Thorax 1996;51:1160–1162

Epstein-Barr virus associated graft failure following heart/lung transplantation

Jim J Egan, James P Stewart, Philip S Hasleton, N Yonan, Paul Bishop, John R Arrand, Ali N Rahman, Kevin B Carroll, Ashley A Woodcock

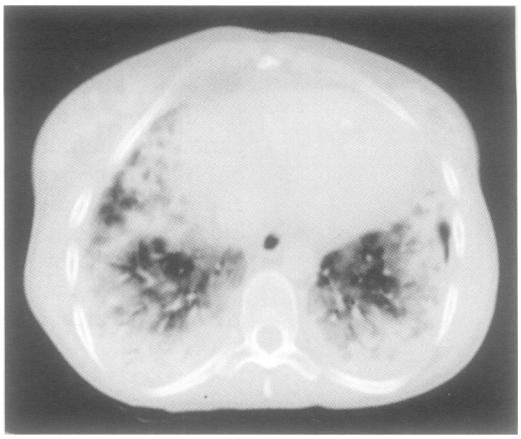

Figure 1 Thoracic CT scan with contrast demonstrating widespread diffuse alveolar shadowing of both lower lobes.

Abstract

A case is described of late pulmonary graft failure in a heart/lung transplant recipient. The major characteristics were alveolar fibrosis and a restrictive physiological deficit. Epstein-Barr virus was implicated as an aetiological agent using immunohistochemical analysis and by a response to treatment with ganciclovir.

(*Thorax* 1996;51:1160–1162)

Keywords: Epstein-Barr virus, pulmonary fibrosis, obliterative bronchiolitis, lung transplant, bronchiolitis obliterans organising pneumonia.

The major limiting factor to the long term success of lung transplantation is the development of obliterative bronchiolitis within the graft which presents as a predominantly obstructive physiological pattern associated with fibrosis and obliteration of the terminal bronchioles of the graft.[1] In contrast, we describe a female patient who developed graft failure with a pure restrictive physiological deficit associated with dominant interstitial fibrosis and the presence of Epstein-Barr virus (EBV) within the lung tissue.

Case report

A 31 year old woman received a heart/lung transplant for Eisenmenger syndrome. She suffered a complete vagotomy at the time of the operation and required a pyloroplasty four weeks following transplantation. A barium study showed slow but satisfactory gastric emptying and no evidence of aspiration. She was maintained on cisapride (Prepulsid) 10 mg at night. Three months following discharge she developed a pyrexia associated with nasal congestion. A routine transbronchial biopsy specimen demonstrated A2a (mild) rejection associated with a marked eosinophilic infiltrate without systemic eosinophilia. She received augmented oral steroids 1 mg/kg for 14 days. A follow up transbronchial biopsy specimen revealed resolution of perivascular cuffing but a persistence of the eosinophilic infiltrate. In the absence of demonstrable infection she then received 500 mg methylprednisolone intravenously on three consecutive days. Because of a progressive decline in lung volumes and a persistent pyrexia she was maintained on high dose oral prednisolone (1 mg/kg reduced by 5 mg per week), in addition to cyclosporin and azathioprine. An autoantibody screen was negative. A thoracic computed tomographic (CT) scan 16 weeks after transplantation demonstrated an acinar infiltrate suggestive of infection. Four consecutive bronchoalveolar lavages (BAL) and transbronchial biopsy failed to reveal an infective agent or evidence of aspiration but demonstrated progressive pulmonary fibrosis. In the presence of ongoing pyrexia and a restrictive physiological defect suggestive of bronchiolitis obliterans organising pneumonia (BOOP), she underwent a repeat CT scan followed by an open lung biopsy of the left lung (10 months after transplantation). The CT scan demonstrated widespread alveolar opacification (fig 1). Histologically, the open lung biopsy specimen showed severe interstitial and alveolar fibrosis (fig 2) as well as a few obliterated bronchioles associated with intimal fibrosis, occlusion of arteries and especially veins.

Lymphoproliferative disease was considered a possibility and the open lung biopsy specimen was examined by immunohistochemistry for the presence of EBV transformed B cells. The antibodies used were specific for EBV latent membrane protein and EBV nuclear antigen 2 (EBNA 2) (CS1-4 and PE2, respectively; Dakopatts) using methods previously described.[2] These EBV specific markers were absent but further immunohistochemical analysis was performed using monoclonal antibody directed against the EBV membrane antigen gp340/220 (72A1; a gift from S D Hayward) which is a marker of productive EBV replication.[3] This reagent identified numerous foci of EBV production (fig 3) further characterised as being within epithelial cells and not infiltrating B cells using appropriate markers (epithelial membrane antigen and CD20; Dakopatts). There was no serological evidence of EBV reactivation (EBNA2/IgG capsid serology) or cytomegalovirus infection (CMV antigenaemia negative, BAL DEAFF test negative, and lung tissue immunohistochemistry negative) and light microscopy also failed to demonstrate evidence of herpes simplex virus infection. The patient then received a trial of

North West Lung Centre
J J Egan
K B Carroll
A A Woodcock

Department of Cardiothoracic Surgery
N Yonan
A N Rahman

Department of Pathology
P S Hasleton
P Bishop

Wythenshawe Hospital, Manchester M23 9LT, UK

Department of Molecular Biology, Paterson Institute for Cancer Research, Christie Hospital, Manchester, UK
J R Arrand

Department of Veternary Pathology, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, UK
J P Stewart

Correspondence to:
Dr J J Egan.

Received 24 July 1995
Returned to authors
9 November 1995
Revised version received
22 January 1996
Accepted for publication
28 February 1996

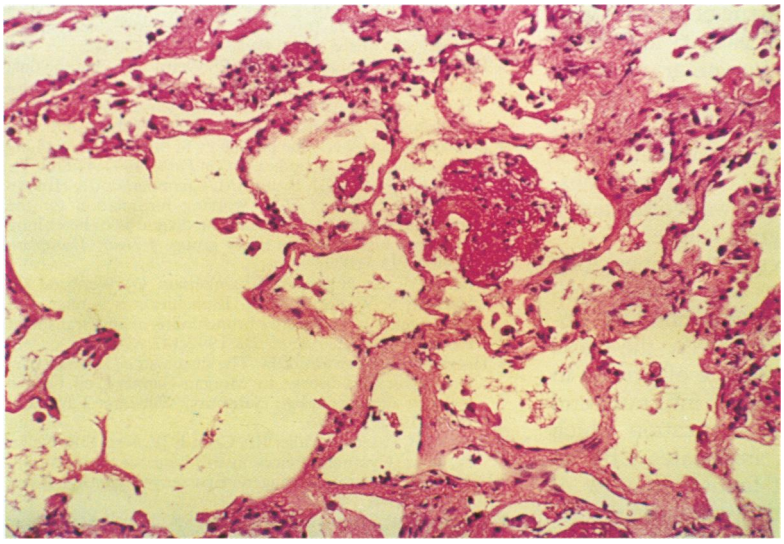

Figure 2 Open lung biopsy specimen showing intra-alveolar and interstitial fibrosis.

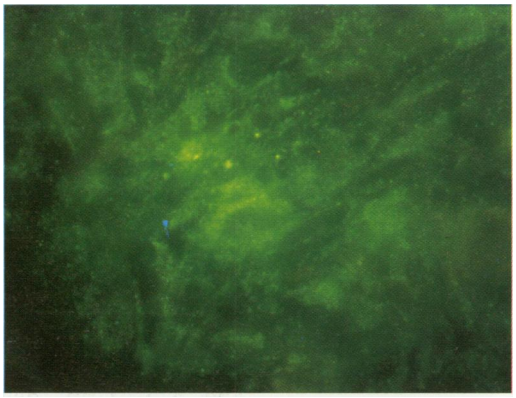

Figure 3 Expression of EBV membrane antigen gp340/220. Sections were stained with the gp340/220-specific monoclonal antibody 72A1 and reactivity was observed using biotinylated rabbit anti-mouse and streptavidin-FITC as previously described.[2] Serial sections from the same block were stained with isotype-matched control serum samples and were negative. The photomicrograph shows gp340/220-specific positive staining under ultraviolet illumination. Original magnification × 800.

ganciclovir 10 mg/kg daily. Over one week her pyrexia resolved.

She was maintained on intravenous ganciclovir until the time of a single lung re-transplant 12 months after her first transplant. The explanted lung of the heart/lung block was re-examined after ganciclovir for the presence of gp340/220-specific staining and was entirely negative. The patient died four weeks later from graft failure, aspergillus infection, and renal failure.

Discussion

The commonest cause of graft failure following heart/lung transplantation is obliterative bronchiolitis.[1] In our patient there were many features atypical of obliterative bronchiolitis and subsequent investigations, together with the therapeutic trial of ganciclovir, suggest that EBV infection may have been causal in the progressive graft fibrosis. The patient was febrile on presentation prior to augmented immunosuppression and this pyrexia persisted. The acinar changes seen on the first CT scan indicated possible infection and this is a common cause of eosinophilic infiltration in a lung graft.[4] Repeated investigations for potential infective organisms were undertaken and EBV replication within the graft tissue was the only organism identified. The recurrent pyrexia, documented for 28 weeks, was only eradicated ultimately with intravenous ganciclovir. This suggests that active EBV infection was responsible for the pyrexia and possibly the eosinophilic infiltration at the time of presentation. An additional finding which implies that EBV was pathogenic in this case was that, when the explanted lung tissue was re-examined after the pyrexia had lysed and following ganciclovir therapy, there was no evidence of EBV replication.

Obliterative bronchiolitis is considered to represent a manifestation of chronic graft rejection. It is typified histologically by scarring of membranous and respiratory bronchioles with obliteration of the bronchiolar lumen and fibro-intimal thickening of the arteries and veins within the graft.[4,5] In our case the dominant feature was that of interstitial fibrosis (fig 2). Physiologically, obliterative bronchiolitis is characterised by progressive small airways obstruction. The physiological pattern in our patient was that of a pure restrictive defect. The typical radiological changes seen with obliterative bronchiolitis are those of hyperinflation and associated bronchiectasis.[6] The CT scans of our patient initially demonstrated acinar changes suggestive of infection which progressed in severity to confluent alveolar opacification located predominantly in the lower lobes (fig 1) without evidence of bronchiectasis.

The restrictive flow volume loop in combination with the CT scan appearances were suggestive of BOOP. However the open lung biopsy specimen did not establish this diagnosis. Detailed histological examination demonstrated severe interstitial and, in areas, confluent fibrosis. The histological features of BOOP, including aggregates of connective tissue within bronchioles and alveolar spaces, were absent.

The clinical picture could have been consistent with lymphoproliferative disease. It was for this reason that the blocks taken from the open lung biopsy specimen were examined for the presence of latent EBV. These was negative but revealed evidence of EBV gp340/220-specific staining located within the epithelial cells of the graft. This antigen is diagnostic of EBV replication where virus particles are produced and is not an antigen expressed during viral latency.[3] The absence of serological evidence of EBV reactivation does not exclude active viral replication, as serological change is known to be a poor marker for EBV infection in an immunocompromised host.[7]

Augmented immunosuppression often leads to opportunistic infection. The finding of EBV within the lung tissue of this patient may therefore be viewed as representing passenger virus rather than a pathogen. Examples of this type of controversy are seen with cytomegalovirus

(CMV) in the pulmonary tissue of patients with AIDS[8] and human herpes virus 6 associated interstitial pneumonitis in bone marrow transplant patients.[9] Although a number of features of this suggest that EBV may be pathogenic, it is important to note that, because of the presence of immunosuppression, it remains possible that the EBV replication was coincidental rather than causal to the graft failure.

The histological features of the open lung biopsy specimen suggest that neither obliterative bronchiolitis nor bronchiolitis obliterans organising pneumonia were the cause of the graft failure. The presence of pyrexia in association with an eosinophilic infiltrate, and the demonstration of EBV replication which responded to ganciclovir, implicates EBV in the aetiology of the graft failure. In view of this case and recent reports documenting EBV within lung graft tissue,[10] EBV may be an important occult pathogen in the setting of lung graft failure.

1 Burke CM, Theodore J, Baldwin JC, Tazelaar HD, Morris AJ, McGregor C, *et al.* Twenty eight cases of human heart/lung transplantation. *Lancet* 1986;i:517–9.
2 Stewart JP, Arrand JR. Expression of the Epstein-Barr virus latent membrane protein in nasopharyngeal carcinoma biopsy specimens. *Hum Pathol* 1993;**24**:239–42.
3 Hoffman GJ, Lazarowitz SG, Hayward SD. Monoclonal antibody against a 250 000-dalton glycoprotein of Epstein-Barr virus identifies a membrane antigen and a neutralising antigen. *Proc Natl Acad Sci USA* 1980;**77**:2979–83.
4 Steward S, Carey N. The pathology of heart and heart/lung transplantation – an update. *J Clin Pathol* 1991;**44**:803–11.
5 Yousem SA, Berry GJ, Brunt EM, Chamberlain D, Hruban RH, Sibley RK, *et al.* A working formulation for the standardisation of rejection in the diagnosis of heart/lung rejection; lung rejection study group. *J Heart Transplant* 1990;**9**:593–601.
6 Granton J, de Hoyas A, Chamberlain D, Weisbrod G, Herman S, Winton T, *et al.* Bronchiectasis is related to the presence of obliterative bronchiolitis in lung transplant recipients. *Am Rev Respir Dis* 1992;**142**:A700.
7 Hotchin NA, Crawford DH. The diagnosis of Epstein-Barr virus-associated disease. In: Morgan-Capner P, ed. *Current topics in clinical virology.* Salisbury, Wiltshire: Laverham Press, 1991: 115–40.
8 Millar AB, Patou G, Miller RF, Grundy JE, Katz DR, Weller IV, *et al.* Cytomegalovirus in the lungs of patients with AIDS. Respiratory pathogen or passenger? *Am Rev Respir Dis* 1990;**141**:1474–7.
9 Carrigan DR, Drobyski WR, Russler SK, Tapper MA, Know KF, Ash RC. Interstitial pneumonitis associated with human herpesvirus-6 infection after marrow transplantation. *Lancet* 1991;**338**:147–9.
10 Hoffman DG, Gedebou M, Jimenez A, Nichols WS, Marchevsky A. Detection of Epstein-Barr virus by polymerase chain reaction in transbronchial biopsies of lung transplant recipients: evidence of infection? *Mod Pathol* 1993;**6**:555–9.

Thorax 1996;**51**:1162–1164

Pregnancy following a single lung transplant

Diane Parry, Andrew Hextall, V P Robinson, N R Banner, M H Yacoub

Abstract

Successful pregnancy in a single lung transplant recipient has not been reported previously. The long term effect of pregnancy on graft function and management of deteriorating pulmonary function is not defined. This case describes the management, outcome, and problems encountered when a single lung transplant recipient developed a progressive deterioration in pulmonary function during pregnancy, attributed to accelerated obliterative bronchiolitis.

(*Thorax* 1996;**51**:1162–1164)

Keywords: lung transplantation, pregnancy, obliterative bronchiolitis.

Harefield Hospital NHS Trust, Harefield, Middlesex UB9 6JH, UK
D Parry
N R Banner
M H Yacoub

Hillingdon Hospital NHS Trust, Uxbridge, Middlesex UB8 3NN, UK
A Hextall
P Robinson

Correspondence to:
Dr D Parry.

Received 22 February 1996
Accepted for publication
2 May 1996

There have been a number of reports of pregnancy with successful outcome following solid organ transplantation. Most of the information available pertains to renal and heart transplant recipients.[1 2] With improving survival and functional status after transplantation, more women are able to consider the possibility of starting a family. Single lung transplantation was first performed successfully in 1983.[3] The indications are usually restrictive or obstructive pulmonary disease. This report describes the successful outcome of a pregnancy in a woman following single lung transplantation.

Case report

The patient was a 31 year old white non-smoker who first presented in 1988 at the age of 24 with breathlessness and a dry cough. She had suffered from Raynaud's phenomenon since childhood and had two grand mal fits during adolescence, the last being 10 years previously. A cerebral computed tomographic scan had been normal. A diagnosis of fibrosing alveolitis was made on open lung biopsy and treatment consisted of high dose prednisolone and cyclophosphamide. She continued to receive sodium valproate for her epilepsy.

Her respiratory condition gradually deteriorated and she developed cyclophosphamide related cystitis. She was accepted for a single lung transplant and placed on the waiting list in June 1992. At that stage she was receiving prednisolone 15 mg daily and her forced expiratory volume in one second (FEV_1) was 1.06 l (36% predicted), forced vital capacity (FVC) 1.17 l.

In April 1993 she underwent left single lung transplantation. The donor was a 28 year old female non-smoker with no history of cardiopulmonary disease. Total ischaemic time for the procedure was five hours. In the early postoperative period the patient required one course of methylprednisolone (1 g for three days) for acute rejection, and antibiotics for *Staphylococcus aureus* cultured from sputum. She made a complete recovery.

In April 1994 her exercise tolerance was excellent. She led a full active life and planned marriage. At her annual assessment FEV_1 was