Transplant Int (1992) 5 [Suppl 1]: S242–S245

TRANSPLANT
International

Role of CMV pneumonia in the development of obliterative bronchiolitis in heart-lung and double-lung transplant recipients

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Abstract. Obliterative bronchiolitis (OB) is the main cause of late mortality after lung transplantation. Cytomegalovirus infection has been associated with late graft failure. The aim of this study was to determine whether the development of OB was related to CMV pretransplant serological status and to CMV infections. The study group comprised 36 lung transplant recipients (27 HLT and 9 DLT) who survived more than 4 months, of whom 47% developed OB (defined by the persistence of an unexplained obstructive disease: FEV1/VC < 0,7). OB occurred more frequently: (1) in seronegative recipients with seropositive donors (8/9) than in seropositive recipients (7/19) or seronegative well-matched recipients (2/8); and (2) in patients who experienced CMV pneumonia (11/16) and CMV recurrence (11/16). Since matching seronegative recipients is the best way to prevent CMV infection, we believe that seronegative grafts must be reserved for seronegative recipients.

Key words: Lung transplantation – CMV infection – CMV pneumonia – Obliterative bronchiolitis

Obliterative bronchiolitis (OB), initially described in 1901 by Lange [11], occurred when injury to small conducting airways is repaired by proliferation of granulation tissue. OB has been described in fume exposure, viral infections, adverse drug reaction and connective tissue diseases [3]. A similar pulmonary disorder was reported in 1984 in heart and lung transplant recipients by the Stanford group: five of their first 14 long-term surviving heart-lung transplant recipients developed progressive obstructive airway disease [1]. Post-mortem material and open-lung biopsies, which were available from three recipients, showed a histological pattern of OB. Since this report OB has been observed by all lung transplant teams in the three types of lung transplantation (heart-lung, double and single lung). The incidence of OB varies between 24 and 67% in long-term survivors after lung transplantation [10, 14].

OB has been suggested to occur more frequently in cases of poorly controlled lung rejection [14]. The role of CMV infection has also been implicated as a causative factor in OB [10] and the development of coronary artery disease in the transplanted heart and late renal graft failure [4, 12].

Our aim was to analyse if the development of OB in double-lung (DLT) and heart-lung transplant (HLT) recipients was related to CMV pretransplant serological status and to CMV post-transplant infection.

Patients and methods

Of the 39 HLT and 14 DLT performed at Marie Lannelongue Hospital from June 1986 to November 1990, we studied 36 (27 HLT and 9 DLT) who survived more than 4 months and were at risk of OB. There were 18 males and 18 females; their ages ranged from 9 to 53 years (mean: 33 ± 12 years). The mean follow up was 718 ± 69 days. The original diagnoses were: primary pulmonary hypertension ($n = 10$), respiratory insufficiency ($n = 16$), Eisenmenger's syndrome ($n = 7$), chronic pulmonary embolism ($n = 3$). The immunosuppressive regimen consisted of: cyclosporin (CyA) adjusted to achieve whole blood levels of 150–300 ng/ml, azathioprine 1–2.5 mg/kg per day and prednisone 0–1 mg/kg per day beginning at day 7. Rabbit antilymphocytic globulin was administered for the first 7 postoperative days. Acute allograft rejection (AAR) was treated by the administration of 1 g methylprednisolone for 3 consecutive days.

The CMV serological status of donors and recipients were determined using an ELISA method (D + = seropositive donor; D − = seronegative donor; R + = seropositive recipient; R − = seronegative recipient). CMV seronegative blood products were used for all patients. Mismatched recipients (D + /R −) received anti-CMV immunoglobulins 250 mg/kg per week for 6 weeks (Centre Transfusion Sanguine Lille, France). Symptomatic CMV infections were treated with ganciclovir (10 mg/kg per day for 15–21 days). The immunosuppressive regimen was not altered when active CMV infection was diagnosed.

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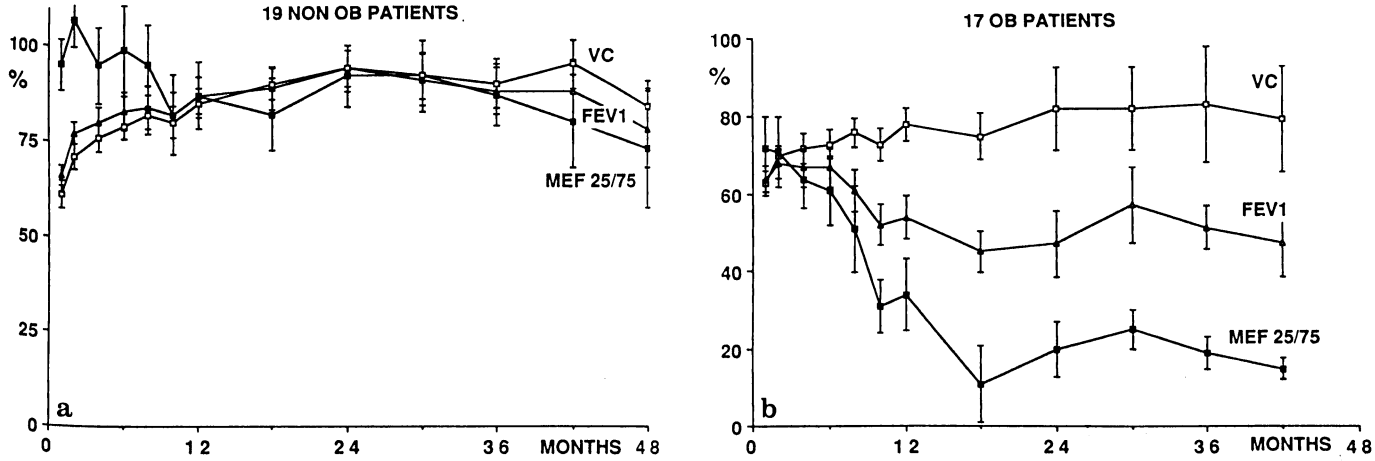

Fig. 1. Evolution of pulmonary function tests (□, VC; △, FEV1; ■, MEF 25/75:) expressed as percentage of predicted values in the two groups of patients (mean ± s.e.m.). *Left panel,* non-OB patients; *right panel,* OB patients

Follow-up

Pulmonary functional tests. Spirometry was performed using computerized Gould equipment (2400, Respiratory Function Laboratory; Gould Instruments, Cleveland, Ohio, USA). Vital capacity (VC) was measured as inspiratory vital capacity. Forced expiratory volume in 1 second (FEV1) and expiratory flow between 25 and 75% of forced vital capacity (MEF 25/75) were read from the largest of three flow-volume curves. Data were expressed as percent of the subjects' own predicted values.

Pulmonary functional tests were performed twice a week during the postoperative course. After their discharge, patients were monitored every 2 weeks for 6 months and then monthly.

Bronchoscopy. Fibreoptic bronchoscopy [bronchial aspiration, bronchoalveolar lavage (BAL) and at least five large trans-bronchial biopsies (TBB)] were routinely performed on days 15, 30, 45, 90 or in the event of symptoms such as fever, chest radiographic abnormalities or decrease in pulmonary function. In addition, TBB were performed during the month following treatment of an episode of confirmed histological AAR. TBB samples were studied for histological patterns of allograft rejection using the Lung Rejection Study Group criteria [18] and for the presence of specific viral inclusions and other opportunistic agents. The presence of virus was assessed in BAL fluid by HES staining, indirect immunofluorescent assay and shell vial culture. Viral culture of blood and urine and serological studies were performed weekly throughout the postoperative stay and every time the patients returned to our institution.

Definitions

CMV infection was defined by the presence of CMV in lung, blood and/or seroconversion. Infection was considered as symptomatic disease when clinical, radiological or biological abnormalities compatible with CMV disease were present: fever, gastrointestinal symptoms, interstitial shadows on chest radiographs, elevated liver enzymes, cytopenia or mononucleosis.

CMV pneumonia was defined by the association of
- the presence of CMV in lung specimens (bronchoalveolar lavage or transbronchial biopsies);
- histological patterns of viral alveolitis on TBB [15]; and
- lung infiltrates on chest radiographs.

OB was diagnosed when the FEV1/VC ratio was less than 70% for more than 3 months in the absence of other causes and independent of the presence of a histological pattern of OB on TBB. A severe OB was considered when FEV1 was lower than 40% of predicted values.

Statistical analysis

Results are expressed as mean ± SD (except for pulmonary functional tests expressed as mean ± s.e.m.). Patients were separated into two groups according to the occurrence of OB. The chi-squared test with Yates' correction and non-paired Student's *t*-test were used to compare the two groups. $P \leq 0.05$ was considered the threshold for significance.

Results

A total of 17 OB occurred in the 36 patients. Their mean age (35 ± 12 years) and sex ratio (8 female, 9 male) did not differ from the non-OB group (37 ± 11 years; 10 female, 9 male). The mean delay to OB occurrence was 271 ± 41 days (range 76–678 days). Ten of the 17 patients with OB became severe and eight of these patients died. OB was confirmed histologically in 13 of the 17 patients (eight post-mortem examinations and five TBB sample examinations). The time-course for the results obtained in the pulmonary function tests are shown in Fig. 1. Initial pulmonary function tests were similar in both groups with a trend for a higher initial MEF_{25-75} in the patients without OB.

Relationships between CMV serological status and OB

The results presented in Table 1 show that among the nine D+/R−, eight developed OB. Only two OB were noted among the eight D−/R−. Six OB occurred in 19 seropositive recipients. OB was more frequently observed in D+/R− patients than in the two other groups D−/R− and R+ (Table 1).

Table 1. Incidence of OB related to CMV serological status

	OB	Non-OB	Total
D−/R−	2	6	8
D+/R−	8*	1	9
R+	7	12	19
Total	17	19	36

OB was more frequently observed in D+/R− patients than in D−/R− and R+.
* $P < 0.01$

Table 2. Incidence of OB related to CMV infection

	OB	Non-OB
Total patients	17	19
No infection	3	6
CMV infection	14	13
Pneumonia	11*	5
Recurrence	11*	5

OB was observed more frequently in patients who experienced CMV pneumonia and CMV recurrence
* $P < 0.01$

Table 3. Incidence of CMV infection related to CMV serological status

	Patients	Pneumonia	Recurrence
D−/R−	8	0	0
D+/R−	9	7*	5
R+	19	9	11
Total	36	16	16

Pneumonia was more frequently observed in D+/R− patients than in D−/R− and R+ patients
* $P = 0.05$

Relationships between CMV infection and OB

Asymptomatic infection was observed in two cases (Table 2). Symptomatic infection was experienced by 25 patients (69%), 16 of whom (64%) had CMV pneumonia. CMV recurrence was observed in 16 patients (64%). No deaths occurred as a result of CMV infection. OB was detected more often in the 16 patients who experienced CMV pneumonia (11 vs 5; $P < 0.01$) and in the 16 patients with recurrence of CMV infection (11 vs 5; $P < 0.01$).

The results presented in Table 3 show that none of the eight D−/R− patients had CMV infection. CMV pneumonia, but not CMV recurrences, were more frequently observed in the D+/R− patients as compared with R+ and D−/R− patients ($P = 0.05$).

Discussion

This study shows that OB is more frequently observed in cases of CMV pneumonia, CMV recurrences and in CMV mismatched recipients (D+/R−). The incidence of OB was 47% in this study while in the literature incidences from 24% to 67% have been reported [16]. This range in the incidence of OB may be due to several factors: (a) differences in duration of follow-up (the longer the follow-up, the higher the risk of OB); (b) differences in the nature of the immunosuppressive regimen or in the patients' management [16]; and (c) the different criteria which were used for the diagnosis of OB (some studies used histological criteria on TBB [10], while others (reference 15 and this report) used pulmonary function tests). Although tissue diagnosis must be considered the 'gold standard', the use of TBB instead of open-lung biopsy increases the chance of missing the diagnosis, since in TBB samples bronchioles are scarce and sometimes absent. Moreover, the distribution of OB is patchy [16] contributing to a decrease in the sensitivity of TBB for diagnosis of OB. Therefore we suggest that pulmonary function tests could be a more sensitive procedure for detecting OB. In this study a persistent decrease in the FEV1/VC ratio (below 70%) was used. The accuracy of this criterion was confirmed by all the autopsy examinations.

CMV infection and CMV pneumonia (69% and 44% in our patients, respectively) were more frequently noted after lung transplantation than subsequent to other organ transplantations [7]. The high incidence of CMV infection was related to the high proportion of CMV seropositive donors and/or recipients leading to a small number of well-matched D−/R− patients (22%). CMV pneumonia was the main visceral CMV disease after lung transplantation (64% of our patients with CMV infection experienced pneumonia). The infection was more frequently observed in D+/R− patients (77%) reflecting the severity of primary infection. None of the patients with CMV pneumonia died but recurrences were frequent (64%). The absence of death was probably due to early diagnosis and early treatment with ganciclovir. The use of an association of sensitive laboratory tests (shell vial culture, immediate fluorescence assay, immunohistochemical staining) allowed early diagnosis of CMV infection. The high incidence of recurrence may be due to the short duration of ganciclovir treatment.

OB was more frequently noted in our patients with CMV mismatch, CMV pneumonia or CMV infection recurrences. Similar results were reported by Keenan et al. who reported that OB was more often observed in patients who had CMV infection (the three D+/R− and nine of the ten R+ developed OB when compared with six of the 14 D−/R−). In addition, seven of the eight patients with CMV pneumonia developed OB. The role of CMV recurrence was not studied. The discrepancies in interpretation of results between Keenan et al. [10] and our results is due to differences in serological status of the two populations [D−/R− patients were 51% (Keenan et al.) vs 22% (this report)]. In contrast, Scott et al. [14] failed to find any association between CMV infection and OB occurrence.

The mechanisms whereby CMV infection favours occurrence of OB remain unclear [2, 5]. We have previously reported that CMV pneumopathy was associated with activation of T lymphocytes and macrophages. Several findings support this notion: (a) serum levels of neopterin (a marker of macrophage activation) and soluble IL2 receptor [9] are increased; and (b) genes coding for IL1β, IL6

and serine esterase B are expressed in BAL cells indicating in situ activation of both macrophages and cytotoxic cells [8]. Substances released by these activated cells (TNFα, IL1β and proteases) may induce lung damage. Furthermore, activated macrophages synthesize growth factors which may account for the fibroblastic proliferation that is observed in OB [6].

CMV infection is obviously not the sole cause of OB since OB was observed in D−/R− patients who remained free of CMV infection. It has been reported that AAR is closely linked to OB [16]. However, CMV infections and AAR may be closely related since: (a) donor-specific alloreactivity assessed by primed lymphocyte testing on BAL cells appeared soon after CMV infection [10] and (b) the expression of MHC class II antigens is increased during CMV infection in infected cells [13, 17].

In conclusion, the prevention of the occurrence of CMV infection after lung transplantation is important. Seronegative donor and recipient matching is the most reliable way to avoid CMV infection in lung transplant recipients. Seronegative grafts must therefore be reserved for seronegative recipients.

References

1. Burke CM, Theodore J, Dawkins KD, et al (1984) Post-transplant obliterative bronchiolitis and other late sequelae in human heart-lung transplantation. Chest 86: 824–829
2. Burke CN, Glanville AR, Theodore J, Robin EU (1987) Lung immunogenicity, rejection and obliterative bronchiolitis. Chest 92: 547–549
3. Epler GR, Colby TV, McLoud TC, Carrington CB, Gaensler EA (1985) Bronchiolitis obliterans organizing pneumonia. N Engl J Med 312: 152–158
4. Fryd DS, Peterson PK, Fergusson RM, et al (1980) Cytomegalovirus as a risk factor in renal transplantation. Transplantation 30: 436–439
5. Grundy JE, Shanley JD, Griffiths PD (1987) Is cytomegalovirus interstitial pneumonitis in transplant recipients an immunopathological condition? Lancet 2: 996–998
6. Hertz M, Henke C, Greenheck J, et al (1991) Pathogenesis of obliterative bronchiolitis after lung and heart-lung transplantation: a possible role for platelet-derived growth factor. Am Rev Resp Dis 143: A468
7. Ho M (1990) Epidemiology of cytomegalovirus infections. Rev Infect Dis 12: S701–S710
8. Humbert M, Cerrina J, Rain B, et al (1991) Interleukin-1β, interleukin-6 and serine esterase B gene expression in bronchoalveolar cells after lung and heart-lung transplantations. Am Rev Resp Dis 143: A600
9. Humbert M, Emilie D, Cerrina J, et al (1991) Soluble interleukin-2 receptor (sIL-2R) and neopterin (N) seric levels after lung and heart-lung transplantations. Am Rev Resp Dis 143: A460
10. Keenan RJ, Lega ME, Dummer JS, et al (1991) Cytomegalovirus serological status and post-operative infection correlated with risk of developing chronic rejection after pulmonary transplantation. Transplantation 51: 433–438
11. Lange W (1901) Ueber eine eigenthümliche Erkrankung der kleinen Bronchien und Bronchiolen. Dtsch Arch Klin Med 70: 324–364
12. Loebe M, Schuler S, Zais O, Warnecke H, Fleck E, Hetzer R (1990) Role of cytomegalovirus infection in the development of coronary artery disease in the transplanted heart. J Heart Transplant 9: 707–711
13. Rubin RH (1990) Impact of cytomegalovirus infection on organ transplant recipients. Rev Infect Dis 12: S754–S766
14. Scott JP, Higenbotham TW, Sharples L, Clelland CA, Smyth RL, Stewart S, Wallwork J (1991) Risk factors for obliterative bronchiolitis in heart-lung transplant recipients. Transplantation 51: 813–817
15. Smith CB (1989) Cytomegalovirus pneumonia: state of the art. Chest 95: 182S–187S
16. Theodore J, Starnes VA, Lewiston NJ (1990) Obliterative bronchiolitis. Clin Chest Med 11: 309–321
17. Von Willebrand E, Petterson E, Ahonen J, Häyry P (1986) CMV infection, class II antigen expression and human kidney allograft rejection. Transplantation 45: 394
18. Yousem SA, Berry GJ, Brunt EM, et al (1990) A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: lung rejection study group. J Heart Transplant 9: 593–601