Virchows Arch (2000) 436:365–369

ORIGINAL ARTICLE

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Mycobacterial DNA in recurrent sarcoidosis in the transplanted lung – a PCR-based study on four cases

Received: 19 August 1999 / Accepted: 11 November 1999

Abstract Sarcoidosis is a systemic granulomatous inflammation, which may be caused by mycobacteria other than *M. tuberculosis* complex (MOTT) in one-third of cases. A few cases of recurrent sarcoidosis in the transplanted lung have been reported. However, mycobacteria have been excluded by acid-fast stains only. We investigated four cases of recurrent sarcoidosis in lung transplant patients. Using PCR for the insertion sequence 6110 of *Mycobacterium tuberculosis* complex and a second PCR for the mycobacterial chaperonin (65-kDa antigen coding sequence), we looked for mycobacterial DNA. In three cases sequence analysis was also performed. One patient was negative for mycobacterial DNA in explanted, but positive for *M. tuberculosis* DNA in transplanted lung, qualifying this case as *M. tuberculosis* infection in the transplant. Three patients were negative for *M. tuberculosis* DNA, but were positive for MOTT-DNA in both explanted and transplanted lungs. In these three patients sequence identity of the amplified sequences before and after transplantation was proven, which rules out mycobacteriosis. Recurrent sarcoidosis does occur, but can only be proven by the exclusion of mycobacterial DNA. In cases of recurrent MOTT-DNA-positive sarcoidosis the diagnosis cannot be confirmed except by proof of sequence identity. Probably MOTT-DNA-positive sarcoidosis is more likely to recur in a transplanted lung.

Key words Recurrent sarcoidosis · Mycobacterial DNA

Parts of this work have been presented as a poster at the Annual Meeting of the US-Canadian Academy of Pathology, Boston, 1998

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Introduction

Sarcoidosis is a systemic granulomatous inflammatory disease for which no common cause can be specified. Recently we have demonstrated MOTT-DNA in one third of cases [17], and others have found *Proprionebacterium* (*Corynebacterium acnes*) in sarcoid granulomas [5]. Since transplantation for sarcoidosis is rare, only single cases with recurrence in different organs have been reported [1, 2, 10–12, 14, 16, 18]. Even large transplant centers have collected only five cases [7, 19]. However, recurrent sarcoidosis appears to be common after lung transplantation [7, 11]. Diagnoses in these reported cases were substantiated by clinical and radiological methods. In all cases granulomas were found in the biopsies. The diagnosis of recurrent sarcoidosis was based essentially on the exclusion of stainable mycobacteria and other organisms, mainly fungi. However, uncertainty remains, because mycobacteria in paucibacillary tuberculosis might escape microscopic detection and mask recurrent sarcoidosis [15]. Mycobacteriosis caused by atypical mycobacteria is another source of mistaken diagnosis, because it can mimic sarcoidosis, presenting as nonnecrotizing epithelioid cell granulomatosis [8]. To test our assumption that MOTT-DNA-positive sarcoidosis might be much more prone to recurrence after lung transplantation, we investigated four cases for the presence of mycobacterial DNA.

Materials and methods

Formaldehyde-fixed and paraffin-embedded lung tissue from four cases of recurrent sarcoidosis were investigated. Noncaseating epithelioid cell granulomas were present throughout the tissue specimens, and histochemical stains for mycobacteria and fungi on serial sections were consistently negative. The sections were processed for PCR for the mycobacterial chaperonin found in all mycobacteria, and for the insertion sequence of *M. tuberculosis* complex with PCR conditions essentially as described elsewhere [17]. An assay for the preservation and integrity of the DNA was done in all tissue samples using a PCR for β-globin [17].

As negative controls we used human lung tissue samples from ten lobectomies performed for carcinoma, six cases of purulent bronchiectasis, twelve of pneumococcal pneumonia, four of viral pneumonia and seven of bronchiolitis obliterans-organizing pneumonia. As positive controls we used biopsies from culture-proven cases of tuberculosis and cultures of *Mycobacterium tuberculosis, kansasii, avium,* and *fortuitum*. To ascertain the presence of granulomas throughout the sections, the first and last ones were stained with H-E and examined. The PCR was performed for sarcoidosis cases and all controls together and repeated three to five times for each case, the limiting factor being either the amount of tissue, i.e., small transbronchial biopsies, or the loss of granulomas.

The PCRs were done using the Gene-Amp PCR System 2400 (Perkin-Elmer, Vienna, Austria). The PCR products were extracted from agarose gel according to the QIAquick gel extraction kit protocol (QIAGENE, Hilden, Germany) to eliminate unbound primers and dNTPs. For the sequence reaction the AutoCycle sequencing kit was used (Pharmacia Biotech, Vienna, Austria). For gel electrophoresis with FITC primer pairs (same as for nested PCR) the ALF-DNA sequence analyzer was used (Pharmacia Biotech, Vienna, Austria).

Owing to the small amount of tissue in two cases, we did not perform multiplex PCR for a subspecification of the mycobacterial DNA as previously demonstrated [9].

Clinical data

Case 1

The patient was a 57-year-old woman in whom sarcoidosis with pulmonary involvement was first diagnosed at age 45. Despite corticosteroid therapy, the disease progressed to end stage and the patient received a right single lung transplant in July 1995. A routine biopsy performed approximately 1 year after the transplant revealed noncaseating granulomata with negative microbiology. No specific therapy was added, and the patient remains well with stable graft function 18 months after recurrence.

Case 2

This 45-year-old woman had sarcoidosis, which had been diagnosed when she was 30 years of age. Clinical disease involved the lungs and skin. Progression of the disease despite therapy resulted in end-stage lung disease with pulmonary hypertension and cor pulmonale. The patient received a right single lung transplant in April 1992. Her course was complicated by multiple episodes of acute rejection. Recurrent sarcoidosis was diagnosed by transbronchial biopsy 1 year after the transplant. She developed bronchiolitis obliterans syndrome at about 18 months after the transplant and died 30 months after the transplant operation.

Case 3

This patient was a 48-year-old man who had had sarcoidosis since 1978. He progressed to end-stage lung disease, without other organ system involvement, and received a bilateral lung transplant in January 1995. The posttransplant course was complicated by prolonged (>30 days) mechanical ventilation and tracheostomy and one episode of CMV pneumonia. Surveillance biopsy 1 year after the transplant showed noncaseating granulomata. His immunosuppression regimen was altered by reduction of the azathioprine dose from 2 mg to 1 mg/kg per day and the addition of methotrexate 10 mg/week. He remains well, with stable graft function, 3 years after the transplant.

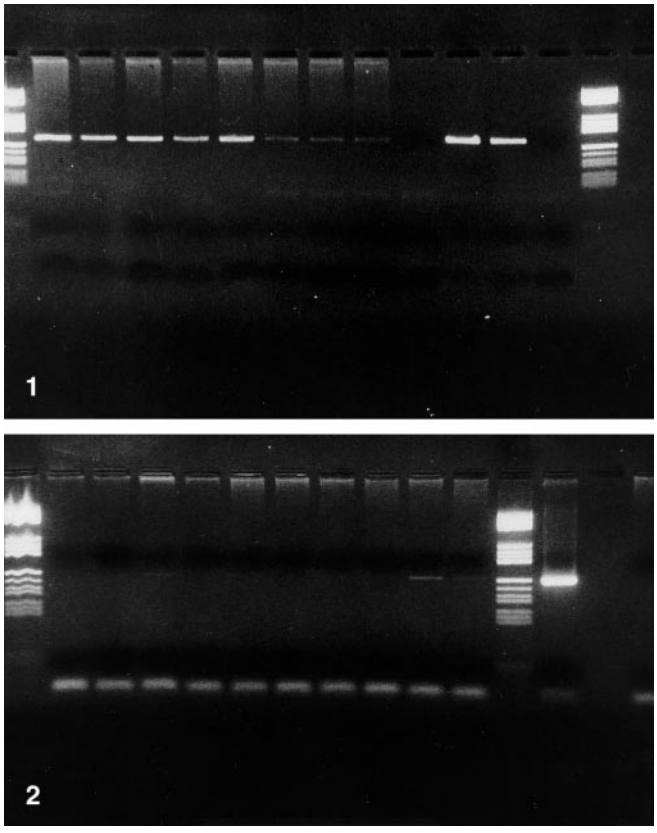

Fig. 1 PCR amplification products of mycobacterial DNA from the chaperonin gene: *lanes 1, 14* molecular weight markers; *lanes 2, 3, 4* PCR product from sarcoidosis granulomas from explanted lungs of patients 1, 2, and 3, respectively; *lanes 5, 6* PCR product from sarcoidosis granulomas from transplanted lungs of patients 1 and 2, respectively; *lanes 7–9* PCR product from sarcoidosis granulomas from transplanted lungs of patient 3, from three different biopsies, taken at different times; in each case the transplant biopsies were taken 1 year after transplantation; *lane 10* PCR product from sarcoidosis granulomas from explanted lungs of patient 4 (negative for mycobacterial chaperonin); *lane 11* PCR product from sarcoidosis granulomas from transplanted lungs of patient 4 (positive for mycobacterial chaperonin); *lane 12* positive control (cultured *M. avium*); *lane 13* negative control

Fig. 2 PCR products from amplified IS 6110: *lanes 1, 12* molecular weight markers; *lanes 2, 3* tissue from granulomas of patient 1 after and prior to lung transplantation; *lanes 4, 5* tissue from granulomas of patient 4 after and prior to lung transplantation, showing a positive signal in *lane 4* from transplanted lung (patient 4) only; *lanes 6, 7* tissue from granulomas of patient 2 after and prior to lung transplantation; *lanes 8, 9* tissue from granulomas of patient 3 after and prior to lung transplantation; *lanes 10, 11* positive control from paraffin-embedded lung tissue of a confirmed case of tuberculosis; *lane 13* positive control from cultured *M. tuberculosis*; *lane 14* negative control

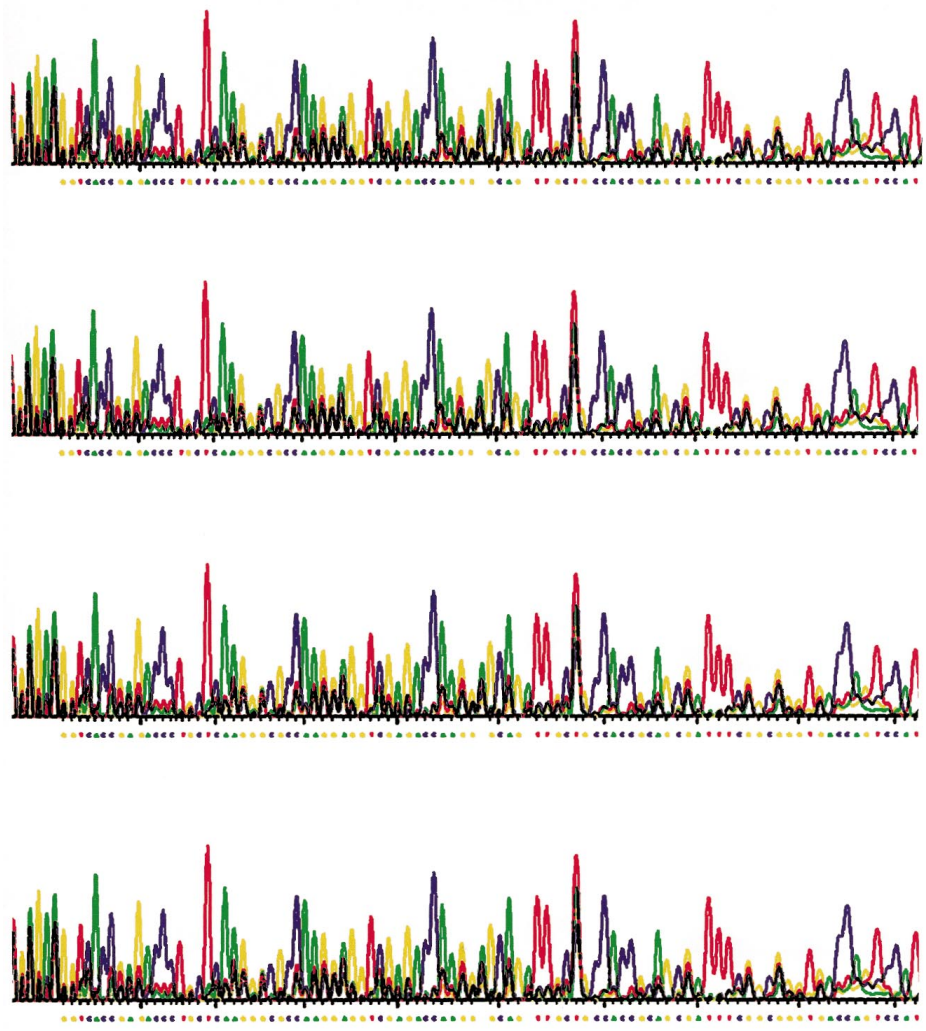

Fig. 3 Comparison of sequences from the chaperonin amplificates of patients 1 and 3, prior and after lung transplantation. Shown are the original output samples from the sequencer including the genetic code below. Lanes 1, 2 (from *top* to *bottom*) are from patients 1 and 3 before, and *lanes 3, 4* are from the same patients after transplantation; nucleotides, *red* T, *green* A, *yellow* G, *blue* C. Sequence identity is clearly evident from these curves

Case 4

This 40-year-old woman with long-standing sarcoidosis received bilateral lung transplants in August 1993 following progression to end-stage lung disease. Between October 1993 and March 1994 she had BAL examinations, which showed *Candida* and *Aspergillus* infection. In addition she had CMV infection during this period. In December and March lung biopsies showed acute rejection, graded 1 and 2–3, respectively. She was treated in March for rejection, and after that had decreased lung function data, but only mild symptoms. She became dyspneic 6 days later and was admitted to hospital with the tentative diagnosis of graft rejection or fungal or viral infection. During her stay in hospital she underwent numerous cultures, but no acid-fast bacteria or *Pneumocystis* species or other fungi were ever seen. CMV infection was verified by PCR and isolated from BAL. The patient was treated with ganciclovir and antibiotics, and also with tuberculostatic drugs because of suspected *Mycobacterium tuberculosis* infection. One month later she died despite all medications. At autopsy the main diagnoses were acute interstitial pneumonia with diffuse alveolar damage, bilateral pulmonary emboli in right and left upper lobes, noncaseating epithelioid cell granulomas negative on acid-fast stains, interpreted as recurrent sarcoidosis in lung graft, with end-stage fibrosis, biventricular cardiac hypertrophy and fibrinous pericarditis and lymphocytic meningitis.

Results

In tissue samples from all four cases a positive amplification was obtained for the β-globin gene, which can be taken as a measurement of DNA integrity.

PCR for the chaperonin gene:

In DNA extracts from sarcoid granulomas derived both from explanted and from transplanted lungs of patients 1–3 the mycobacterial chaperonin could be amplified (Fig. 1). In DNA extracts from sarcoid granulomas of lungs explanted from patient 4 the PCR for the chaperonin gene was negative (Fig. 1). However, a positive amplification was achieved from sarcoid granulomas from autopsy material of the transplanted lungs in this patient.

PCR for insertion sequence 6110

A PCR for IS 6110 was negative in sarcoid granulomas in explanted and transplanted lungs of patients 1–3. IS

6110 was also negative in the explanted lungs of patient 4, but positive in the transplant (Fig. 2).

Sequence analysis:

Chaperonin amplificates from the sarcoid granulomas in explanted and transplanted lungs from patients 1–3 were sequenced and compared against each other. Each of these sequences showed 100% homology between explant and transplant (Fig. 3). There was also 100% homology between the amplified sequences before and after transplantation in all three cases.

Discussion

Two different scenarios have been seen in our four cases:

1. One case of sarcoidosis was negative for mycobacterial DNA before, but was positive after, transplantation (case 4). In this case chaperonin DNA and IS 6110 were amplified, which qualifies this case as *M. tuberculosis* infection in the transplanted lung, but sarcoidosis before transplantation. This case was diagnosed primarily as consistent with recurrent sarcoidosis, because regular stains and culture were negative. However, based on PCR results and the knowledge of antituberculous treatment, this case now qualifies as paucibacillary tuberculosis. This cases highlights the necessity of performing PCR for mycobacteria in certain complex cases.
2. Three cases of sarcoidosis were positive for MOTT-DNA before and after transplantation. Since it would be highly unusual for mycobacteriosis to remain stable for a long time before and after transplantation, we feel that these patients had been suffering from MOTT-DNA-positive sarcoidosis prior to lung transplantation. Further, because clinically evident mycobacterial infection was absent, pre- and posttransplant sequencing of the amplificates was performed. This showed 100% homology between the pre- and posttransplant pairs, but in addition also among the three cases. Since the statistical probability of this occurring by chance is quite small, the most likely explanation for these findings is that MOTT-DNA-positive sarcoidosis existed before transplantation and recurred in these three cases. Laboratory-based contamination could be excluded by the negative controls. In addition, there was an inflammatory response with granuloma formation. But the 100% homology of the chaperonin sequences in all three cases still had to be interpreted. A 100% homology in these cases could be explained in two different ways. Within the region of the open reading frame of the chaperonin gene amplified by PCR there is a high degree of homology between the different mycobacteria [4, 9]. The sequence we have found could belong either to *M. avium* or to *M. fortuitum*, for example. The chaperonin sequences have been analyzed for only a few mycobacteria so far, mostly pathogenic species, while in many others the sequence is unknown. Nonpathogenic mycobacteria have not yet been analyzed. Therefore the 100% homology in our cases could be interpreted as an indication that in these cases all sequences might belong either to *M. avium* or to another MOTT species. However, members of the tuberculosis complex could be ruled out.

We are unable to subspecify the MOTT-DNA further, because in two cases not enough DNA from the granulomas was available. In another investigation a method of subspecification was used [3] that could also have been used in our investigation. A major problem of both methods, including our own [9], is the poor knowledge of genetic sequences among pathogenic and apathogenic mycobacteria: eight different MOTTs could be identified by the method of Cook et al. [3], and five different MOTTs, by the method of Klemen et al. [9]. Many more pathogenic MOTTs and vast numbers of nonpathogenic mycobacteria do exist, for which the genomic sequences including the chaperonin sequence are not known.

Other authors [6] were able to amplify *Mycobacterium tuberculosis* complex DNA fragments (IS 6110) in 64% of sarcoidosis patients investigated. This confirms other, earlier reports (see Discussion in reference [17]) and reflects the probable multifactorial etiology of sarcoidosis. Investigators in Japan have demonstrated DNA of *Corynebacterium acnes* in many of their sarcoidosis patients and discussed the possibility that in these patients *Corynebacterium acnes* might be the causative agent [13].

We have shown that recurrent sarcoidosis in lung transplant recipients has an unusually high positivity for MOTT-DNA. One might speculate that positivity for mycobacterial DNA could be the main cause for recurrence, whereas the chance of recurrence is lower in MOTT-negative cases. This points to a probable mechanism for a hypothetical thesis on the etiology of sarcoidosis. In the background there is probably a genetically based hypersensitivity reaction with abnormal up-regulation of T-H1-lymphocytes, or a loss of suppressor function [4]. An allergic reaction is started by an infection with slow-growing or even apathogenic mycobacteria as well as other organisms, such as *Corynebacteria*, which might have in common the property of being slowly degradable, for example because of their capsule. The allergic reaction is mounted by the antigenicity of different capsule structures, bacterial proteins/polypeptides, and probably also DNA. This hypothesis might explain why MOTT-DNA-positive cases recur after transplantation. However, this must be proven in a multicenter study with larger series. In addition, we have shown that a negative acid-fast stain does not rule out mycobacterial infection in transplant patients.

Acknowledgements This study was supported by a grant (no. 6148) from the Austrian National Bank Jubilee Fund to H.H.P., which is gratefully acknowledged.

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