Mycobacterium smegmatis in Skin Biopsy Specimens from Patients with Suppurative Granulomatous Inflammation

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Formalin-fixed, paraffin-embedded skin biopsy specimens, including 72 suppurative granulomatous inflammation (SGI) and 47 non-SGI controls, were tested for mycobacteria by using a broad-range PCR and a suspension array identification system. Mycobacterium smegmatis was detected in 13 (18.1%) of the SGI skin biopsy specimens, which was significantly more than 2 (4.3%) in the controls (odds ratio, 5.73; 95% confidence interval, 1.21 to 27.06; P = 0.028).

TABLE 1 Comparability of SGI and non-SGI control groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>SGI</th>
<th>Non-SGI</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Female gender (%)</td>
<td>32 (44.4)</td>
<td>29 (61.7)</td>
<td>0.066</td>
</tr>
<tr>
<td>Mean age (yr) ± SD</td>
<td>14.9 ± 19.9</td>
<td>8.5 ± 3.7</td>
<td>0.201</td>
</tr>
<tr>
<td>Seasons of lesion onset</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Springd</td>
<td>17 (23.6)</td>
<td>27 (37.5)</td>
<td>0.693</td>
</tr>
<tr>
<td>Summerd</td>
<td>27 (37.5)</td>
<td>18 (38.3)</td>
<td></td>
</tr>
<tr>
<td>Autumnd</td>
<td>11 (15.3)</td>
<td>4 (8.5)</td>
<td></td>
</tr>
<tr>
<td>Wintere</td>
<td>17 (23.6)</td>
<td>14 (29.8)</td>
<td></td>
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<tr>
<td>Mean lesion duration (mo) ± SD</td>
<td>4.98 ± 4.87</td>
<td>3.77 ± 4.12</td>
<td>0.093</td>
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</table>

119 skin biopsy specimens obtained from Beijing Children’s Hospital, Beijing, China, between January 2001 and May 2010, including 72 SGI and 47 non-SGI specimens that served as controls. The non-SGI controls were selected from cases of erythema nodosum (n = 38) and erythema induratum (n = 9). The histologic features of these control cases include histiocytic proliferation, the presence of eosinophils, and vasculitis with no suppurative granulomatous inflammation. Clinically and histopathologically, these control cases can be well differentiated from SGI cases that arise following the contamination of traumatic or surgical wounds with contaminated water or other materials (5, 6).

To date, about one-third of the species of nontuberculous mycobacteria (NTM) described have been associated with human disease (5). Clinical presentations include infections of the skin and soft tissue, lymph nodes, joints, and lungs. Disseminated infections may arise following the contamination of traumatic or surgical wounds with contaminated water or other materials (5, 6). Most species of NTM have been associated with skin and soft tissue infections, but rapidly growing mycobacteria (Mycobacterium fortuitum, M. chelonae, and M. abscessus), M. marinum, and M. ulcerans have been the ones most frequently described. Some of these organisms exhibit geographic restrictions. The timely and accurate diagnosis of cutaneous NTM infections is important in these control cases because of their potential for increased morbidity. Tissue culture is the diagnostic gold standard, but when a mycobacterial infection is responsible, cultures often take weeks to grow (4, 5).

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We have developed a MycoID assay that incorporates broad-range PCR amplification with suspension array detection to identify 17 mycobacterial complexes, groups, and species in a single reaction with a detection limit of 50 to 500 copies/g of tissue (8). In this study, we have employed the MycoID procedure to detect and identify mycobacterial species in formalin-fixed, paraffin-embedded (FFPE) skin biopsy specimens exhibiting SGI and non-SGI controls.

(Received 26 December 2012 Accepted 28 December 2012 Published ahead of print 9 January 2013)

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doi:10.1128/JCM.03421-12
Nucleic acid extraction and mycobacterial detection. Nucleic acids were extracted from FFPE biopsy specimens with a QIAamp DNA FFPE Tissue kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions (9). A human β-actin real-time PCR assay was used for internal quality control of extracted DNA (10). The mycobacterial 16S-23S rRNA gene internal transcribed spacer region was amplified and detected by genus- and species-specific nucleotide probes in the MycoID procedure as previously described (8). Specimens reactive to genus-specific probes but not to species-specific probes were listed as containing “unidentified” mycobacteria.

Statistical analysis. The difference between the study groups was determined by chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated, and P values of ≤0.05 were considered statistically significant.

The comparability of the SGI and control groups is indicated in Table 1. Mycobacterium-specific DNA was detected in 40 (33.6%) of the 119 skin biopsy specimens tested. The Mycobacterium species detected included M. smegmatis (n = 15), M. tuberculosis complex (n = 4), M. gordonae (n = 3), M. kansasi (n = 3), M. intracellulare (n = 2), and a single case each of M. szulgai, M. holsaiticum, M. haemophilum, and M. vanbualeni. Identification to the species level was not possible in nine cases. Mycobacterial species were detected in 41.7% (30 of 72) of the SGI patient specimens, about twice the percentage of the control specimens (21.3% [10 of 47]; OR, 2.64; 95% CI, 1.14 to 6.129; P = 0.024). M. smegmatis was detected at a significantly higher frequency in SGI specimens (18.1%) than in the controls (4.3%; OR, 5.73; 95% CI, 1.21 to 27.057; P = 0.028) (Table 2). Five in the SGI group were positive by AFB staining, and three of these were identified as M. tuberculosis complex positive while two were identified as NTM positive by AFB staining, and three of these were identified as M. smegmatis complex positive while two were identified as NTM positive.

SGI exhibits an intradermal and occasionally subcutaneous aggregation of histiocytes, multinucleated giant cells, and granulocytes, predominantly neutrophils (1, 2). Depending on the etiology and age of the lesion, plasma cells and eosinophils may also be noted. When confronted with this inflammatory pattern, it is important to consider infectious causes. Mycobacterial infections are believed to be a common cause of SGI; however, as noted in our findings, staining for these organisms is often negative and although tissue culture increases the chances of identifying a specific infectious agent, it is time-consuming. Bartralot et al. have stressed that infections caused by different species of NTM cannot be differentiated on histopathologic grounds (1).

Because of its high sensitivity and specificity, the PCR is now widely employed to amplify NTM-specific nucleic acids in a variety of clinical and environmental specimens (4, 6, 7, 11). The suspension array system has been used for the characterization and assessment of mycobacterial infections, as previously reported (12–14). The MycoID system is capable of identifying mycobacterial isolates to the species level by a combination of broad-range PCR amplification and Luminex xMAP suspension array detection and identification directly from positive mycobacterial liquid culture medium (8). With a detection limit of 50 to 500 copies/g of tissue, this assay is expected to be more sensitive than AFB staining for the diagnosis of mycobacterial skin infections. This system has recently been reported to promote the diagnosis of an M. avium-M. intracellulare-caused skin infection by detection and identification to the species level of mycobacterial pathogens in FFPE skin biopsy specimens (15).

M. smegmatis is found in normal human genital secretions (smegma), as well as in animals, soil, dust, and water (16). It was recognized as a human pathogen by Vonmoos et al. in 1986 (17), and since then, fewer than 50 additional cases have been reported, with 56 to 76% involving soft tissue infections. Disseminated infections caused by M. smegmatis are commonly associated with immunosuppression (18–20). Alvarado-Esquiel and coworkers have opined that exposure to M. smegmatis might occur through intraoperative contamination or application of adulterated lipid creams to open wounds (21). This organism is also capable of inducing chronic cellulitis with fistula formation, usually as a result of traumatic inoculation of contaminated material (19).

Our observation that M. smegmatis was the organism responsible in cutaneous biopsy specimens exhibiting SGI was unexpected and speaks to the difficulty of correctly identifying NTM infections of the skin by traditional staining and culture methods. M. smegmatis exists universally in the environment, and contamination may happen during specimen processing and testing. Whether a causal relationship between M. smegmatis and SGI can be established merits further investigation. The MycoID procedure can be completed within 5 h and may provide an effective means of diagnosing mycobacterial infections in FFPE skin biopsy specimens. Expanded evaluations employing these assay techniques to detect and identify M. smegmatis and other mycobacterial pathogens in cutaneous infectious lesions are under way.

ACKNOWLEDGMENTS

This study was supported in part by grants from the Beijing Bureau of Health for Talent Training in Medicine and Technology (2009–3–39), the Beijing Municipal Program for Talent Training (20051A0300823), and the Beijing Natural Science Foundation (7092031 and 7122064). We thank Xiong Wan and Wun-Ju Shieh for critically editing and reviewing the manuscript.

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### Table 2: Mycobacterium species detected in SGI and non-SGI control groups

<table>
<thead>
<tr>
<th>Organism</th>
<th>SGIa (%)</th>
<th>Non-SGIb (%)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any Mycobacterium sp.</td>
<td>30 (41.7)</td>
<td>10 (21.3)</td>
<td>2.643 (1.140–6.129)</td>
<td>0.024</td>
</tr>
<tr>
<td>M. smegmatis</td>
<td>13 (18.1)</td>
<td>2 (4.3)</td>
<td>5.726 (1.212–27.057)</td>
<td>0.028</td>
</tr>
<tr>
<td>M. tuberculosis complex</td>
<td>3 (4.2)</td>
<td>1 (2.1)</td>
<td>2.643 (0.263–26.517)</td>
<td>0.409</td>
</tr>
<tr>
<td>Non-M. smegmatis NTM species</td>
<td>14 (19.4)</td>
<td>7 (14.9)</td>
<td>1.762 (0.642–4.834)</td>
<td>0.271</td>
</tr>
</tbody>
</table>

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b n = 47.
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