Prevalence of Streptococcus Invasive Locus (sil) and Its Relationship with Macrolide Resistance among Group A Streptococcus Strains

A recent study by Bidet et al. (1) reported the molecular epidemiology of the streptococcal invasive locus (sil) in the group A streptococcus (GAS), an organism which caused invasive infections in French children. The authors demonstrated the prevalence of emm type toxin genotypes among 74 invasive GAS isolates from French children. The authors PCR amplified and characterized the locus DNA of sil from invasive isolates, but there were no data concerning noninvasive isolates. It seems that the invasive locus was present not only in invasive isolates but possibly also in noninvasive isolates. Therefore, we conducted a study in which our aims were (i) to examine the prevalence of Streptococcus pyogenes exotoxins in relationship to the sil gene in invasive and noninvasive isolates of GAS, (ii) to define whether sil was predominantly present only in invasive isolates or also in noninvasive isolates of GAS, and (iii) to characterize the relationship between GAS and macrolide resistance.

To set up our hypothesis, we examined 242 noninvasive isolates (tonsillitis, 170 isolates; rhinosinusitis, 51 isolates; and acute otitis media, 21 isolates) and 13 invasive isolates (septicemia, 5 isolates; purulent arthritis, 4 isolates; meningitis, 2 isolates; necrotizing fasciitis, 1 isolate; and peritonal abscess, 1 isolate) of GAS, which were isolated from individual patients. emm typing of GAS strains was performed by DNA sequencing according to the recommendations of the Division of Bacterial and Mycotic Diseases, the Centers for Disease Control and Prevention, and the emm sequence database (http://www.cdc.gov/ncidod/biotech/strep/strepindex.htm). Multiplex PCR was used for toxin gene (speA, speB, speC, speF, speG, speH, speJ, ssa, and smeZ) profiling, as described by Schmitz et al. (5). PCR detection of the sil locus was performed according to the method described by Bidet et al. (1). Macrolide resistance genes of GAS were determined by the PCR methods described by Weber et al. (6). To study the degree of macrolide resistance, MICs of azithromycin to all strains were determined by broth microdilution, using the standard method (2). All the experiments were conducted in duplicate.

Among the 242 noninvasive isolates, 11.98% (29/242) harbored the sil gene in their genomic DNA. The emm types and the toxin gene profiles of sil-positive isolates are shown in Table 1. In noninvasive strains, the sil locus was detected in 9 out of 33 emm type isolates found in the collection (27.27%), and 41.4% (12/29) of the sil-positive isolates belonged to emm type 4. emm type 4 (12 isolates), emm type 48 (3 isolates), and emm type 94 (6 isolates) represented 72.41% (21/29) of the sil-positive isolates. All of the sil-positive noninvasive isolates carried speB alleles, but 68.96% of strains carried speC. There were no significant differences between the toxin gene profile of the sil-positive isolates and that of the sil-negative isolates, except for smeZ, which was 10.3% of the sil-negative isolates but 31% of the sil-positive noninvasive isolates. Seventy-five percent of emm type 4, 75% of emm type 48, 100% of emm type 94, 100% of emm type 53, 100% of emm type 54, and 100% of emm type 102 isolates harbored the sil gene in their DNA.

Although we used limited numbers of invasive isolates, 15.4% of the invasive GAS isolates harbored the sil gene, which is consistent with data from a previous study of invasive strains, which showed that 16% carried the sil gene (1). One hundred percent of emm type 87 and 100% of emm sequence type 1732 were positive for the invasive locus. Thirty percent of the sil-negative invasive isolates carried speC alleles, but all sil-positive isolates were negative for the speA gene. All strains were positive for the speB gene. Fifty percent of the sil-positive isolates were positive for speC, but 30% of the sil-negative isolates were positive for speC. There is no statistical significance in the prevalence of the sil gene among invasive and noninvasive isolates (Fishier’s exact test, P = 0.499).

Among 255 invasive and noninvasive isolates, 16.86% (3 were invasive, and 40 were noninvasive; total, 43/255) of the isolates were azithromycin resistant and were positive for macrolide-resistant genes (Table 2). Among these strains, 65.12% (28/43), 13.95% (6/43), and 20.93% (9/43) of the strains possessed the mef(A), erm(B), and erm(TR) genes, respectively. All sil-positive isolates were sensitive to azithromycin and were negative for macrolide resistance genes (Fishier’s exact test, P ≤ 0.006).

From these results, we concluded that sil is present not only among invasive isolates but also among noninvasive isolates, with similar prevalences (15.4% versus 11.98%, respectively). To our knowledge, this is the first report to show the prevalence rates of sil in both invasive and noninvasive isolates of GAS in Japan. The predominant emm types that harbored sil were emm type 4, emm type 94, and emm type 48. Hidalgo-Grass et al. identified sil in the invasive serotype M14 clone, the organism that caused necrotizing fasciitis in Israel (3). In our study, sil was absent from emm type 3 isolates, a finding comparable to that in a previous study and associated with GAS invasive diseases worldwide (3). The sil locus was confirmed by direct sequencing of several representative PCR-

| TABLE 1. Characteristics of streptococcal toxin gene profile of invasive and noninvasive sil-positive isolates

<table>
<thead>
<tr>
<th>Isolate type</th>
<th>emm type</th>
<th>Sequence type</th>
<th>No. of isolates</th>
<th>Pyrogenic exotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>speA speB speC speH smeZ</td>
</tr>
<tr>
<td>Noninvasive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>+ - + +</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>+ + + -</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>+ + - +</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>+ + + +</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>+ + + +</td>
</tr>
<tr>
<td>48</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>+ + + +</td>
</tr>
<tr>
<td>53</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>- + + +</td>
</tr>
<tr>
<td>54</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>- + + +</td>
</tr>
<tr>
<td>75</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>- + + +</td>
</tr>
<tr>
<td>94</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>+ + + +</td>
</tr>
<tr>
<td>94.1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>- + + +</td>
</tr>
<tr>
<td>102.2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>- + + +</td>
</tr>
<tr>
<td>Invasive</td>
<td>87</td>
<td>1732</td>
<td>1732</td>
<td>+ + + +</td>
</tr>
</tbody>
</table>

*Characteristics of streptococcal toxin gene profile indicating the presence (+) and absence (−) of invasive and noninvasive sil-positive isolates.
amplified products and comparing those with the previous sequence. The overall prevalence of the sil locus in invasive isolates was the same as that from a previous study (16% versus 15.4%, respectively) (1). Up to now, there was no study which showed the status of noninvasive strains with the sil gene. When we examined noninvasive strains, the sil gene was found in 12% of isolates, which is not a remarkably different rate from that found in invasive isolates. All sil-positive isolates were negative for macrolide resistance genes, which were irreversibly important for clinical practice. Future studies should focus on a better understanding of the role of sil in the pathogenesis of GAS infection and its relationship with macrolide resistance. A recent candidate vaccine based on the M protein failed to elicit antibodies to serotype M4, and sil-encoded proteins might represent alternative vaccine targets for this serotype (4). The results of this study should contribute to a better understanding of the pathogenesis of GAS, as well as the epidemiology of GAS-associated disease, and to the establishment of methods for the prevention of diseases caused by GAS in Japan.

REFERENCES


Dewan Sakhawat Billal
Muneki Hotomi
Jun Shimada
Keiji Fujihara
Department of Otolaryngology
Wakayama Medical University
811-1 Kimidera
Wakayama, Japan

Kimiko Ubukata
Kitasato University
Tokyo, Japan

Rinya Sugita
Sugita ENT Clinic
Chiba, Japan

Noboru Yamanaka*
Department of Otolaryngology
Wakayama Medical University
811-1 Kimidera
Wakayama, Japan

*Phone: 81-73-441-0651
Fax: 81-73-446-3846
E-mail: ynobii@wakayama-med.ac.jp

* Published ahead of print on 20 February 2008.