Absence of Ribosomal RNA of Mycobacterium tuberculosis Complex in Sarcoidosis

Joaquim Marcoval, MD; Miguel A. Benítez, MD; Fernando Alcaide, MD; Juan Mañá, MD

Objective: To determine whether Mycobacterium tuberculosis ribosomal RNA (rRNA) is present in fresh tissue specimens from patients with sarcoidosis.

Design: A prospective study.

Setting: A university-based hospital.

Patients: Thirty-five patients diagnosed as having sarcoidosis at the University Hospital of Bellvitge, Barcelona, Spain, were included in the study. Fresh tissue samples with granulomatous inflammation were prospectively collected between 1997 and 2001 from all patients. For each sample tested, approximately 1 negative control was included.

Main Outcome Measures: Mycobacterium tuberculosis rRNA was detected using an isothermal enzymatic amplification system of target rRNA of M tuberculosis complex via DNA intermediates. Smears for acid-fast staining and mycobacteriological cultures were also obtained.

Results: A total of 78 biopsy specimens (57 skin, 10 lymph node, 3 lacrimal gland, 2 spleen, 2 lung, 2 muscle, 1 bone, and 1 nerve) collected from 74 patients (35 patients with sarcoidosis and 39 control patients) were included in the study. Stains for acid-fast bacilli and mycobacteriological cultures were negative for organisms in all cases. Mycobacterium tuberculosis rRNA was not detected in the specimens from any patients with sarcoidosis or in those from control patients whose cultures were negative for organisms. Ribosomal RNA was detected in 6 tissue specimens from patients with cultures that were positive for M tuberculosis and that were processed in parallel to the samples included in the study.

Conclusions: Although previous studies have reported that mycobacterial antigens may play a role in granuloma formation in some patients with sarcoidosis, our results suggest that M tuberculosis cannot be considered to be the etiologic agent of the disease.

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Sarcoidosis is a multisystemic disease that is defined by the formation of noncaseating granulomas in different organs. Although the etiology of sarcoidosis remains uncertain, the apparition of clusters of the disease in some communities and the existence of seasonal variations in incidence suggest that environmental or infectious agents may play a role in its development. Moreover, similarities in the clinical, pathologic, and immunologic abnormalities in patients with sarcoidosis and in certain patients with tuberculosis have raised the suspicion that mycobacterial infection could be associated with the pathogenesis of this disorder.

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In recent years, some studies have focused on the detection of mycobacterial DNA by polymerase chain reaction (PCR) in patients with sarcoidosis, with quite divergent results. Mycobacterial DNA has been found in approximately half of the samples in some studies, while it was not detected at all in other studies. Most of these studies were retrospective and were carried out in paraffin-embedded tissues. Only 1 study, which was performed using liquid-phase hybridization, looked for mycobacteria-specific rRNA in fresh samples from a small number patients with sarcoidosis (n=5).

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Author Affiliations:
Departments of Dermatology (Dr Marcoval), Microbiology (Drs Benítez and Alcaide), and Internal Medicine (Dr Mañá), Hospital de Bellvitge, University of Barcelona, Barcelona, Spain.
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vitge (a 1000-bed teaching institution in Barcelona, Spain). The diagnosis of systemic sarcoidosis was made according to the classic criteria: a compatible clinical and radiologic picture; histologic demonstration of noncaseating granulomas involving 1 or more tissues or a positive Kveim test result; and exclusion of other granulomatous diseases. Half of the specimen from each patient was fixed in 10% buffered formaldehyde and processed for histologic evaluation to determine whether granulomas were present in the samples.

CONTROLS
At least 1 negative control was included for each test sample. The negative control samples, which were analyzed in the same manner as the test samples, consisted of fresh specimens of normal tissue obtained from patients without evidence of sarcoidosis or tuberculosis. Most cutaneous specimens were obtained from redundant normal skin acquired during minor surgical procedures.

SPECIMEN PROCESSING, MICROSCOPY, AND CULTURE
All biopsy specimens were homogenized in 3 mL of 0.9% sodium chloride solution within 24 hours of specimen collection. Part of this suspension was used for the preparation of smears for acid-fast staining with auramine-rodamine fluorochrome and for culture. The remaining suspension was stored at -80°C for subsequent use in the genetic amplification procedure. An equal volume (0.5 mL) of the processed specimens was inoculated in 2 culture media: a liquid medium bottle (MB/BacT; bioMérieux SA, Marcy l’Etoile, France) and a Lowenstein-Jensen slant (MAIM, Barcelona, Spain) as a solid medium. All cultures were incubated at 35°C to 37°C for up to 6 weeks. The bottles were registered and processed by means of a nonradiometric system for incubation and continuous monitoring of mycobacterium growth (BacT/ALERT 3-dimensional instrument; bioMérieux SA). The Lowenstein-Jensen media were incubated in 3% carbon dioxide and examined for colonies on the slant once a week.

EXTRACTION OF NUCLEIC ACIDS
The material was extracted with a commercially available kit (RNA/DNA Maxi Kit; Qiagen, Hilden, Germany) that allows rapid isolation of RNA and DNA from small numbers of bacteria in clinical samples.

GENETIC AMPLIFICATION PROCEDURE
A nucleic amplification test (AMDT; Gen-Probe Inc, San Diego, Calif) was used to detect M tuberculosis RNA. This test is an isothermal amplification system based on the reverse transcription of mycobacteria-specific tRNA targets via DNA intermediates. It uses 2 enzymes (RNA polymerase and reverse transcriptase) and 2 primers, one of which contains a promoter sequence for RNA polymerase. Detection of RNA amplicons is achieved with an acridinium ester-labeled DNA probe. The entire process (amplification and detection) is performed in a single tube, which helps to reduce the risk of carryover contamination. In the present study, the amplification test was performed and the results were interpreted according to the manufacturer’s recommendations. Six clinical samples (3 lymph node specimens and 1 biopsy specimen each of spleen, liver, and lung) with cultures that were positive for M tuberculosis were used as quality control for extraction and amplification techniques.

RESULTS
A total of 78 specimens obtained from 74 patients (35 patients with sarcoidosis and 39 control patients) were included in the study. The 37 specimens obtained from specific granulomatous lesions of 35 patients with sarcoidosis were skin (n=28), lymph node (n=5), muscle (n=2), lung (n=1), and lacrimal gland (n=1). The 41 specimens obtained from 39 control patients were skin (n=29), lymph node (n=5), spleen (n=2), lacrimal gland (n=2), lung (n=1), bone (n=1), and nerve (n=1).

All the specimens included in the study were negative for acid-fast bacilli. Likewise, all the cultures were negative for M tuberculosis after at least 6 weeks of incubation. The 37 samples from the 35 patients with sarcoidosis and the 41 specimens from the 39 subjects in the control group were all negative for M tuberculosis rRNA. However, rRNA was detected in 6 tissue specimens with cultures that were positive for M tuberculosis and that were processed in parallel to the samples included in the study.

COMMENT
In the present study, we were not able to detect the presence of M tuberculosis rRNA in fresh tissue samples of granulomatous lesions prospectively collected from patients with systemic sarcoidosis. The smears for acid-fast bacilli and mycobacteriological cultures were also negative for organisms. Clinical and pathologic similarities to pulmonary tuberculosis have suggested a potential role of mycobacteria in the etiology of sarcoidosis. The histopathologic features of the granulomas and the occasional presence of caseation necrosis also indicate the possibility of a mycobacterial pathogenesis in sarcoidosis. However, the absence of caseation in most instances, the negativity of mycobacterial cultures, and the absence of a response to antituberculous treatment are factors that contradict this theory. Likewise, over the past century, while there has been a marked reduction in the incidence of tuberculosis, the incidence of sarcoidosis has not decreased.

During the last decade, several studies have used PCR analysis in an attempt to detect mycobacterial DNA in a number of biopsy specimens from patients with sarcoidosis. The findings of these studies have been quite inconsistent, with positive results ranging from 0% to 80%. The surprising variability of these data may be explained by several technical factors, including the choice of target sequence and DNA-retrieval variability, depending on which technique is used to obtain the DNA. An alternative explanation for these divergent results is inadvertent contamination of samples in some series. It is noteworthy that the studies showing the highest proportion of positive results for samples from patients with sarcoidosis also reported a relatively high proportion of positive results for samples from control subjects and/or from individuals with prior tuberculous infections. Moreover, most studies about the detection of mycobacterial DNA using PCR in sarcoidosis were performed in formalin-fixed, paraffin-embedded specimens from retrospective pools of pathology departments. In such studies, there is a greater possibility of both contamination of the paraffin-
embedded specimens and more DNA fragmentation. However, the present study was prospective and only fresh tissue was used. For each patient, half of the biopsy specimen was processed for routine histologic evaluation to confirm the presence of granulomas in the samples included in the study. The use of fresh tissue allowed us to apply a technique of rRNA amplification that has several advantages. It is more sensitive than DNA amplification because there are more than 2000 copies of rRNA for 1 DNA molecule. Another advantage of the nucleic acid amplification system that was used in the present study is the low level of cross contamination, because the entire process is performed in a single tube. The only study analyzing the presence of rRNA of mycobacteria on fresh samples of sarcoidosis included only 5 patients and used a technique in which the samples were more easily exposed to exogenous contamination (liquid-phase hybridization).

To establish epidemiologically that an infective agent is the cause of disease requires consistent isolation of the agent from cases and production of disease in an organism when the agent is introduced. These points have not been demonstrated consistently to date. Polymerase chain reaction detection of mycobacterial DNA in sarcoidosis does not indicate whether the detected mycobacteria are still viable and susceptible to antituberculous therapy or whether they represent nonviable remnants of prior mycobacterial colonization. The presence of mycobacterial DNA in tissues involved with sarcoidosis only suggests that mycobacterial vestiges may be present in some lesions in a variable proportion of patients. However, the absence of mycobacterial rRNA, despite the higher sensitivity compared with DNA detection, and the negativity of the cultures rule out the presence of viable mycobacteria in the sarcoidosis lesions.

We suggest that vestiges of mycobacteria may act as a stimulus for granuloma formation in some patients who are developing sarcoidosis, as can occur when there are foreign bodies in the skin. This hypothesis may explain why mycobacteria have never been recovered from sarcoidal tissue, why tuberculostatic therapy of sarcoidosis has generally not been successful, and why reactivation of tuberculosis has not been observed in sarcoidosis despite the use of corticosteroids or other immunosuppressive agents.

In conclusion, although mycobacterial antigens may be responsible for granuloma formation in some patients, our results suggest that M. tuberculosis cannot be considered the etiologic agent of sarcoidosis.

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Correspondence: Joaquim Marcoval, MD, Department of Dermatology, Hospital de Bellvitge, c/Feixa Llarga s/n, Hospital de Llobregat, 08907 Barcelona, Spain (jmarcoval@csub.cs.es).
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