Identification of a novel glycoprotein-binding activity in *Streptococcus pyogenes* regulated by the *mga* gene

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The interaction between *Streptococcus pyogenes* and the host cell surface is not completely understood. Characterization of the adhesion mechanisms of the bacterium to the host cell surface is needed in order to develop new vaccines and anti-adhesion drugs. The presence of glycoprotein-binding activities among streptococcal strains was investigated. An activity binding to thyroglobulin, fetuin, asialofetuin and mucin but not non-glycosylated proteins was found to be present in the majority of the *S. pyogenes* strains studied. Cross-inhibition experiments suggested that the glycoproteins share a common structure recognized by the bacteria. The glycoprotein-binding activity was found to be proteinaceous, tightly attached to the bacterial surface and it also mediated the adherence of bacteria to solid surfaces coated with glycoproteins. The activity was found by transposon mutagenesis and complementation to be regulated by the multiple-gene regulator Mga, which has been implicated as a regulator of *S. pyogenes* virulence factors.

**Keywords:** *Streptococcus pyogenes*, bacterial adhesion, transposon mutagenesis, glycoproteins

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**INTRODUCTION**

*Streptococcus pyogenes* is an important human pathogen causing a wide variety of infections ranging from tonsillitis, impetigo and erysipelas to severe life-threatening infections like necrotizing fasciitis and sepsis, which have increased in number during the last few years (Chelsom et al., 1994). This drives us to look for new preventive methods and therapies. Characterization of the molecular basis of interactions between *S. pyogenes* and the host could provide us with new candidate vaccines. Furthermore, novel anti-adhesive drugs could be developed using analogues of receptor molecules to prevent the bacteria from binding to host cells (Karlsson, 1995; Beuth et al., 1996; Zopf & Roth, 1996).

The adherence of bacteria to host cells is in many cases mediated by lectin-like adhesins on the bacterial surface binding to carbohydrate receptors (Beachey, 1981; Ofek & Sharon, 1990), which are present on the host cell surface as part of the membrane glycoproteins and glycolipids. *Escherichia coli* is known to bind at least to eight different carbohydrate structures via its surface proteins (Karlsson, 1995; Sharon, 1996; Sharon & Lis, 1997). Some of the carbohydrate structures have been used to inhibit the binding of *Esc. coli* to the host intestinal epithelia (Mouricout et al., 1990). Likewise, streptococcal infections of the human pharyngeal epithelia, where local administration of the drug to the infection focus would be easy to carry out, would be a suitable target for anti-adhesive therapy. Unfortunately, very little is known about the streptococcal receptors on host cells.

A number of *S. pyogenes* surface proteins interacting with the human host have been identified. Some of these, like the fibronectin-binding proteins (Talay et al., 1991, 1992; Courtney et al., 1992, 1994; Hanski & Caparon, 1992; Kreikemeyer et al., 1995; Rakonjac et al., 1995; Jaffe et al., 1996; Rocha & Fischetti, 1999), are adhesins, while others, for example M-like proteins (Cleary & Retnoningrum, 1994) and C5a peptidase (Chen & Cleary, 1990), are protective against the host immunodefence system. M-protein has a dual role,
being the major anti-phagocytic factor of *S. pyogenes* and also an adhesin that binds fibronectin, fibrinogen and albumin, among others (Fischetti et al., 1988; Schmidt et al., 1993; Gubbe et al., 1997). The ability of *S. pyogenes* to infect and to persist in varied locations of the human host is in part controlled by the multiple-gene regulator of *S. pyogenes*, Mga, which controls the expression of the M-protein (*emm*), M-like proteins (*fcrA, emm, sph*) and C5a peptidase (*scpA*) genes (Podbielski et al., 1996a). These genes are located in one region where the transcription of the genes downstream of the *mga* gene is monocistronically regulated. The genes encoding the opacity factor (*sof*), secreted inhibitor of the complement cascade (*sic*) and streptococcal cysteine protease (*speB*) are also regulated by Mga (Podbielski et al., 1996a).

The purpose of the present study was to characterize glycoprotein-binding specificities of streptococci to understand the interaction between the bacteria and the potential carbohydrate receptor structures of the host cell surface. A glycoprotein-binding adhesion activity was shown to be present in the majority of the *S. pyogenes* strains studied. Using transposon mutagenesis, the novel glycoprotein-binding activity was shown to be regulated by Mga.

**METHODS**

**Bacterial strains and culture conditions.** *Streptococcus pyogenes* clinical isolate A8173 (type M2) and group D streptococci (D1213, D1263, D1448, D1470, D7637, D7883) were provided by K. Kunnas, National Department of Health, Kuopio, Finland. *S. pyogenes* (Tyks 2/8-114, Tyks3/7-150, Kuo 36/5623, OHI R5/65G and SAN R67/32), group C (C-strept 5/95, C-strept 13/95, C-strept 21/95, C-strept 120/95, C-strept 164/95 and C-strept 200/95) and group G streptococci (G-strept 8-114, G-strept 8-150, Tyks 2-7-150, Tyks3/7-150, Tyks8-114, Tyks3-7-150, Tyks3-9/95, G-strept 5/95, G-strept 38/95 and G-strept 74/95) were isolated from throat, wound or blood and were provided by P. Huovinen, National Department of Health, Turku, Finland (Kataja et al., 1996a, b). *S. pyogenes* M2-71-676 wild-type and mutant strains (Podbielski et al., 1996b; Schmidt et al., 1997) were kindly provided by A. Podbielski, University Hospital Ulm, Ulm, Germany and *S. pyogenes* CS101 and mutant strains by M. Boyle, Medical College of Ohio, Toledo, USA (Podbielski et al., 1996b). *Escherichia coli* JM109 was obtained from Promega.

Streptococcal strains and *Enterococcus faecalis* were grown on Todd–Hewitt (Difco) plates or media supplemented with 0·5% yeast extract (THY; Biokar Diagnostics). *E. coli* was grown in Luria broth. All cultures were incubated at 37 °C in ambient air unless otherwise stated. All bacteria were grown in Luria broth. All cultures were incubated at 37 °C, except where otherwise stated.

**Trypsin treatment and extractions.** Bacteria grown and harvested under standard conditions were digested with trypsin at concentrations of 0·1–100 μg ml⁻¹ for 30 min at 37 °C, after which the digest was terminated with trypsin inhibitor. In other experiments, the bacteria were suspended in PBS containing 2 M NaCl, 1% Triton X-100 or 10 mM DTT and incubated at room temperature for 1 h, or resuspended in PBS and kept at 80 °C for 30 min. After the treatments the bacteria were pelleted by centrifugation, washed twice and resuspended in PBS.

**Binding of radiolabelled streptococci to thyroglobulin on microtitre plates.** Bacteria were grown in 15 ml THY broth containing 0·5 mM (185 MBq) *Tran*³⁵S-label at 37 °C overnight. The bacteria were concentrated by centrifugation (3000 g for 10 min at 4 °C) to 1 ml PBS and the unbound label was removed by centrifuging the bacteria as above through 5 ml 6% BSA in PBS. Microtitre plate wells were coated with 0·1 ml of the ligand at 10 μg ml⁻¹ at 37 °C for 2 h. The wells were washed with PBS and saturated with 2% BSA, 0·1% Tween 20 in PBS for 1 h at room temperature. The wells were overlaid with 100 μl bacteria in 0·1% BSA, 0·05% Tween 20 in PBS at a concentration that gave an OD₅₆₀ of 0·3 (≃ 4 × 10⁹ c.f.u. ml⁻¹), incubated for 1·5 h at 37 °C with gentle agitation and washed three times for 10 min with 200 μl PBS per well. In inhibition experiments, the bacteria were preincubated with excess ligand at 1 mg ml⁻¹ in 0·1% BSA,
A novel binding activity in *S. pyogenes*

0.05% Tween 20 in PBS for 30 min and then added to the wells. Binding of the bacteria to wells coated with 2% BSA was used as control. Experiments were carried out in 12 parallel wells. The bound bacteria were detected using a liquid-scintillation counter.

**Conventional DNA techniques.** Plasmid DNA preparation from *E. coli*, restriction enzyme digestions, ligations, transformations, agarose gel electrophoresis and Southern blotting were performed as described by Sambrook *et al.* (1989). Chromosomal *S. pyogenes* DNA was prepared as described by Caparon & Scott (1991).

**Transposon mutagenesis and analysis of the mutants.** Transposon mutagenesis was performed as described by Caparon & Scott (1991). Briefly, a spontaneous streptomycin-resistant *S. pyogenes* strain (A8173sr) was grown overnight in 100 ml THY broth containing 1 mg streptomycin ml⁻¹ at 37 °C. Donor strain *Ent. faecalis* CG110 (provided by D. Clewell, University of Michigan, Ann Arbor, USA) was grown similarly in 10 ml THY broth containing 10 μg tetracycline ml⁻¹. Bacteria were harvested and A8173sr was resuspended in 1 ml THY. *Ent. faecalis* was resuspended in 10 ml THY. One millilitre of both suspensions were mixed together, plated on THY plates (no antibiotics) and grown overnight at 37 °C. Bacteria were collected from the overnight culture, plated on THY plates containing 1 mg streptomycin ml⁻¹ and 5 μg tetracycline ml⁻¹ and grown for 48 h at 37 °C. Colonies were blotted onto nitrocellulose membranes and were analysed by the glycoprotein-binding assay using dianimobenzidine as substrate. The number of transposons integrated into the genomes of the non-binding mutants was determined by Southern hybridization of HindIII-digested genomic DNAs using [α-³²P]dCTP-labelled plasmid pAM120 (which carries the whole Tn916 transposon; provided by D. Clewell) as the probe.

**Cloning of the Tn916-chromosomal DNA junction fragment.** Chromosomal DNA from *Streptococcus pyogenes* mutated with Tn916 was cleaved with HindIII and the fragments were separated by agarose gel electrophoresis. A 14 kb fragment containing the 5' end of Tn916 was extracted from the gel, cleaved with HinII and ligated to pBS13+ (Promega). The ligation mixture was transformed into *E. coli* JM109, the bacteria were grown overnight and the colonies transferred to a nylon membrane. The membrane was probed with [γ-³²P]ATP labelled oligonucleotide Tn1 (5'-GAGTGGTTTGG-ACTTGATA-3') which binds to the 5' end of Tn916 at position 53–72. Plasmid DNA from hybridizing colonies was prepared and the DNA sequenced.

**DNA sequencing.** DNA was sequenced with a Sequenase 2.0 kit according to the instructions of the manufacturer. Primer Tn1 was used in sequencing reactions. The products were analysed by gel electrophoresis and visualized by autoradiography. The sequence data were analysed with DNAStar software.

**Generation of an mga-complemented strain.** Plasmid pJRS2050 (Andersson *et al.*, 1996; carries mga and associated regulatory sequences in pLZ12-Spec; provided by J. Scott, Emory University Health Sciences Center, Atlanta, USA) was electroporated into A8173-1 to generate A8173-1(pJRS2050). The electroporation method described by Simon & Ferretti (1991) was used with one modification: THY medium was used instead of Todd–Hewitt supplemented with horse serum. As a control, the plasmid pLZ12-Spec (an *E. coli–S. pyogenes* shuttle vector) was introduced into A8173-1 to generate A8173-1(pLZ12-Spec).

**RESULTS**

**Binding of glycoproteins to streptococci**

To characterize the interaction between streptococci and carbohydrates, an assay to study the binding of glycoproteins to streptococci was developed. The assay is based on the binding of biotinylated ligands to bacterial dots on a nitrocellulose membrane and the detection of the bound ligand by streptavidin–HRP and ECL autoradiography. The glycoproteins used in the assay containing a wide selection of N-linked complex, high-mannose and O-linked glycans were bovine thyroglobulin, bovine fetuin and asialofetuin, and bovine submaxillary mucin (Spiro & Bhyroo, 1974; Yet *et al.*, 1988; Strous & Dekker, 1992; Rawitch *et al.*, 1993). Horse myoglobin and bovine erythrocyte carbonic anhydrase were used as controls representing non-glycosylated proteins.

Six *S. pyogenes* strains representing different infection foci and different geographical origins, six streptococcal strains from the Lancefield groups C and G (wound and throat infections) and six strains from group D were analysed for binding to glycoprotein ligands. Thyroglobulin, fetuin and asialofetuin bound to several *S. pyogenes*, group C and group G strains, and submaxillary mucin to *S. pyogenes* and group C strains (Fig. 1). No binding of the control non-glycosylated proteins horse myoglobin and carbonic anhydrase to the bacteria was detected.

Inhibition assays were carried out using excess ligand to inhibit the binding of the glycoproteins to a dilution series of *S. pyogenes* A8173 on nitrocellulose membranes (Table 1). As expected, the binding of biotinylated thyroglobulin to A8173 was abolished when excess bovine thyroglobulin was used as inhibitor, and similar inhibition was obtained with human and porcine thyroglobulin (data not shown). However, fetuin, mucin and asialofetuin inhibited the binding of thyroglobulin to half or less. Binding of fetuin to the bacteria was in turn abolished with excess thyroglobulin, and reduced to half or less when incubated with excess fetuin, mucin or asialofetuin. Likewise, binding of mucin was abolished when excess thyroglobulin was used as inhibitor. Fetuin had no effect on the binding of mucin, but asialofetuin and mucin reduced the binding to half or less. Binding of asialofetuin inhibited the binding of thyroglobulin to A8173.

**Extractability of the binding activity**

To characterize the properties of the glycoprotein-binding molecule, bacteria were subjected to various treatments, and the binding of thyroglobulin to a
Fig. 1. Binding of glycoproteins to streptococcal strains. Six identical membranes containing six different S. pyogenes (A), group C (C), group D (D) and group G (G) strains were prepared by applying 1 µl aliquots of bacterial suspensions (OD₆₀₀ 0.5) to the membrane. Strain A8173 is in the upper right corner of each membrane. The biotinylated glycoproteins (thyroglobulin, fetuin, asialofetuin, submaxillary mucin) and control non-glycosylated proteins (myoglobin, carbonic anhydrase) were added at concentrations of 0.3–1.0 µg ml⁻¹ and the bound ligands were detected after washings with streptavidin-HRP and ECL autoradiography. The films were scanned with a Kodak DC 120 Digital Camera and the data processed with the Kodak Digital Science 1D image analysis system.

Table 1. Cross-inhibition of the binding of glycoproteins to streptococci

<table>
<thead>
<tr>
<th>Inhibitory ligand</th>
<th>Binding ligand</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Thyroglobulin</td>
</tr>
<tr>
<td>None</td>
<td>32</td>
</tr>
<tr>
<td>Thyroglobulin</td>
<td>–</td>
</tr>
<tr>
<td>Fetuin</td>
<td>8</td>
</tr>
<tr>
<td>Asialofetuin</td>
<td>16</td>
</tr>
<tr>
<td>Submaxillary mucin</td>
<td>8</td>
</tr>
</tbody>
</table>

Membranes containing dilution series of S. pyogenes A8173 were preincubated with 1 mg ml⁻¹ of the inhibitory ligand before addition of the biotinylated binding ligand at 0.3 µg ml⁻¹ (thyroglobulin) or 1.0 µg ml⁻¹ (fetuin, asialofetuin, and submaxillary mucin). Results are given as the reciprocal of the binding titre (–, no binding).

dilution series of the bacteria was analysed (Table 2). Trypsin digestion of the bacteria using different enzyme concentrations either reduced or abolished the binding activity, which suggests that the interaction is mediated by a protein on the bacterial surface. Incubation of the bacteria at 80 °C or in the presence of 2 M NaCl, 10 mM DTT or 1% Triton X-100 had only a minor effect on the thyroglobulin-binding activity, which indicated that the protein is heat stable and tightly attached to the surface of the bacteria.

Adhesion of bacteria to immobilized ligands

Adhesion of bacteria to thyroglobulin immobilized on microtitre plate wells was investigated to find out whether the glycoprotein-binding activity could support the adhesion of bacteria to glycan-containing surfaces. Metabolically labelled bacteria adhered to the wells coated with thyroglobulin, and the adhesion was inhibited with excess ligand (Fig. 2a). Essentialiy, no binding was detected to the control BSA-coated surface. These results indicate that S. pyogenes can adhere to surfaces coated with thyroglobulin.

Transposon mutagenesis and analysis of the mutants

Transposon mutagenesis of wild-type strain A8173 was carried out in an attempt to identify the gene that encodes the binding activity. Transposon-harbouring mutants (~4000 colonies) were screened by blotting the mutant colonies onto nitrocellulose membranes and probing them for their binding activity using thyro-
Table 2. Extractability of the glycoprotein-binding activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Binding activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64</td>
</tr>
<tr>
<td>Trypsin (µg ml⁻¹)</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>64</td>
</tr>
<tr>
<td>0.1</td>
<td>32</td>
</tr>
<tr>
<td>1.0</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>Heat (80 °C, 30 min)</td>
<td>32</td>
</tr>
<tr>
<td>NaCl (2 M)</td>
<td>32</td>
</tr>
<tr>
<td>Triton X-100 (1%)</td>
<td>32</td>
</tr>
<tr>
<td>DTT (10 mM)</td>
<td>32</td>
</tr>
</tbody>
</table>

Fig. 2. Binding of radiolabelled streptococci to thyroglobulin on microtitre plates. The wells were coated with 10 μg ml⁻¹ thyroglobulin (Tg) and saturated with 2% BSA, 0.1% Tween 20 in PBS. Tran³⁵S-labelled bacteria were added and incubated with gentle agitation at 37 °C for 1.5 h. In the inhibition experiment the bacteria were preincubated with excess thyroglobulin prior to addition to the wells. Binding of the bacteria to wells coated with 2% BSA was used as control. Results are given as the mean values ± SD of 12 parallel wells. (a) Binding of S. pyogenes A8173 to thyroglobulin and BSA and the inhibition of binding with excess thyroglobulin. (b) Binding of the mga mutant A8173-1 to thyroglobulin. White bar, A8173; black bars, A8173-1.

Fig. 3. The glycoprotein-binding activity of wild-type, transposon mutant and mga-complemented bacteria. A membrane containing aliquots of serially diluted (OD₆₀₀ 4.0 to 0.125) wild-type S. pyogenes A8173, the corresponding transposon mutant A8173-1, control strain with vector only and strain complemented with the mga gene was incubated with biotinylated thyroglobulin and the binding was detected as in Fig. 1. Similar results as with thyroglobulin were also obtained with fetuin, asialofetuin and submaxillary mucin.

Fig. 4. Determination of the number of the transposons integrated into the genome of A8173-1. HindIII-digested genomic DNAs of the wild-type A8173 and the corresponding mutant A8173-1 were probed with pAM120 in a Southern hybridization experiment. The two signals arise from the single cleavage site in Tn916. The positions of reference markers (in kb) are indicated.

HindIII-digested genomic DNA with plasmid pAM120 (Fig. 4). A 14 kb fragment of the A8173-1 genomic DNA that contained the 5' end of Tn916 was cloned. The DNA was sequenced and the insertion locus was found to be in the mga gene at position 2076 in the previously published sequence (Podbielski et al., 1995).

Binding activity in an mga-complemented strain and mga regulon mutants

To confirm that the mutation in the mga gene was responsible for the loss of S. pyogenes A8173 glycoprotein-binding activity, the mutant strain was complemented for mga expression with the plasmid pJRS2050, which carries all of mga and the regulatory region upstream of mga in the Esc. coli–S. pyogenes shuttle vector pLZ12-Spec (Andersson et al., 1996). The binding of the glycoproteins to dilution series of the complemented strain A8173-1(pJRS2050) was compared with the binding to the wild-type A8173 and the mutant A8173-1 strains (Fig. 3). Introduction of the mga gene

globulin as the ligand. Two colonies that did not bind to thyroglobulin (Figs 2b, 3) were identified. The mutant designated A8173-1 was found to harbour one transposon in the genome by Southern hybridization of the

A novel binding activity in S. pyogenes
partly restored the thyroglobulin binding activity, while the control plasmid pLZ12-Spec had no effect.

Further evidence for the dependence of the S. pyogenes A8173 glycoprotein-binding activity on Mga was obtained by analysing independent, previously characterized mga mutants for their binding to thyroglobulin. As with the A8173 strain, thyroglobulin bound to the M2 71-676 and CS101 wild-type strains. Thyroglobulin showed no binding to the mga gene insertion mutants of these strains (Table 3). In contrast, binding of thyroglobulin to the emm and fcr mutants of M2 71-676, and to a mrp mutant of CS101 was as strong as to the parental strains, which indicated that neither the M-

protein nor the IgG-binding protein, which are regulated by the mga gene, is responsible for the binding activity.

**Prevalence of the binding activity**

Prevalence of the binding activity among S. pyogenes was investigated by studying the binding of thyroglobulin to 46 S. pyogenes strains. The activity was present in at least 32 of the 46 strains studied (Fig. 5). There appeared to be no major difference in the presence of the activity between bacteria from various infection foci (throat, skin and blood), opacity factor positive and negative strains or strains of different T serotypes (not shown), or from different geographical origins (Finland, USA, Spain).

**DISCUSSION**

The issue of Streptococcus pyogenes adherence to human epithelial cells is only just beginning to be understood. Some of the S. pyogenes surface components have been identified that mediate the binding of the bacteria to the host epithelial cells, but little is known about the host cell-surface receptors recognized by S. pyogenes. The fibronectin-binding adhesin SfbI/protein F1 has proven to be important in the adherence of the bacteria to host respiratory epithelial cells and to extracellular matrix fibronectin. In addition, these proteins have been demonstrated to promote the invasion of S. pyogenes into host cells (Hanski et al., 1992; Talay et al., 1992; Sela et al., 1993; Ozeri et al., 1996, 1998; Molinari et al., 1997; Jadoun et al., 1998). Some studies suggest that M-protein mediates adherence of S. pyogenes to epithelial cells and that the receptor structure would be a fusoc-containing oligosaccharide of the type found on HEp-2 cells (Tylewska et al., 1988; Wang & Stinson, 1994a, b). Other studies demonstrate no difference in the binding of an S. pyogenes strain and its M-protein defective derivative to human buccal and tonsillar epithelia (Caparon et al., 1991). It has also been proposed that the S. pyogenes hyaluronic acid capsule, which is present on the surface of the most of S. pyogenes strains, may serve as a universal mediator of attachment to human keratinocytes of the pharyngeal mucosa and the skin (Schrager et al., 1998). A hypothetical two-step model where bacterial surface lipoteichoic acid (LTA) has a key role has been proposed for the interaction of streptococci and host cells (Hasty et al., 1992). This model combines the LTA-mediated weak and reversible adherence of the bacteria to the cells with the strong and irreversible binding between fibronectin-binding proteins and fibronectin, or M-protein and an M-protein receptor.

Cell adhesion mechanisms characterized for other streptococci include the interactions between Streptococcus pneumoniae and the oligosaccharide structures Galβ1-4GlcNAcβ1-3Galβ1-4Glc (Andersson et al., 1983), GalNAcβ1-4Galβ1- (Krivian et al., 1988), GalNAcβ1-3Galβ1-4Galβ1-4Glc-Cer (Cundell & Tuomanen, 1994) and NeuAcβ2-3Galβ1- (Barthelson et al., 1998) of

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**Table 3. Glycoprotein binding activity of mga-regulon mutant strains**

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Characteristics</th>
<th>Binding activity†</th>
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</thead>
<tbody>
<tr>
<td>M2 71-676</td>
<td>Wild-type strain, M-type 2</td>
<td>64</td>
</tr>
<tr>
<td>M2 mga†</td>
<td>Mga- deficient mutant</td>
<td>–</td>
</tr>
<tr>
<td>M2 emm†</td>
<td>M-protein deficient mutant</td>
<td>64</td>
</tr>
<tr>
<td>M2 fcr†</td>
<td>IgG-binding-protein deficient</td>
<td>64</td>
</tr>
<tr>
<td>CS101</td>
<td>Wild-type strain, M-type 49</td>
<td>32</td>
</tr>
<tr>
<td>CS101 mga†</td>
<td>Mga- deficient mutant</td>
<td>–</td>
</tr>
<tr>
<td>CS101 mrp†</td>
<td>IgG-binding-protein deficient</td>
<td>32</td>
</tr>
</tbody>
</table>

*Described by Podbielski et al. (1996b) and Schmidt et al. (1997).
† Binding activity of biotinylated thyroglobulin on serially diluted bacteria is expressed as the reciprocal of the binding titre (–, no binding).

**Fig. 5. Prevalence of glycoprotein-binding activity among S. pyogenes strains.** Bacterial suspensions (OD600 0·5) of 46 S. pyogenes from various infection foci (throat, skin and blood; Finnish strains) and from other geographical origins (USA, Spain) were pipetted as 1 µl aliquots onto nitrocellulose membranes. Binding of thyroglobulin to the bacteria was detected as in Fig. 1 and compared to binding to A8173.
human respiratory epithelial cells. The oligosaccharides were used as inhibitors of binding of pneumococci to epithelial cells. The binding of the meningitis-associated Streptococcus suis to Galβ1-4Gal and to sialic acid-containing oligosaccharides are other well characterized interactions of streptococci with oligosaccharide receptors (Luukkonen et al., 1992; Haataja et al., 1993, 1994). Streptococcus suis were used as inhibitors of binding of pneumococci to containing salivary glycoproteins (Demuth et al., 1988, 1990). Anti-adhesion experiments have been carried out in animal models using lacto-N-neo-tetraose and its derivatives to attenuate the course of pneumococcal pneumonia and to prevent colonization of the nasopharynx (Idänpää-Heikkilä et al., 1997; Barthelson et al., 1998).

The results of the present study suggest the presence of a novel glycoprotein-binding activity in S. pyogenes. The presence of the activity is demonstrated both by the binding of soluble glycoproteins to immobilized bacteria, and by the binding of bacteria to immobilized glycoproteins. The activity is different from the S. pyogenes fibronectin-binding activity, since the binding of thyroglobulin to A8173 was not inhibited by fibronectin (J. Hytönen & J. Finne, unpublished results). The glycoprotein-binding activity is present in the majority of S. pyogenes strains studied. A similar activity is also found in group C and G streptococci.

There are no major protein sequence similarities between the glycoproteins studied. The cross-inhibitions of the glycoproteins and the absence of binding to nonglycosylated proteins therefore suggest that the receptor structure may be a carbohydrate. On the other hand, the observation that the binding of thyroglobulin is only partially inhibited by fetuin, asialofetuin and mucin may suggest the presence of one or more additional binding activities. Bovine thyroglobulin contains only N-linked glycans (Rawitch et al., 1993) and bovine submaxillary mucin mainly O-linked glycans (Strous & Dekker, 1992). Fetuin is glycosylated with both N- and O-linked sialic acid-containing oligosaccharides (Spiro & Bhoyroo, 1974; Yet et al., 1988). Preliminary experiments using a selection of mono-, di- and trisaccharides as inhibitors of binding did not reveal the binding specificity of the adhesin (P. Isomäki & J. Finne, unpublished results). The receptor structure could therefore be a more complex or extended structure included in the N- and/or O-glycans of these glycoproteins, or a structure not apparent in gross peptide sequence comparison analysis.

As indicated by the resistance of the binding protein to solubilizations by salt, detergent, reducing agent and heat treatment, it appears to be tightly anchored to the bacterial surface. Only trypsin digestion removed the activity from the bacteria. These physical characteristics parallel those described for some other streptococcal surface proteins like streptococcal surface dehydrogenase, which was shown to be resistant to 2% SDS and 2 M NaCl treatments but sensitive to trypsin digestion (Pancholi & Fischetti, 1992). Unfortunately, analysis of the trypsin extract of streptococcci revealed that the activity was rapidly inactivated and the molecule was therefore not amenable to further purification.

In transposon-mutagenesis experiments, the adhesion-inactivating insertion was found to be in the mga gene. The finding that the S. pyogenes binding-activity expression is controlled by the mga gene was confirmed by studying the adhesion of the glycoproteins to independent mga mutant strains, and by complementation with a wild-type mga gene. That the complementation was partial may be related to the fact that the mga gene used originated from another strain of S. pyogenes. On the other hand, there was binding of the glycoproteins to mutant strains deficient in M-protein and IgG-binding protein, which indicates that these proteins do not mediate the binding activity. The fact that the mga gene is a regulator of important S. pyogenes virulence factors, together with the high prevalence of the glycoprotein-binding activity among S. pyogenes and group C and G strains, suggests that it may have a role in the pathogenicity of streptococci. Further studies including the cloning of the gene are needed in order to characterize the molecular properties of this ‘streptadhesin’ activity, its receptor specificity and its role in streptococcal virulence.

ACKNOWLEDGEMENTS

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