Detection of Genes Encoding Internalization-Associated Proteins in Streptococcus pyogenes Isolates from Patients with Invasive Diseases and Asymptomatic Carriers*†

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A total of 161 Streptococcus pyogenes isolates from patients with invasive infections or from asymptomatic carriers were examined for genes (prtF1, prtF2, and fba) coding for fibronectin-binding proteins to evaluate their involvement in the pathogenesis of different streptococcal manifestations. We found no significant differences in the presence of these three genes between the two groups. Overall, the prtF2 gene was present in similar percentages among strains from both sources (61% versus 63%). Strains carrying the gene fba were slightly more common among those isolated from asymptomatic carriers (72.6% versus 65%). Also, the prtF1 gene was present in a higher, but not significant, percentage among strains from throat swabs than among isolates from invasive infections (75% versus 64.9%). However, this more detailed characterization of the genes encoding fibronectin-binding proteins allowed us to identify a strong association of genes of the erm class, coding for macrolide resistance, with prtF1 and prtF2 rather than with prtF1 alone. Since macrolide resistance was significantly associated with throat swab isolates, it may be hypothesized that proteins coded by prtF1 and prtF2 genes may be synergic in providing support for cell invasion and/or colonizing or persistence efficiency.

Streptococcus pyogenes causes a variety of clinical syndromes, from less-severe manifestations such as pharyngitis and skin infections to invasive ones such as bacteremia, arthritis, rheumatic fever, streptococcal toxic shock-like syndrome, and necrotizing fasciitis (9). Although once considered to be an extracellular pathogen, S. pyogenes has been shown to be able to invade epithelial cells and survive for a limited time (2, 23). The ability of S. pyogenes to invade epithelial cells has been suggested to be a trait connected to the propensity of strains to cause invasive infections (22), although more recent studies have either demonstrated the opposite (i.e., efficiency to penetrate cells is more pronounced among isolates from noninvasive infections) (13, 17, 23) or observed no differences between strains from various sources of isolation (16).

In the last decade, more than a dozen S. pyogenes surface proteins that may be involved in cell adherence and invasion have been identified (5, 7, 19). Fibronectin-binding proteins (FBPs), particularly PrtF1, have been demonstrated to mediate internalization within epithelial cells (24). A peculiar association of PrtF1 with resistance to macrolides mediated by erm class genes has been indicated by Facinelli et al. (11), suggesting that erythromycin-resistant S. pyogenes escapes antimicrobial treatment and the host immune response by invading epithelial cells by means of the prtF1 gene. This gene has also been indicated to be prevalent among persisting group A streptococci from asymptomatic carriers (27), although no difference was found in the frequency of prtF1 in asymptomatic carriers and children with pharyngitis (25); these authors detected prtF2 more frequently in S. pyogenes isolates from asymptomatic carriers. The only study examining the prevalence of FBPs among isolates from invasive infections is that by Delvecchio et al. (10), which analyzed a collection of isolates from a population living in the Northern Territory of Australia. These authors found that PrtF2, rather than other FBPs, was overly represented among isolates from invasive diseases. However, the primary site of infection for invasive diseases in the Australian Northern Territory is the skin; this is in contrast to Europe and North America, where the primary site is usually the throat (4, 12). As for other FBP genes, not much is known about a differential distribution, except for a few scattered studies (29, 30). In all of these studies, collections of isolates comprised, at most, fewer than 100 strains. Subgrouping for the source of isolation (i.e., throat swabs, pharyngitis cases, or invasive infections) left only small number of isolates from each group, possibly biasing the results.

To verify a possible association between a specific FBP pattern and the site of infection, we examined a collection of S. pyogenes isolates from patients with invasive diseases and from asymptomatic carriers. The relationship with the emm type and antibiotic resistance was also investigated.

MATERIALS AND METHODS

Bacteria. Seventy-eight S. pyogenes strains isolated during a national enhanced surveillance developed under the auspices of the Fifth Framework Strep-Euro project on invasive S. pyogenes infections (2003 to 2005) were analyzed, together with eighty-three strains isolated from throat swabs of patients without clinical symptoms (8). All isolates were from Italy. In particular, isolates from asymptomatic patients were all collected during an ongoing investigation of children affected by neurological disorders of various types; some of these disorders were possibly associated with S. pyogenes colonization or infection (8).

The clinical criteria for the definition of S. pyogenes invasive infections were in
accordance with those published by the Working Group on Severe Streptococcal Infections (31), i.e., the isolation of S. pyogenes from a normally sterile site such as blood, cerebrospinal fluid, joint aspirate, pericardial or peritoneal fluids, bone, deep tissue, or abscess at operation or necropsy. In the case of toxic shock-like syndrome, S. pyogenes could also be isolated from a nonsterile site (such as the skin, throat, or vagina).

All strains were emm typed by molecular methods as previously described (8). Isolates were maintained in glycerol at −80°C and subcultured twice on sheep blood agar before testing. Todd-Hewitt broth was used for routine culture.

**DNA isolation and PCR.** Total bacterial DNA was prepared by the Chelex-based procedure using the InstaGene matrix (Bio-Rad) as previously described (1, 8). Samples were amplified on a DNA thermal cycler (MJ Research) at different annealing temperatures depending on the gene to be amplified. The presence of the FBP genes fba (29), prtF1 (27), and prtF2 (18) was investigated by using the primers listed in Table 1. Genes mediating macrolide resistance—ermA subclass ermA (TR), ermA(B), and mefA—were detected as previously described (8). Products were analyzed by gel electrophoresis in a 2% (wt/vol) agarose gel.

**Statistical analysis.** Proportional differences in the frequency of the genes between the two isolation sources were analyzed by the Fisher exact test.

**RESULTS**

Genes encoding FBPs were present among isolates from different sources as shown in Table 2. Overall, the prtF2 gene was present in comparable percentages among strains from both sources (61% versus 63%). Strains carrying the gene fba were slightly more common among those isolated from asymptomatic carriers (65% versus 72.6%); also, the prtF1 gene was present in a higher percentage among strains from throat swabs than among isolates from invasive infections (75% versus 72.6%). Strains carrying the gene fba were slightly more common among those isolated from asymptomatic carriers (65% versus 72.6%); also, the prtF1 gene was present in a higher percentage among strains from throat swabs than among isolates from invasive infections (75% versus 72.6%).

The association of the FBP genes in the two groups was as shown in Table 3, strains belonging to a given emm type carried almost exclusively the same gene pattern. Strains belonging to emm types 4, 5, 6, 9, 11, 12, 22, 24, 28, 44/61, 75, 77, 78, 89, and 118 were always positive for prtF1. Strains belonging to emm types 1, 2, 4, 6, and 75 were always negative for prtF2. fba appeared to have a more widespread distribution (Table 3).

**DISCUSSION**

FBPs represent important virulence factors for a number of pathogens, including group A streptococci, since the ability to bind extracellular matrix proteins supports the colonization of mucosal surfaces and the persistence of bacteria either on the cell surface or within epithelial cells. In particular, proteins of the series F have been demonstrated to have a role in the pathogenesis of group A streptococcal infections. The proportion of prtF1 gene reported in other studies varies greatly, ranging from more than 70 to 80% (11, 25) to 30 to 40% (6, 20, 27). The only study examining strains from invasive infections

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**TABLE 1. Primers used in this study for the detection of genes encoding FBPs**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>fba</td>
<td>GGT GAT TCA ACA TCA GTT AC (forward)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>CGT TTT GTG ACT AAA AGA CT (reverse)</td>
<td>30</td>
</tr>
<tr>
<td>prtF1</td>
<td>TTT TCA GGA AAT ATG GTT GAG ACA (forward)</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>TCG CCG TTT CAC TGA AAC CAC TCA (reverse)</td>
<td>11</td>
</tr>
<tr>
<td>prtF2</td>
<td>GAA GAA AAG CTT CCA GAC GAG CAA (forward)</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>GGA ATC TCA GAG TTA CTT TCT GGT TCC (reverse)</td>
<td>15</td>
</tr>
</tbody>
</table>

**TABLE 2. FBP genes carried by S. pyogenes isolated from invasive infections or asymptomatic carriers**

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of strains (%) carrying gene:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>prtf1</td>
</tr>
<tr>
<td>Invasive infections</td>
<td>50 (64.9)</td>
</tr>
<tr>
<td>Asymptomatic carriers</td>
<td>63 (75)</td>
</tr>
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</table>

**TABLE 3. FBP genes carried by S. pyogenes examined in this study in relation to the source of isolation and emm allele type**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of strains from:</th>
<th>emm type(s)</th>
<th>Total no. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>prtf1</td>
<td>Patients with invasive infections</td>
<td>Asymptomatic carriers</td>
<td></td>
</tr>
<tr>
<td>prtf2</td>
<td>5 9 6.0</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>fba</td>
<td>14 8 1.0, 2.0</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>prtf1/prtf2</td>
<td>11 16 2.0, 4.0, 75.0</td>
<td></td>
<td>27</td>
</tr>
<tr>
<td>prtf2/fba</td>
<td>2 2 3.1, 3.2, 5.3, 70.0, 78.0</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>prtf1/prtf2/fba</td>
<td>10 6 12.0</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>prtf1/prtf2/fba</td>
<td>24 34 3.1, 5.3, 9.0, 11.0, 22.0, 28.0, 44/61.0, 77.0, 78.0, 89.0, 118.5</td>
<td></td>
<td>58</td>
</tr>
<tr>
<td>Total</td>
<td>78 83</td>
<td></td>
<td>161</td>
</tr>
</tbody>
</table>

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**FBPs IN S. PYOGENES FROM INVASIVE DISEASES 1285**
found that 50% of the S. pyogenes isolates obtained from an Australian Aboriginal population were prtF1 positive (10). The percentage of prtF1-carrying strains in our collection of isolates from patients with invasive diseases was ca. 75% and was thus more similar to the percentage reported by studies that examined collections of strains from European and North America locations, suggesting the spread of clones with different properties in the different continents. Vlaminkx et al. (30) reported an extremely low incidence of prtF1 among isolates from invasive infections in Europe, limited to emm6 type isolates; however, we found that the forward primer they used was specific for emm6 and could not detect prtF1 sequences that differed in the specific segment in other emm types (data not shown). These authors (18) also found that ca. 63% of their strains carried the gene fba; however, since only the characteristics of the predominant emm types were reported, a conclusive comparison of data cannot be done.

The gene encoding PrtF2 was demonstrated to be present in 36 to 80% of clinical S. pyogenes isolates (14, 21), and in our collection PrtF2 was also present in ca. 60% of the isolates. Also, the percentages of strains carrying prtF1/prtF2 simultaneously within the two groups examined is in agreement with previous studies that reported values ranging from 31 to 100% of the S. pyogenes strains tested (14, 21). In genomes possessing both FBP genes, prtF1 and prtF2 either resided within a potential pathogenicity island (fibronectin-binding, collagen-binding, T-antigen island) in the serotype M12 (3) or at separate loci of the genome of serotypes M3 and M18 (26, 28) as a potential consequence of genome-scale recombination events. In the present study, the gene combination prtF1/prtF2 was found in a number of emm types, regardless of the isolation source, including emm3 and emm12. However, none of the six emm18 strains examined carried both genes.

The higher percentage of macrolide resistance among isolates from throat swabs confirms data already reported by our group (8). In this case, it was interesting that the peculiar association of genes of emm class with genes, especially prtF1 and prtF2, encoding FBPs. Facinelli et al. (11) observed a strong association of the gene emm with prtF1 and a less consistent association with mefA. Our results indicates that a strong association exists between prtF1/prtF2 and the emm gene rather than the latter with prtF1 alone. As a matter of fact, 100% of the emm-positive isolates also possessed prtF1 and prtF2 genes; it should be noted that this group comprised strains of different emm types, thus excluding a possible clonal origin. On the other hand, prtF1 alone was present only in macrolide-susceptible isolates. Of the mefA-positive isolates, we also observed 6 of 29 isolates carrying the fba gene alone, confirming a less-consistent association of prtF1 with mefA as already described (11). An even weaker association was observed for mefA and prtF2, since only a few isolates carried these two genes simultaneously.

On the whole, these data suggest that no specific association of FBP genes is evident among S. pyogenes isolates from different sources. This finding confirms data already reported on the apparent lack of association of a specific virulence trait with a clinical manifestation of S. pyogenes infection. However, the collection examined here encompasses a much larger number of strains, which definitely adds to the statistical significance of the findings. Moreover, a more detailed characterization of strains on the basis of the FBP pattern allowed us to identify the strong association between the genes prtF1/prtF2 and macrolide resistance mediated by emm class genes rather than with prtF1 alone as suggested by others (11). Since macrolide resistance, including that obtained through methylation of the 23S rRNA, is significantly associated with throat swab isolates or, more generally, with strains from patients with noninvasive diseases, it may be hypothesized that the proteins these genes encode may be synergic in providing support for cell invasion, colonization or persistence, and/or efficiency. Indeed, Gorton et al. (15) have recently suggested that prtF2-positive isolates have an increased internalization capacity compared to prtF1-positive strains. Also, we have preliminary data indicating that strains carrying the prtF2 gene, alone or in combination with other genes, appear to be more efficient in intracellular survival (unpublished data). It is important to remember, however, the observation that most properties of S. pyogenes isolates have been associated with specific emm types; thus, the association of a particular trait to the isolation source may be due to the association of a predominant emm type with a specific infections rather than to the trait itself. Studies are in progress to evaluate whether the association prtF1/prtF2 and/or emm genes displays a differential efficiency in cell penetration.

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REFERENCES


