an elevated CD_4/CD_8 ratio and PBL, and especially in the BAL and subsequent development of chronic rejection.

Response to Augmented Immunosuppression

All recipients who developed chronic rejection were believed to have received a reasonably therapeutic level of immune suppression that equaled the treatment re-

TABLE 3. *Sequential Pulmonary Function in Recipients who Developed Chronic Rejection*

	Before PLT and/or Increased CD_4/CD_8	Initial PLT and/or Increased CD_4/CD_8	Time of First Clinical Symptoms	Diagnosis of Obliterative Bronchiolitis
Postoperative Day	202 ± 134	258 ± 140	356 ± 157	555 ± 450
FVC (%)†	72 ± 20	75 ± 24	69 ± 20	66 ± 23
No.	8	8	11	11
FEV_1 (%)	81 ± 20	83 ± 27	69 ± 24	66 ± 27
No.	8	8	11	11
FEV_1/FVC	92 ± 6	90 ± 6	79 ± 12	79 ± 13
No.	8	8	11	11
FEF_{25-75} (%)	105 ± 45	91 ± 35	61* ± 37	57 ± 39
No.	8	8	11	11
FRC (%)	75 ± 15	81 ± 22	88 ± 17	92 ± 19
No.	7	6	9	8
DLCO (%)	72 ± 18	68 ± 24	75 ± 26	73 ± 28
No.	7	7	10	9
PaO_2 (RA)	93 ± 5	96 ± 13	82 ± 12*	75 ± 14
No.	6	6	9	10

* Significantly lower ($p \leq 0.05$) as compared with the previous value by the Wilcoxon signed rank test.

† Percent predicted. Data expressed as mean ± 150.

TABLE 4. *Cellular Profile of BAL in Recipients who Developed Chronic Rejection*

	Before Increase of CD_4/CD_8	Time of Initial Increase of CD_4/CD_8	Time of First Biopsy	Diagnosis of Obliterative Bronchiolitis
No. of total cells	46 ± 37†	66 ± 64	122 ± 234	42 ± 31
Macs (%)	60 ± 21	49 ± 21	51 ± 24	51 ± 26
Lymphs (%)	25 ± 22	38 ± 20	15 ± 12*	17 ± 11
PMN (%)	15 ± 21	13 ± 27	33 ± 30	31 ± 33
CD_4 (%)	27 ± 10	43 ± 13*	32 ± 12	35 ± 13
CD_8 (%)	40 ± 14	38 ± 25	43 ± 22	42 ± 21
CD_4/CD_8	0.8 ± 0.3	2.7 ± 3.6*	1.0 ± 0.5	1.0 ± 0.4

* Significantly different from previous value by the Wilcoxon signed rank test.

† Data expressed as mean ± 150.

ceived by those who did not have obliterative bronchiolitis (Table 1). Of the eleven recipients with rejection, two died before our current understanding of the process and did not receive additive immune suppressive treatment; three other early recipients had established obliterative bronchiolitis with recurrent infections and constant purulent sputum from severe associated bronchiectasis, and azathioprine has recently been added, with caution, to their regimen; and the remaining five recipients are now receiving azathioprine and have received additional therapy with methylprednisolone (1 g daily for 3 days) and oral prednisone (taper from 100 mg) alone and in combination with either ATG or OKT_3. An initial improvement in pulmonary function and/or biopsy occurred in these five recipients (Figs. 5A–C), but did not last; all patients required additional treatments within 1–3 months. Two of these patients have died, one patient of myocardial infarction 18 months after transplantation, and the other of sepsis and severely impaired pulmonary function (FEV_1 20%). The three remaining recipients with chronic rejection appear stable with adequate pulmonary function but little reserve (FEV_1 between 50 and 62%). After treatment, spirometry has improved but has not reached previous levels, and the PLT has been suppressed. Relapse has been associated with an increase in PLT and a decline in pulmonary function. Recently, we have detected ongoing bronchiolitis by transbronchial biopsy in one patient whose spirometry had improved but whose PLT was elevated.

Discussion

Obliterative bronchiolitis after cardiopulmonary transplantation was first reported in 1984 by Burke et al.[4] This airway obstruction was also associated with occasional bronchiectasis and recurrent infections. At that time, the disease of the airway had not been noted in experimental animals, and subsequently, has still not been recognized in chronically surviving subhuman primates that provided the impetus for human cardiopulmonary transplantation. Obliterative bronchiolitis had previously been recognized as a common response to pulmonary injury from toxic exposure and infection, and had also been linked to immune-mediated mechanisms in autoimmune diseases and as a form of chronic graft *versus* host reaction after the transplantation of bone marrow tissue.[7–12]

In our series, obliterative bronchiolitis has been common in patients who survived for more than 4 months and has affected eleven of 36 recipients. It has been associated with the deaths of four of those afflicted. An immune basis for this process is likely, because it has been predicted by donor-specific alloreactivity of lung and blood lymphocytes and has been at least temporarily responsive to augmented immune suppression, and also because no infection has been detected when the PLT was observed or when the diagnosis of chronic rejection was established. Although a slight relationship between obliterative bronchiolitis and human leukocyte antigen matching has been suggested,[13] we have not been able to make similar observations in a larger group of our recipients. The histopathology of obliterative bronchiolitis suggests that activated lymphocytes infiltrate and damage the airway and are seemingly attracted to epithelial targets that express Class II antigens. This results in dilatation of bronchi by loss of smooth muscle and obstruction to the small bronchioles by organization of intraluminal polypoid granulation tissue.

Prop et al. have shown that rat lung allografts that reject promptly contain large amounts of bronchial-associated lymphoid tissue, which likely stimulate prompt and strong allogeneic responses.[14] Our own studies have documented the persistence of donor lymphocytes and macrophages within the transplanted lung, and it is also possible that this persistence of donor cells within the abundant reticuloepithelial repositories of the lung may contribute to a limited graft *versus* host reaction within the lung that may augment a rejection process through the release of various lymphokines and other complex cellular mechanisms.[15] It is of interest that obliterative bronchiolitis has not as yet been recognized after single or double lung transplantation (J. D. Cooper, personal communication, January 1988). In the fewer patients at risk, its absence might be due to good fortune, but it

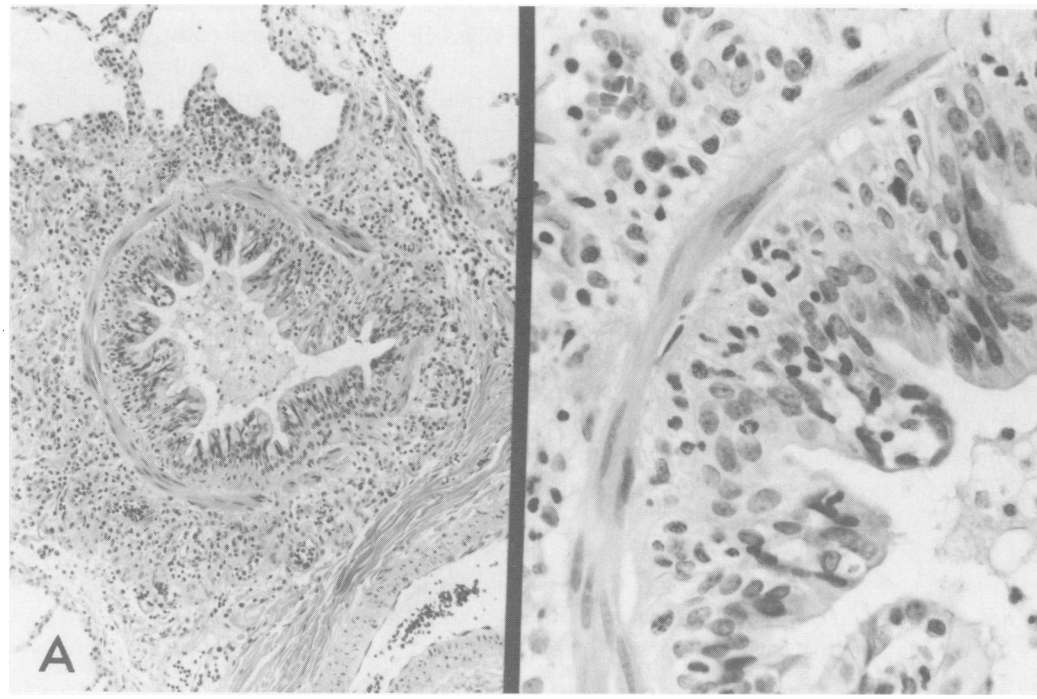

FIG. 5A. Original biopsy. A marked lymphocytic bronchiolitis was noted with prominent intraepithelial lymphocytes (right) (left, H&E, ×125; right, H&E, ×500).

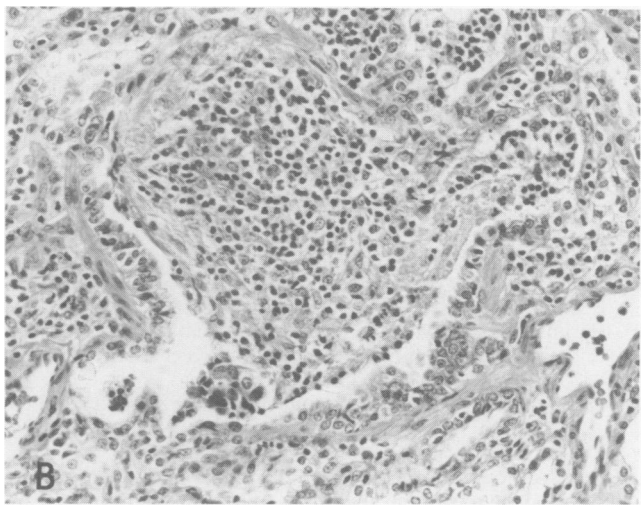

FIG. 5B. Second biopsy. Organizing plugs of granulation tissue and chronic inflammation were more exuberant after OKT_3 therapy (H&E, ×315).

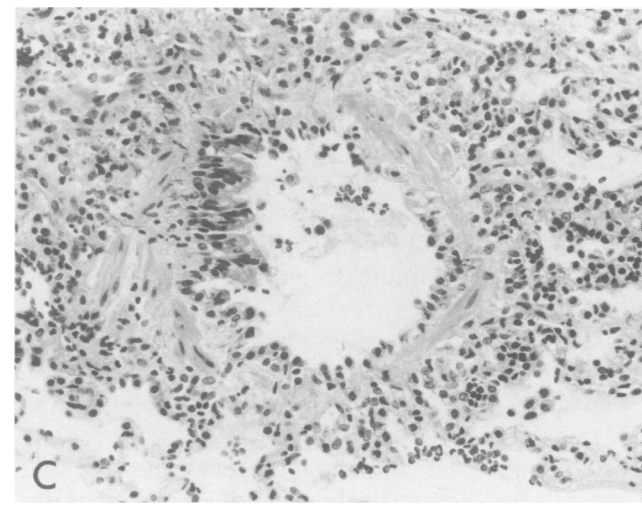

FIG. 5C. Third biopsy. Obliterative bronchiolitis resolved with a reduced inflammatory infiltrate. Residual regeneration of epithelium is seen (H&E, 315×).

might also be related to the fact that significantly less donor-related lymphatic tissue is transplanted with the single or double lung block.

Romaniuk et al. have shown that during rejection of rat single lung allografts, Class II histocompatibility antigens were expressed on infiltrating lymphocytes and were also induced on the bronchiole epithelium.[16] After treatment with CsA, signs of rejection were restricted to the epithelium of the airways that continued to express Class II antigen. It is known that Class II antigen expression in the airway can also result from infection, and it is intriguing that donor-specific alloreactivity (PLT) was frequently detected in our patients after infections from *P. carinii,* CMV, and EBV, and always antedated the early clinical signs of cough and dyspnea and fall in expiratory lung volumes. Because for all the recipients, rates of infection are high after cardiopulmonary transplantation, the usual finding of Class II antigen on the bronchial epithelium has not been a predictor of those who will develop chronic rejection. If these infections can incite a rejection response by inducing the expression of Class II antigen, their prevention might also decrease the prevalence of chronic rejection. Routine immune-peroxidase staining for Class II antigen on se-

quential transbronchial biopsy specimens should, in the future, help clarify the relative importance of Class II antigen.

It has previously been suggested that pulses of methylprednisolone and increased maintenance immune suppression with cyclosporine, prednisone, and azathioprine can partially reverse and reduce the rate of decline of airflow in patients with established obliterative bronchiolitis.[17,18] We have found that it is difficult to maximize maintenance triple drug therapy because recipients have often required adjustments, due to individual toxicities of the drugs and the presence of intercurrent infections. We now use a triple drug regimen but experience suggests that intermittent and, we hope, earlier augmented immune suppression with therapy that depletes lymphocytes, such as rabbit antithymocyte globulin or OKT_3, will also be required.

If the detection of a PLT represents an alloreactive process that results in an injury to the airways, it becomes evident that standard pulmonary function studies do not detect this early phase of chronic rejection. Our data indicate that, on average, 3 months elapse between the development of a PLT and the onset of symptoms or pulmonary function changes. It is quite likely that substantial injury to the allograft is occurring undetected. Such a possibility was exhibited in one of our patients whose pulmonary function had improved after augmented immune suppression, but who continued to have a PLT and histologic evidence by transbronchial biopsy of persisting bronchiolitis obliterans. Our experience, as well as that of others,[4,17] is that chronic rejection diagnosed when symptoms or pulmonary function deteriorates is difficult to treat effectively, whereas augmented immune suppression may stabilize or modestly improve pulmonary function.

The data from BAL confirms that the increased CD_4/CD_8 ratio is secondary to an increase in the percentage of CD_4 lymphocytes, and that this occurs when the PLT is first detected. Interestingly, when PLT is present, the percentage of lymphs is at its highest, and when symptoms or abnormal PFT occur, the total cell count is at its highest due to an influx of neutrophils. This suggests that the neutrophils play a major role in injuring the lung and in initiation of cough and shortness of breath. Later, when the diagnosis is made, the total cell count has decreased, suggesting that the major inflammatory and destructive process has passed and that treatment at this stage is ineffective.

No patient with obstructive airway disease has ever experienced complete recovery, and most follow a relentless downhill course. It may well be, however, that earlier treatment at the peak of the alloreactive process, when the PLT is first present, might be more effective. Earlier detection and earlier treatment of chronic rejection represents our current strategy designed to reduce the toll exacted by chronic rejection.

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Discussion

DR. RICHARD E. WILSON (Boston, Massachusetts): I wonder if changes in systemic host reactivity might be able to be determined, along with standard tests, so that it could be possible to begin therapy for rejection earlier than just depending on finding reactive lymphocytes in the serum?

DR. BARTLEY GRIFFITH (Closing discussion): The uniqueness of pulmonary transplantation is that our reactive cells can be lavaged directly from the affected organ. We have measured PLT in peripheral blood lymphocytes. It is almost universally present when the PLT is present within the lung lavage cells. However, the level of PLT is always much higher within the lung lavage, and therefore we choose that as the most specific measure.