Detection of Mycobacterial DNA in Papulonecrotic Tuberculid Lesions by Polymerase Chain Reaction

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Tuberculids are a heterogenous group of cutaneous lesions. Recent discoveries of *M. tuberculosis* DNA in these lesions by PCR suggest that *M. tuberculosis* could play a role in their pathogenesis. The aim of this study was to demonstrate the presence of *M. tuberculosis* DNA by polymerase chain reaction in papulonecrotic tuberculid lesions. Skin biopsy specimens from ten patients with papulonecrotic tuberculid lesions (histopathologic features) were studied. All of them tested solidly positive in a tuberculin intradermal test. A gene-amplification PCR, using primers capable of amplifying

DNA in the *M. tuberculosis* complex, was performed to detect *M. tuberculosis* DNA in the lesions. A 285-bp sequence specific of *M. tuberculosis* complex was amplified and confirmed by Southern-blot hybridation with a 32 p 5'-labelled internal probe. No inhibitors were detected in the negative PCR samples. The PCR technique makes the detection of mycobacterial DNA in tuberculids a possibility, and therefore provides a rational basis for antituberculous therapy and for the clinical management of these disorders. J. Clin. Lab. Anal. 14:133–135, 2000. © 2000 Wiley-Liss, Inc.

Key words: tuberculids; PCR

INTRODUCTION

Tuberculous skin lesions are rare entities and so often are not suspected or detected (1–4). On the other hand, the diagnostic identification of *M. tuberculosis* and the related organisms of the *M. tuberculosis* complex (*M. bovis* and *M. africanum*) is often a complicated process (due to slow bacterial growth, ineffectiveness of antibody investigation, and limited value of intradermoreaction) and it requires a great deal of supposition and the performance of differential diagnoses (5,6).

The ability to demonstrate mycobacteria in tuberculid lesions by either microscopy or culture has been explained as being due to the presence of only small numbers of bacilli, which may be rapidly destroyed by a local delayed hypersensitivity reaction.

Recently, a different approach to the diagnosis of mycobacterial disease has been developed based on DNA analysis by polymerase chain reaction (PCR) whose sensitivity is even better than culture techniques (7–11).

We have studied the *M. tuberculosis* presence in tuberculids, a group of skin eruptions whose tuberculous origin has remained controversial, since mycobacteria cannot usually be cultured from these lesions.

MATERIALS AND METHODS

Patients

We have studied skin biopsy specimens from ten patients in whom cutaneous tuberculosis was a possibility. Skin bi-

opsy specimens presented histopathologic features consistent with papulonecrotic tuberculid (dermal necrosis with granulomatous inflammation), and all patients tested positive when given the tuberculin intradermal test (response greater than 20 mm). Clinical features were characterized by symmetrically distributed erythematous papules often ulcerated and healed that primarily affected the extremities. Tests on skin specimen cultures in Lowenstein–Jensen medium were negative in all cases. One patient had a medical history of tuberculosis but no active focus of tuberculosis was found. Chest radiographs were normal in all cases.

PCR Procedure

One formaldehyde solution-fixed, paraffin-embedded block from each patient was studied. Two sections from each biopsy specimen were treated and submitted for PCR amplification as previously described (12,13). Briefly, after xylene treatment and proteolytic digestion in 200 µl of buffer, 20 µl of samples were incubated in a PCR buffer (10 nmol TRIS pH 8.3; 50, nmol of potassium chloride, 4 nmol of magnesium chloride) with 2 U of Taq DNA polymerase (Promega Corporation, Madison, WI) and 50 pmol of sense (5'-CAA-GGC-TTC-AAT-TCC-GGT-GAT-GCC-3') and

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antisense (5'-TGG-TCC-GGT-TCA-TAC-TCG-GGC-TGG-3') primers make up to 60 µl of total volume for 5 minutes at 95°C (denaturation) and subjected to PCR amplification for 40 cycles of 94°C and 70°C (1 minute at each temperature). Usual precautions to prevent contamination problems were taken, and positive and negative controls were performed in each experiment. For the positive control, 10, 5, and 1 µ/ml of DNA from a pulmonary tuberculosis patient's *M. tuberculosis* positive culture were used; the negative controls were DNA from *M. avium*, *M. paratuberculosis* (obtained from positive culture), lymphonode biopsy (positive culture) from a patient infected with *M. scrofulaceum*, skin biopsy from a *M. leprae* lesion, and tissue samples from a patient with sarcoidosis and a patient with lupus erithematosus. (One µg/ml DNA was always added, and for DNA extraction and amplification the same protocol was used.) Detection of presence of inhibitors into negative samples was performed by a second PCR after adding purified *M. tuberculosis* DNA (50 pgr) to the tissue extracts.

An aliquot of PCR product was analyzed by electrophoresis in 2% agarose gels. Bands (285 bp) were visualized by ethidiumbromide staining.

The specificity of the amplicons was verified by Southern-blot hybridization with a P³² 5'-labeled (about 1 × 10⁶ cpm/ml) internal oligonucleotide probe (5'-TCA-GCG-GAG-AGC-CGG-TGA-ACT-TGT-C-3'). Briefly, samples were transferred to Hyband N⁺ nylon fillers (Amersham, Little Chalfont, UK) incubated with 5 × SSC, 5 × Denhardt's solution, 0.5% of SDS, and 100 ngr/ml denatured salmon sperm DNA for one hr at 58°C, then hybridized for 18 hr at 58°C with a p32 5'-end labelled internal probe as previously described (14). All chemical reagents were purchased from Sigma Diagnostics (St. Louis, MO).

RESULTS

Ten biopsy specimens from ten patients were included (female-male ratio, 7:3). A chest X-ray examination was performed in all patients and there was no evidence of previous pulmonary tuberculosis; also, culture of the sputum was negative for mycobacteria. Only two patients (20%) had a family history of tuberculosis.

Eight samples (80%) and positive *M. tuberculosis* controls gave a PCR and Southern-blot hybridization positive results. These results were confirmed in a second independent experiment starting from a new slice of specimen as showed in Figure 1. Inhibitors were not found in the two specimens with negative result.

The primer/probe system used was highly sensitive and specific for *M. tuberculosis* complex organisms: PCR gave negative results in all specimens, including the lymphonode biopsy from patients infected with *M. scrofulaceum*, the skin biopsy from a *M. leprae* lesion, the sarcoidosis skin biopsy, and the lupus erithematosus bi-

Fig. 1. Results from the investigation of *M. tuberculosis* complex by PCR and Southern-blot in 10 papulonecrotic tuberculid lesions.

opsy. Purified DNA from *M. avium* or from *M. paratuberculosis* also gave a negative PCR result.

DISCUSSION

Mycobacterium tuberculosis can induce a wide spectrum of cutaneous lesions (1-6,13). Mucosal and orificial tuberculosis has been well described as have been the chronic forms of cutaneous tuberculosis; but less clear are the relationships between the cutaneous tuberculids and the presence of *M. tuberculosis* within these skin lesions. The subject has been a controversial one because micobacterial DNA has never been cultured from lesions (15-17). The availability of newer molecular diagnostic techniques permits examination into the cause of these poorly defined lesions and such techniques can also be applied to archival tissues (18).

With regard to cutaneous manifestations of tuberculosis, there are several reports indicating the usefulness of PCR. In a previous analysis of 20 patients with various skin conditions of possible tuberculous etiology, 85% were found to contain *M. tuberculosis* complex DNA (19). The controversy about the origin of tuberculids has been going on for many years; it is thought that they represent hypersensitivity reactions to hematogenously disseminated bacilli or mycobacterial antigens. We have demonstrated the presence of mycobacterial DNA in the lesions of 80% of our patients. This finding has been confirmed by other investigators (15,20,21) with percentages between 50 and 100% positive. The detection of mycobacterial DNA in tuberculids suggests not only the presence of bacterial fragments or proteins as has generally been assumed, but also the presence of bacte-

ria that are structurally intact, since nascent DNA rapidly degrades.

PCR appears to be a promising tool in the diagnosis of skin tuberculosis, although false-negative results may be obtained by different causes. (Degraded target DNA, the formalin used as fixative substance for tissues, has been found to diminish the PCR amplification efficiency, or, insufficient target DNA, due to the presence of mycobacteria, is hard to lyse because of its lipid-rich cell wall) (22–24). Therefore, this method not only facilitates an improved diagnosis but also provides a rational basis for antituberculous therapy of tuberculids and for the clinical management of these disorders.

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