CASE STUDY

Recurrent sarcoid granulomas in a transplanted lung derive from recipient immune cells

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ABSTRACT: From 1992–2004, single lung transplantation has been performed in seven patients with end-stage pulmonary sarcoidosis at the Danish National Centre for Lung Transplantation. The objective was to assess whether recurrent sarcoid granulomas in the lung graft are derived from recipient or donor immune cells.

Three patients had sarcoid recurrence in the lung graft, but none had clinically overt extrathoracic sarcoidosis. Graft sex-mismatch was present in one patient, a 52-yr-old female having a lung graft from a male donor. In order to discriminate between recipient and donor cells fluorescence in situ hybridisation (FISH), using probes for both X- and Y-chromosomes, was applied on transbronchial lung biopsies (TBB) from the lung graft containing sarcoid granulomas.

The recipient’s explanted lung contained multiple active sarcoid granulomas. TBB from the implanted donor lung 5 months after transplantation showed sarcoid granulomas. FISH showed that the immune cells in the granulomas were X-chromosome positive and Y-chromosome negative and, therefore, were derived from the recipient.

In conclusion, the results indicate that recurrent sarcoid granulomas in the transplanted lung are derived from recipient’s immune cells, having colonised the lung allograft.

KEYWORDS: Donor, granuloma, in situ hybridisation, lung transplantation, pulmonary, sarcoidosis

The aetiology of sarcoidosis is unknown. Most likely, antigen(s) of infectious origin play a role in the pathogenesis of the disease, in combination with a genetic predisposition [1, 2]. The histological hallmark of sarcoidosis is the formation of noncaseating epithelioid cell granulomas resulting from antigen stimulation. Pulmonary involvement is frequent in sarcoidosis, and in the majority of patients the lung appears to be the principal target organ [3–5].

Lung transplantation has been established as an effective therapeutic procedure in terms of survival in end-stage sarcoid pulmonary disease [6]. Recurrence of sarcoidosis in the transplanted lung occurs in ~50% of Caucasian patients [6]. In theory, epithelioid cell granulomas in the transplanted lung could be formed from immune cells derived either from the recipient and/or the donor. The objective of the present study was, therefore, to examine the genetic origin of recurrent epithelioid cell granulomas in the lung allograft using fluorescence in situ hybridisation (FISH) technique.

PATIENT AND METHODS

Lung transplantation has been performed at the Danish National Centre for Lung Transplantation since 1992. The immunosuppressive regimen has consisted of induction treatment with rabbit derived antithymocyte globulin or daclizumab preparations, and triple agent maintenance immunosuppression with cyclosporine A, azathioprine and low dose prednisolone of 5 mg·day⁻¹. Surveillance flexible bronchoscopy (FFB) with bronchoalveolar lavage (BAL) and transbronchial lung biopsy (TBB) was routinely scheduled at 0.5, 1, 1.5, 3, 6, 12, 18 and 24 months after transplantation. Using FFB, 3–4 TBB specimens were obtained under fluoroscopic guidance. The same pathologist examined all biopsy specimens, and acute cellular rejection was graded A0–A4 according to international guidelines [7]. Acute rejection episodes (A2–A4) were treated with intravenous methylprednisolone.
1 g·day\(^{-1}\) for 3 days followed by high dose prednisolone, subsequently tapered to the maintenance dose.

From 1992–2004, a total of 379 lung transplantations (excluding seven re-transplantations) had been performed. Seven (1.9%) patients had single lung transplantation (SLTx) due to end-stage pulmonary sarcoidosis. This has been described previously in a series [6]. None of the patients had clinically overt extra-thoracic sarcoidosis. Follow-up of these seven transplanted sarcoidosis patients was a median of 31 months (range 12–136 months). One patient died 22 months after transplantation. Three patients had histological evidence of recurrent sarcoidosis in the lung allograft, and only one of these patients had radiological appearances consistent with pulmonary sarcoidosis (i.e. adenopathy and/or reticulo-nodular parenchymal infiltrates).

Graft-sex mismatch was present in one female patient having a right-sided lung allograft from a 20-yr-old male donor “M”. Macroscopic inspection of the donor lung at the time of surgery was unremarkable. The female recipient was 52 yrs old at the time of SLTx. The duration of sarcoidosis until SLTx was 25 yrs. Examination prior to transplantation showed chest radiograph results compatible with stage III pulmonary sarcoidosis and severely reduced lung function values: forced expiratory volume in one second (FEV\(_1\)) 24% predicted, forced vital capacity (FVC) 47% pred and carbon monoxide diffusing capacity of the lung (DL\(_{\text{CO}}\)) 60% pred. Functional status was New York Heart Association class III, with a 6 min walk distance of 80 m.

Histological examination of the recipient’s explanted lung revealed multiple active sarcoid granulomas. After transplantation, the peri-operative course was uncomplicated. Within 4 months after transplantation the patient had three acute cellular rejection episodes (A2–A3) treated with corticosteroids. Maximum pulmonary function was attained at 4 months post-transplant: FEV\(_1\) 66% pred, FVC 71% pred and DL\(_{\text{CO}}\) 67% pred. During this period, there had been no radiological or histological evidence of sarcoidosis in the donor lung. TBB from the donor lung 5 months after transplantation showed recurrence of sarcoid granulomas. At this time, chest radiographical and computerised tomography (CT) scanning of thorax demonstrated mediastinal lymphadenopathy and parenchymal nodules throughout the right lung allograft, consistent with sarcoidosis. Radiological appearances of the patient’s native lung remained unchanged; specifically, there was no evidence of pulmonary sarcoidosis relapse. Immunosuppressive treatment remained unchanged. Two subsequent bronchoscopies with TBB at 6 and 9 months post-transplant, demonstrated peribronchial fibrosis without evidence of sarcoid granulomas or acute cellular rejection. A follow-up chest radiograph and CT scan of the thorax demonstrated progressive volume reduction and diffuse increase in parenchymal density in the lung allograft, with regression of mediastinal lymphadenopathy. In the absence of concomitant infection and/or acute rejection, a diagnosis of bronchiolitis obliterans syndrome (BOS) was made [8]. The last lung function test was severely reduced: FEV\(_1\) 21% pred, FVC 35% pred and DL\(_{\text{CO}}\) 37% pred. BOS was resistant to therapy and the patient died 22 months after transplantation, an autopsy was not performed.

Donor M’s left lung was unsuitable for transplantation as a result of contusion injury; however, the liver was used for transplantation of a 48-yr-old male recipient with end-stage liver disease secondary to sclerosing cholangitis. The liver recipient had no symptoms or radiological appearances consistent with sarcoidosis, and no evidence of granulomas in liver biopsies taken 2 weeks and 4 yrs after transplantation.

**HISTOLOGY AND FLUORESCENCE IN SITU HYBRIDISATION**

TBB specimens were formalin fixed and processed immediately by a microwave oven technique to paraffin, from which serial sections (3–4 μm in thickness) were cut to obtain full coverage of the tissue. Samples were stained with haematoxylin-eosin, Periodic-acid-Schiff, silver staining and Masson-Trichrome. Ziehl-Nielsen staining was performed to detect mycobacteria and immunohistochemical studies were made to detect cytomegalovirus infection.

FISH was applied on TBB from the lung allograft containing sarcoid granuloma using probes for the X- and Y-chromosomes. Biocolour FISH was performed on 1–2 μm thick paraffin sections using specific α-satellite DNA-probes for the X-chromosome (CEP\(_X\) (DXZ1, α-satellite) and SpectrumGreen\(^\text{TM}\) (Vysis, Downers Grove, IL, USA)), and the Y-chromosome (CEP\(_Y\) (DY3, α-satellite) and Spectrum-Orange\(^\text{TM}\) (Vysis)) according to a standard protocol recommended by DakoCytomation (Copenhagen, Denmark), using heating in pre-treatment-solution followed by proteolytic digestion by cold ready to-use-pepsin at room temperature. Denaturation was performed for 1 min at 82°C and hybridisation took place over night at 42°C. The number of hybridisation signals for each probe was assessed in the cells constituting the granulomas and the surrounding tissue. Normal human male and female lung tissue was used as a control.

**RESULTS**

Staining, PCR and culture for mycobacteria, as well as immune staining for Cytomegalovirus was negative in all TBB specimens.

In the explanted lung, the majority of cells in the sarcoid granulomas and the adjacent lung tissue displayed positive signals for the X-chromosomes, but no signal for the Y-chromosome (figure not shown). FISH findings in the transplanted lung are shown in figure 1. In the lung allograft, cells constituting the sarcoid granulomas display positive signals for the X-chromosomes, but no signal for the Y-chromosome. In contrast, the adjacent lung tissue displays positive signals for both the X- and the Y-chromosome.

The pattern of distribution of the X- and Y-chromosome signals indicates that immune cells originating from the female recipient form the sarcoid granulomas in the transplanted lung.

**DISCUSSION**

The lung is the most commonly involved organ in sarcoidosis [3, 4]. Pulmonary involvement shows regression or stability in >90% of patients [5]. Severe pulmonary dysfunction occurs in a small percentage of the patients and accounts for most cases of morbidity and mortality [3, 5].
Non-necrotising epithelioid cell granulomas are the histological hallmark of sarcoidosis. Most likely, these granulomas are formed in response to a persistent inflammatory antigen [1–3]. The dominant cellular constituents of mature epithelioid cell granulomas are non-lymphoid mononuclear cells, which include blood monocytes and tissue macrophages (histiocytes) [15]. In epithelioid cell granulomas, cells belonging to the mononuclear phagocyte system are represented by the macrophages, epithelioid cells and giant cells of the Langhans type [15]. Studies of the Kveim test show that epithelioid cell granulomas can develop in 10–12 days [16]. Accordingly, recurrent sarcoidosis in the lung allograft has been reported as early as two weeks after transplantation [13]. In the three patients within this study, recurrence was observed at 2, 5 and 6 months after transplantation, respectively [6].

It has been suggested that the evolution of allograft rejection and sarcoid granulomas may share a common pathogenesis via activated T-lymphocytes [10, 17]. However, it is conceivable that the formation of sarcoid granulomas and the mechanism of allograft rejection are governed by different immunological pathways [18]. Sarcoid granulomas are considered to be a CD4 T helper 1-type T-lymphocyte mediated process [19, 20], whereas allograft rejection displays characteristics of a lytic CD8 cytotoxic T-lymphocyte response to allograft cells [18, 21]. These differences between the two processes are emphasised by the different responses to immunosuppressive treatment. Cyclosporine A is effective in controlling allograft rejection, but ineffective in controlling the clinical course of sarcoidosis [22]. The observation that recurrence of sarcoid granulomas is common in the transplanted donor lung despite treatment with cyclosporine A, is in accordance with this interpretation.

The transplanted donor lung harbours variable amounts of immune cells (lymphocytes, monocytes, alveolar macrophages) from the donor. These cells will be exposed to the putative antigen involved in the pathogenesis of sarcoidosis, and might, in theory, respond by formation of epithelioid cell granulomas. After blood perfusion has been re-established in the transplanted lung, immune cells from the recipient colonise the graft and start to replace the donor immune cells. Although a few cases of graft versus host disease have been described in association with lung transplantation [23], in the majority of transplanted patients, the donor lymphocytes disappear from the BAL fluid within three months, and the donor alveolar macrophages disappear from the BAL fluid within 6 months of transplantation [24]. This is either due to apoptosis or due to the deleterious effect of the recipient immune response to the allograft. Donor alveolar macrophages may be the cell type with the longest “survival” time in the lung allograft, as in a few patients, they have been recovered in BAL fluid several years after transplantation [24]. In the patient in the present study, the sarcoid granulomas in the donor lung were discovered 5 months after transplantation, i.e. donor immune cells might still have been present in the lung allograft.

In theory, the donor could have had undiagnosed, subclinical pulmonary sarcoidosis. The left donor lung had a contusion and was unacceptable for transplantation; however, the donor liver was transplanted. The recipient did not show evidence of sarcoid disease and surveillance biopsies of the liver did not...
show any granulomas. Therefore, it appears unlikely that unrecognised sarcoid disease could have been transmitted with the donor lung.

The genetic predisposition involved in the pathogenesis of sarcoidosis may also influence the reaction of the immune cells following exposure to the unidentified sarcoidosis antigen. The recipient immune cells are known to carry the genetic predisposition. The donor immune cells probably do not have a similar genetic predisposition and, therefore, may not react by granuloma formation when exposed to the same antigen.

In conclusion, the present results show that recurrent sarcoid epithelioid cell granulomas in the transplanted lung allograft are derived from recipient immune cells; however, additional studies are required to substantiate these findings.

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