Vaccination with FimA from *Streptococcus parasanguis* Protects Rats from Endocarditis Caused by Other Viridans Streptococci

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Received 26 June 2001/Returned for modification 15 August 2001/Accepted 16 October 2001

The FimA protein of *Streptococcus parasanguis* is a virulence factor in the rat model of endocarditis, and immunization with FimA protects rats against homologous bacterial challenge. Because FimA-like proteins are widespread among the oral streptococci, the leading cause of native valve endocarditis, we evaluated the ability of this vaccinogen to protect rats when challenged by other streptococcal species. Here we report that FimA vaccination produced antibodies that cross-reacted with and protected against challenge by the oral streptococci *S. mitis*, *S. mutans*, and *S. salivarius*. FimA thus has promise as a vaccinogen to control infective endocarditis caused by oral streptococci.

Viridans streptococci that inhabit the mouth account for 45 to 80% of all native valve endocarditis in humans (3, 21). The morbidity and mortality of streptococcal endocarditis justify the development of safe and effective vaccines or other interventional strategies to prevent or control this disease (9). While investigating viridans streptococcal pathogenesis in a rat model of endocarditis, we discovered that a surface protein called FimA was a major virulence determinant (5). Subsequently, we showed that immunization with recombinant FimA protected rats challenged with the *Streptococcus parasanguis* strain from which the *fimA* gene was cloned (22). FimA is a member of a family of lipoprotein receptors found in several species of gram-positive cocci, including the viridans streptococci, pneumococci, the enterococci, and others (14, 20). These proteins have been termed “lipoprotein receptor antigens” (Lra1), although lipid modification has been demonstrated for only a few members (6, 11, 12). The predicted fatty-acylated amino-terminal cysteine is believed to allow membrane anchoring.

To be an ideal vaccinogen, FimA from *S. parasanguis* should afford cross-protection against other viridans streptococci carrying related Lra1 antigens. Using DNA hybridization, PCR amplification, and Western blot analysis, we previously demonstrated that a *fimA* homolog was present in and expressed by a variety of viridans species, including *S. sanguis*, *S. mutans*, *S. mitis*, and *S. salivarius* (15, 22). In this study, we used FimA from *S. parasanguis* to vaccinate rats, which were subsequently challenged with the oral species *S. mutans*, *S. mitis*, or *S. salivarius*, or the distantly related organism *Enterococcus faecalis*.

FimA protein was produced as a histidine-tagged recombinant protein by using the pQE30 expression vector system as previously described (22). Male Sprague-Dawley rats were immunized by a previously published protocol (22), with animals receiving an initial dose of 100 μg of FimA in complete Freund’s adjuvant followed in 3 weeks by a second dose of 100 μg of FimA in incomplete Freund’s adjuvant. The rat model of endocarditis used here was as described by Munro and Macrina (18) and involved insertion of an indwelling catheter through the aortic valve to induce valve roughening. Immunized rats and nonimmunized control rats were challenged via tail vein inoculation with 10⁷ organisms 1 to 2 days after cardiac catheterization. Animals were euthanized 2 days later by carbon dioxide inhalation. The aortic valve region and any visible vegetations were removed, homogenized, and spread on brain heart infusion agar plates for bacteriological analysis. All animal experiments were done under the authorization of Virginia Commonwealth University IACUC protocol no. 9410-2082. The challenge strains *S. mitis* V2483 and *S. salivarius* V2499 were human blood isolates identified to the species level by the API 20 Strep Test (bioMerieux); their identification was confirmed by the diagnostic microbiology laboratory, Medical College of Virginia Hospitals, Virginia Commonwealth University. *S. mutans* ATCC 25175 was purchased from the American Type Culture Collection. Two additional *S. mutans* strains isolated from cases of human endocarditis were provided by Richard Facklam, Centers for Disease Control and Prevention (designations 1381-90 and 2192-81). All three *S. mutans* strains gave indistinguishable DNA fragment profiles when examined by contour-clamped homogeneous electric field gel analysis or Southern blot analysis with 16S rRNA probes (data not shown). Thus, data from our studies with *S. mutans* were pooled and analyzed together.

Table 1 reports the results of the challenge experiments with these three species. Rats were judged as infected if bacteria were recovered from homogenized heart valves. The significance of differences in infectivity between vaccinated and unvaccinated animals was calculated by Fisher’s exact test. Inci-
TABLE 1. Endocarditis in FimA-immunized and control rats

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. (%) of rats infected/ total no.</th>
<th>Control</th>
<th>Immunized</th>
<th>P value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mitis</td>
<td>5/7 (71)</td>
<td>10/15 (67)</td>
<td>7/32 (22)</td>
<td>0.0196</td>
</tr>
<tr>
<td>S. mutans</td>
<td>10/15 (67)</td>
<td>7/32 (22)</td>
<td>0.0042</td>
<td></td>
</tr>
<tr>
<td>S. salivarius</td>
<td>5/8 (62)</td>
<td>2/24 (8)</td>
<td>0.0048</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Surgical survivors with correct catheter placement at necropsy.  
<sup>b</sup> Fisher’s exact probability test.

dence of endocarditis resulting from heterologous streptococcal challenge was reduced in rats that had received immunization with FimA compared with naive control animals similarly challenged. Endocarditis infections were significantly reduced for all three challenge organisms.

Since protection presumably results from cross-reactivity between anti-FimA antibodies and the FimA homologs present in these species, the amino acid sequences of these proteins were compared. A FimA homolog has been detected in S. salivarius (22), but its sequence has not been determined. The deduced amino acid sequences of the FimA homologs from S. mutans (SloC) and S. mitis (PsaA) have been reported (accession no. AAF81674 and AAF64229, respectively). The GAP program (Genetics Computer Group, Madison, Wis.) was used to compare the FimA sequence to these sequences. The first 19 to 20 amino acids of each sequence, which would be removed by signal peptidase II if these proteins are processed to lipoproteins (20), were not included in the analysis. The FimA sequence shared 82% identity with S. mitis PsaA. This value is comparable to those obtained by comparison of FimA with the LraI members from S. sanguis (SsaB; accession no. AAC98426; 83% identity), S. gordonii (ScaA; AAA71947; 82% identity), S. anginosus (PsaA; AAF64228; 86% identity), and S. oralis (PsaA; AAF64230; 85% identity). SloC from S. mutans is less similar to FimA, sharing only 75% identity.

Because the vaccine protected against challenge with S. mutans, whose LraI homolog is more divergent from FimA than that of any of the other species listed above, we looked outside of the viridans streptococci for the next challenge organism. The distantly related endocarditis pathogen Enterococcus faecalis was chosen. The FimA homolog previously identified in this species, EfaA (accession no. AAA70056) (17), shares 61% identity with FimA when compared as described above. This experiment also employed sham vaccination with an irrelevant fusion protein similar in length to the FimA fusion to control for any protective effects produced by the adjuvant, contaminating E. coli proteins, or any other aspect of the vaccination process. The sham vaccinogen was constructed from nucleotides 2569 to 3492 of the kgp gene (formerly called prfP) (7) from Porphyromonas gingivalis (1) and was purified in the same manner as the FimA fusion. The challenge strain of E. faecalis for the experiment was a rat-passaged derivative of strain V2437, a human blood isolate shown previously to express a protein reactive against anti-FimA antiserum, presumably EfaA (22). The results of the study were as follows: 4 of 10 (40%) unvaccinated rats were infected, 6 of 15 (40%) FimA-immunized rats were infected, and 3 of 15 (20%) FimA-immunized rats were infected. Protection by the FimA vaccine was incomplete at best (P = 0.43), suggesting that the FimA vaccinogen would not be effective in its current form for prevention of enterococcal endocarditis. Importantly, however, there was no evidence of any protective effect imparted by sham vaccination, suggesting that the protection demonstrated in the previous experiments was FimA dependent.

To further investigate the immune response generated by the vaccine, immunoblots were performed with sera pooled from four rats vaccinated with FimA and four rats vaccinated with the sham antigen. These sera were used without adsorption to see the effects of vaccination in the context of total antibodies present in the sera. The top panel of Fig. 1 shows a Coomassie blue-stained polyacrylamide gel with duplicate loadings of protein lysates from the challenge strains used in this study, prepared as described previously (22). Ten micrograms of total protein was loaded into each lane, as measured by bichinchoninic acid protein assay with bovine serum albumin as a standard (Pierce Chemical Co.). The figure confirms that although the banding pattern for each strain is unique, the total protein content of each lane is similar. In the bottom panels, proteins from a duplicate gel were transferred to a nitrocellulose membrane, reacted with one of the pooled antisera, and then visualized following reaction with alkaline

FIG. 1. Immunoblot analysis with sera from immunized rats. Protein lysates from the challenge strains indicated (with ATCC 25175 employed as the S. mutans strain) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained with Coomassie blue or transferred to a nitrocellulose membrane for immunoblot analysis. The molecular masses of marker proteins (Kaleidescope; Bio-Rad Laboratories) electrophoresed in adjacent lanes are indicated. (Top panels) Coomassie blue-stained gel. (Bottom panels) Immunoblots reacted with pooled sera from rats immunized with FimA (α-FimA) or the sham antigen (α-Sham). The position of the FimA protein is indicated by an arrow.
phosphatase-conjugated secondary antibody and colorimetric detection (22). The molecular masses of marker proteins are indicated to the left of the gel and the blots.

A number of proteins are recognized in each lane, as would be expected when bacterial lysates are reacted with unadsorbed sera. However, the FimA protein of *S. parasanguis* (indicated by an arrow) and proteins in the other species with similar apparent molecular masses (34 to 39 kDa) are detected in the blot reacted with the anti-FimA antisera, but not in the blot reacted with the anti-sham antigen sera. Presumably, these additional proteins are FimA homologs. It is interesting to note that two such proteins appear to be present in the *E. faecalis* lysate. The incomplete genomic sequence of *S. mutans* (available at The Institute for Genomic Research web site at http://www.tigr.org) was searched with the FimA amino acid sequence. The sequence of the previously identified LraI protein, EfaA, was found as expected. However, another putative protein with greater similarity to FimA (80% identity, measured as described above) was also found. It is possible that this protein is one of the bands apparent in the anti-FimA blot. It is not clear why the vaccine provides less protection against infection by *E. faecalis* than by the oral streptococci, despite the cross-reactivity of the enterococcal proteins to the FimA sera. One possibility is simply that *E. faecalis* was more virulent than the other challenge strains, perhaps due to its animal passage. This seems unlikely, however, given that infection rates in unvaccinated animals were not higher with this strain than with the viridans streptococci. Resolution of this question awaits determination of the mechanisms by which the FimA vaccine protects against infection, whether through antibody-mediated immune clearance, interference with one or more functions of LraI proteins, or other means.

To date, three properties have been associated in various combinations with LraI proteins in streptococci—adhesion, metal ion uptake, and virulence. LmbI, an LraI member from *S. agalactiae*, was shown to mediate attachment to human laminin, suggesting an adhesion function for this protein (19). FimA was shown to bind to fibrin and to be required for virulence in the rat model of infective endocarditis (5). Metal uptake was suggested initially by the finding that FimA was encoded within an operon whose products had homology to ATP-dependent transport systems in general (10) and metal uptake systems in particular (2), although metal uptake by FimA was not examined. Since then, the *sca* (16), *mts* (13), and *adc* (8) LraI operons have been shown to encode metal uptake systems. The *slo* operon was shown to be necessary for virulence of *S. mutans* for endocarditis, but metal uptake and adhesion were not investigated (15). Finally, adhesion, metal ion uptake, and virulence have all been associated with the *psa* operon in *S. pneumoniae* (4, 8).

Evidently relating any of these functions to specific domains of the FimA protein is lacking. Such information could explain why FimA is a protective antigen in the endocarditis model. FimA may have separate metal binding and adhesion domains. Alternatively, the adhesin-like properties of FimA may be a manifestation of binding to metal-protein complexes. In the first case, anti-FimA antibodies might function solely to block fibrin binding, an action that has been demonstrated in vitro (22). However, a noncompeting argument is that such antibodies could interfere with metal acquisition. This could result in nutritional insufficiency for bacteria in vivo, putting them at a selective disadvantage manifesting itself as reduced virulence. A related effect might be the inability to accumulate essential metals crucial to the genetic expression of virulence genes or the functionality of their gene products. Any or all of these possibilities could account for the immunological protection afforded by the FimA vaccinogen.

We thank Lisa Weymouth for confirming the identification of the streptococcal strains used in this study. We are grateful to Richard Facklam (Centers for Disease Control and Prevention) for providing *S. mutans* strains used in this study.

This work was supported by USPS grant DE04224 and a grant from MedImmune, Inc.

REFERENCES


