**CASE STUDY**

**Tumour necrosis factor-α gene expression by alveolar macrophages in human lung allograft recipient with recurrence of sarcoidosis**

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End-stage fibrosis due to sarcoidosis is an unusual, but previously reported, indication for lung transplantation [1]. Recurrence of sarcoidosis has been demonstrated in renal [2] and cardiac [3] transplantation, and two recent studies have noted it in lung transplantation [4, 5]. Such cases offer a unique occasion to monitor the development of sarcoid granulomas in the lung and of local biological abnormalities potentially relevant to the pathogenesis of sarcoidosis. Although mechanisms regulating this pathogenesis remain poorly understood, a number of mediators have been involved, such as proteases, oxygen metabolites, arachidonic acid metabolites and cytokines [6]. Among these cytokines, tumour necrosis factor-alpha (TNF-α) is likely to play a role. Alveolar macrophages from patients with active sarcoidosis have an increased in vitro secretion of TNF-α and an increased TNF-α messenger ribonucleic acid (mRNA) expression when compared to healthy subjects [7–9]. A similar increase has also been observed in other granulomatous diseases [10]. TNF-α has been shown to stimulate T-lymphocyte proliferation, expression of class II histocompatibility antigens and release of cytokines, such as interleukin-1 and interleukin-6 (IL-1 and IL-6) [11]. As such, it has been involved in the pathogenesis of sarcoidosis and also in acute rejection in experimental models of lung transplantation [12].

We report a case of recurrence of sarcoidosis in the allograft 2 years after a single lung transplantation. Sequential monitoring allowed us to demonstrate a lack of increase in the expression of TNF-α gene by alveolar macrophages at the time of recurrence. In contrast, we observed an increased expression occurring later in the disease process when granulomas were associated with acute rejection.

**Case report**

In November 1990, a 25 year old man underwent a right single lung transplantation for end-stage lung fibrosis attributed to stage IV sarcoidosis. Histological diagnosis of sarcoidosis was obtained on a prescalenic node biopsy performed 2 yrs previously. The explanted lung demonstrated extensive fibrosis with honeycomb and no active granulomatous disease. Post-transplantation immunosuppression included cyclosporin, prednisone and azathioprine. In 1991, no complications occurred. Nine months after surgery, the forced expiratory volume in one second (FEV1) was 3 L (FEV1/forced vital capacity (FVC) 89%). At this time, the patient developed a febrile bronchitis due to *myxovirus influenzae*. Twenty two months after lung transplantation (September 1992), a grade 2 rejection episode occurred. The patient received the same steroid regimen as had been prescribed in January 1992.

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In November 1992, transbronchial biopsies (TBB) demonstrated a few noncaseating granulomas consistent with recurrence of sarcoidosis. This finding was associated with a 0.5 L drop in FEV1 (FEV1/FVC 89%). At this time,
gradually tapered to 30 mg·day⁻¹; respiratory functional
66%). From February to September 1993, prednisone was
jection was still observed, whilst there was no more gra-

Granulomas; D: acute rejection grade 1; E: bronchiolitis obliterans syn-

recurrence of sarcoidosis (granulomas); C: acute rejection grade 1 and

sion (ratio±SEM) over a 2 year period. A: acute rejection grade 2; B:

acid-fast stains were negative and no fungal elements or
cysts were identified. Chest radiographic image was nor-
mal but computed tomographic (CT) scan demonstrated
 discrete ground-glass opacities, without significant medi-
stinal nodal enlargement. Serum angiotensin converting
enzyme was slightly elevated to 28.4 units (normal range
11–23 units). Prednisone was increased from 15 to 40
gmg·day⁻¹. One month later (December 1992), granulomas
were no longer observed in TBB and the prednisone dos-
age was gradually reduced to 20 mg·day⁻¹ in the follow-

ing month.

In January 1993, prominent granulomas associated with
a grade 1 rejection were observed in TBB and no asso-
ciated infectious process could be identified. The patient
was treated with a steroid pulse and then remained on
prednisone, 40 mg·day⁻¹. In February 1993, a grade 1 re-
jection was still observed, whilst there was no more gra-

Rulomas. At this time, FEV₁ was 1.45 L (FEV₁/FVC 66%). From February to September 1993, prednisone was
ggradually tapered to 30 mg·day⁻¹; respiratory functional
status remained stable in this period. In November 1993,
a further decrease in FEV₁ led to transbronchial biopsies
and since no acute rejection, granulomas or infectious
process could be identified, this functional deterioration
was attributed to bronchiolitis obliterans syndrome (BOS).
Two subsequent transient increases of the prednisone dos-
age have permitted the respiratory functional status
to be stabilized. Today, the patient is still alive 54 months
after transplantation, with a FEV₁ of 0.85 L (BOS stage
3a).

Measurements of TNF-α and β-actin gene expression
by alveolar macrophages were made in seven broncho-
alveolar lavages (BAL) from this patient, using tech-
niques described previously [10, 13]. The first BAL was
performed in November 1991, when no complications
had yet occurred. The second BAL was performed in
February 1992, 1 month after the clinical rejection episode.
The subsequent lavages were carried out in September
1992 (grade 2 rejection), November 1992 (granulomas),
January 1993 (grade 1 rejection and granulomas), February
1993 (grade 1 rejection) and November 1993 (BOS). As
shown in figure 1, formation of granulomas in November
1992 was not associated with an increased expression of
the TNF-α gene by alveolar macrophages, but TNF-α
gene expression increased later in January 1993 when
granulomas were associated with a grade 1 rejection.
The gene expression returned to baseline after increase
of the steroid dosage and disappearance of the granulo-
as. At the time of BOS, TNF-α gene expression sub-
sequently peaked. Values in January 1993 and November
1993 are not significantly different from each other but
are significantly higher (p<0.05, unpaired t-test) than the
five other values.

Discussion

The incidence of recurrence of sarcoidosis in a lung
allograft is not known but is likely to be frequent. Indeed,
in the two other reports published so far on this topic,
sarcoid granulomas developed in the allograft of five of
the seven patients transplanted for end-stage pulmonary
sarcoidosis [4, 5]. This phenomenon is an interesting
model to study the pathogenesis of sarcoidosis. In the
follow-up of this patient, we observed an increase in TNF-
α gene expression by alveolar macrophages; this over-
expression was not observed when granulomas were first
found in November 1992 but later, in January 1993, when
granulomas were associated with a grade 1 acute rejec-
tion. Although quantification of granulomas on trans-
bronchial biopsies remains difficult, it is notable that
granulomas were prominent in January 1993 but not in
November 1992. We can, therefore, hypothesize that
TNF-α is not implicated in the early events of forma-
tion of granulomas in lung sarcoidosis but rather plays
a role in perpetuating and increasing the granulomatous
process.

Another potential explanation would be that there is a
synergia for TNF-α gene expression when sarcoidosis
and rejection are associated. Indeed, an overexpression of
TNF-α gene has been demonstrated not only in sar-
coidosis [10] but also in experimental models of lung
allograft rejection [12]. Against this hypothesis, there
was no increase of TNF-α gene expression by alveolar
macrophages at the time of a grade 2 rejection episode
in September 1992. This is apparently in contrast with
observations made in rejection of human liver and renal
allografts, where in situ expression of TNF-α gene using
in situ hybridization was shown to be increased [14, 15].
However, in rejection of human lung allografts, only one
report showed an increase in spontaneous in vitro TNF-
α production by alveolar macrophages; two other stud-
ies did not observe this phenomenon and even showed
a decrease in stimulated TNF-α production by alveolar
macrophages when compared to healthy lung recipi-

ents [16–18].

Finally, TNF-α gene expression by alveolar macrophages
subsequently peaked when our patient developed BOS.
Little is known about the role of cytokines in the patho-
genesis of obliterative bronchiolitis. Fibrogenic growth factors seem to be implicated, since Hertz et al. [19] demonstrated an increased concentration of platelet-derived growth factor in lavage fluid from lung allograft recipients with obliterative bronchiolitis. As we have shown [20], gene expression of inflammatory cytokines, such as interleukin-8 (IL-8) is elevated in the course of other small airway diseases, such as bronchiolitis obliterans with organizing pneumonia. No study has yet investigated the role of TNF-α in the pathogenesis of obliterative bronchiolitis. However, this cytokine has been shown to be involved in fibroproliferative disorders [21].

This case report shows that the follow-up of the behaviour of alveolar cells in lung transplant recipients can be relevant to the study of the pathogenesis of complications such as BOS and also, in the case of transplantation for end-stage pulmonary sarcoidosis, to the study of the potential role of these cells and cytokines in the pathogenesis of this disease, since it seems to recur quite often. It also provides new information about the potential role of tumour necrosis factor-α in the orchestration of lung inflammation in the transplanted lung and in the pathogenesis of sarcoid granulomas.

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References