Mycobacterium leprae is identified in the oral mucosa from paucibacillary and multibacillary leprosy patients

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Abstract

In leprosy, the nasal mucosa is considered as the principal route of transmission for the bacillus *Mycobacterium leprae.* The objective of this study was to identify *M. leprae* in the oral mucosa of 50 untreated leprosy patients, including 21 paucibacillary (PB) and 29 multibacillary (MB) patients, using immunohistochemistry (IHC), with antibodies against bacillus Calmette-Guérin (BCG) and phenolic glycolipid antigen-1 (PGL-1), and polymerase chain reaction (PCR), with *MntH*-specific primers for *M. leprae*, and to compare the results. The material was represented by 163 paraffin blocks containing biopsy samples obtained from clinically normal sites (including the tongue, buccal mucosa and soft palate) and visible lesions anywhere in the oral mucosa. All patients and 158 available samples were included for IHC study. Among the 161 available samples for PCR, 110 had viable DNA. There was viable DNA in at least one area of the oral mucosa for 47 patients. *M. leprae* was detected in 70% and 78% of patients using IHC and PCR, respectively, and in 94% of the patients by at least one of the two diagnostic methods. There were no differences in detection of *M. leprae* between MB and PB patients. Similar results were obtained using anti-BCG and anti-PGL-1 antibodies, and immunoreactivity occurred predominantly on free-living bacteria on the epithelial surface, with a predilection for the tongue. Conversely, there was no area of predilection according to the PCR results. *M. leprae* is present in the oral mucosa at a high frequency, implicating this site as a potential means of leprosy transmission.

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Introduction

There has been a notable decline in the global prevalence of leprosy, which has been primarily attributed to the use of multidrug therapy. However, 130 countries and territories submitted reports of leprosy to the World Health Organization (WHO) by the beginning of 2011, specifically from regions in Southeast Asia, North and South America, Africa and the Western Pacific. Worldwide, a total of 228 474 new cases were detected in 2010, and the global registered prevalence for the first quarter of 2011 was 192 246 cases [1]. Early diagnosis and the appropriate treatment of patients are the key elements for blocking disease transmission [2].

The mode of transmission of leprosy remains unclear; however, the upper respiratory tract, particularly the nasal mucosa, is considered as the primary route of entry and elimination of *M. leprae* [2]. There have been many studies showing the role of the nasal mucosa in the transmission of *M. leprae*; however, few studies have been conducted in the oral mucosa. Some reports indicated the participation of the oral mucosa in leprosy transmission [2,3], particularly when there are leprosy-specific lesions [4].

Such lesions have been described in multibacillary (MB) patients in advanced stages of the disease [5–8]. Paucibacillary (PB) patients or those patients with incipient disease rarely present lesions in the oral mucosa [9].

Recent studies have described the rarity of oral lesions in leprosy [9–14], possibly due to the effectiveness of multidrug therapy. However, alcohol-acid-resistant bacilli (AARB) have been detected in the clinically normal oral mucosa of MB patients [7,13,15–17]. Recently, *M. leprae* DNA was amplified from oral mucosa samples [3,18] in MB and PB patients using polymerase chain reaction (PCR), showing that sensitive techniques can detect the presence of bacilli even when they are undetectable by routine examination.

Immunohistochemistry (IHC) with antibodies that are directed against *M. leprae* antigens is another sensitive technique for detecting the bacillus, and this technique preserves tissue integrity. Among the various antibodies, anti-BCG [19] and anti-PGL-I [20,21] are the most widely used.

Our aim was to investigate the presence of *M. leprae,* using IHC and PCR, in the oral mucosa from leprosy patients and to compare the results from PB and MB patients, the preferred location of the bacillus, and the efficacy of the methods in detecting *M. leprae* antigens and DNA.

Methods

This was a cross-sectional retrospective study approved by the Research Ethics Committee of São Paulo Federal University (CEP 0609/04 and amended on 10 January 2010). The study analysed 163 oral mucosa biopsy samples included in paraffin blocks from 50 leprosy patients before starting treatment. The biopsy samples were systematically obtained from clinically normal mucosa, including the buccal mucosa, soft palate and tongue at pre-established points, totalling 150 samples, and 13 additional biopsy samples of clinically suspected leprosy-specific lesions that were noted in any part of the oral cavity. Histopathology revealed no specific impairment of the oral mucosa in any of the samples, excluding two normal mucosa samples from the same MB patient in which granulomas and AARB were evident [9,13,14].

The patient charts were reviewed, and their ages, gender and clinical forms of leprosy according to the WHO operational classification [22] were recorded. For inclusion in the study, the paraffin blocks for each patient must have contained sufficient material to generate the IHC sections and extract genomic DNA.

Immunohistochemistry

Immunohistochemistry was performed using the method of third-generation polymers labelled with immunoglobulins and peroxidase [23] with anti-BCG (rabbit anti-BCG, code B0124; Dako A/Sm, Glostrup, Denmark) and anti-PGL-I (anti-*M. leprae* produced in rabbits, Institute of Tropical Medicine, College of Medicine, University of São Paulo, Brazil) polyclonal primary antibodies.

The microscopic analysis of immunoreactivity was performed in the cytoplasms of the epithelial cells, within macrophages and nerves in the corium and on free-living bacteria on the epithelial surface. The presence of a brown precipitate at the site indicated a reaction between the antigen and the primary antibody and was considered positive. Skin from an MB patient was used as a positive control, and the primary antibody was omitted for the negative control.

Polymerase chain reaction

For the PCR analysis, two 5.0-μm sections from each sample were deparaffinised with xylene, hydrated in decreasing concentrations of ethanol, and lysed using a buffer (1 M Tris, pH 8.0, 0.5 M EDTA, 10% SDS, 1 M NaCl, and sterile water) to which 500 μg/mL proteinase K was added every 24 h for 3 day. DNA was extracted using a 4-M ammonium acetate solution and precipitated with isopropanol. After quantification, 5.0 μL of DNA was used per reaction.

A previously designed pair of primers that was specific to a 336-bp internal sequence of the manganese ion transporter gene (*MntH*) was used to detect *M. leprae* bacilli, according to a standardized protocol for PCR [24].

The specificity of these primers was confirmed using cultured samples of *Mycobacterium tuberculosis* and *Mycobacterium avium* complex, in which no amplification was detected, thus excluding any cross-reactivity between the *M. leprae MntH* primers and sequences from other mycobacteria. In addition, 10 samples with positive PCR products for the *M. leprae MntH* gene were randomly selected, and PCR was performed using primers to amplify a 383-bp sequence for other *Mycobacterium* spp., which yielded a negative result for all of the tissue samples and positive amplification only for cultured *M. tuberculosis* [24].

For all of the oral mucosa samples that yielded negative PCR results for the *M. leprae MntH* gene, an additional PCR was performed with keratin-specific primers, which amplified a 343-bp fragment, to confirm that human genomic material was present [24].

To confirm the sequences of the PCR products that were amplified using the *M. leprae MntH* primers, six samples were sequenced, and they indicated identity and homogeneity to a *M. leprae MntH* gene sequence in GenBank (AL583924 and GI: 13093618).