International Congress Series 1289 (2006) 246–249

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The contribution of serum opacity factor to group A streptococcal epithelial cell invasion

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Abstract. Serum opacity factor (SOF) is a bifunctional cell surface protein expressed by 40–50% of group A streptococcal (GAS) strains comprised of a C-terminal domain that binds fibronectin and an N-terminal domain that mediates opacification of mammalian sera. SOF is co-transcribed in a two-gene operon with another fibronectin-binding protein, SfbX. We compared the ability of an SOF(+) wild-type (WT) serotype M49 GAS strain and isogenic mutants lacking SOF or SfbX to invade cultured Hep-2 human pharyngeal epithelial cells. Elimination of SOF led to a significant decrease in Hep-2 intracellular invasion while loss of SfbX had minimal effect. The hypoinvasive phenotype of the SOF(−) mutant could be restored upon complementation with the *sof* gene on a plasmid vector, and heterologous expression of *sof*49 in M1 GAS or *Lactococcus lactis* conferred marked increases in Hep-2 cell invasion. Studies using a mutant *sof*49 in which the fibronectin-binding domain had been deleted in-frame confirmed that the C-terminal domain of SOF contributes to cellular invasion independent of the fibronectin-binding domain.

Keywords: Group A *Streptococcus*; Serum opacity factor; SOF; SfbX; Cellular invasion; Fibronectin binding; Epithelial cell

1. Introduction

Group A *Streptococcus* (GAS) causes a wide spectrum of human disease. The serum opacity factor operon is found in 40–50% of GAS strains and consists of two genes, serum opacity factor (*sof*) and the fibronectin-binding protein X (*sfbX*) [1,2].

SOF is a 110-kDa protein that is expressed both extracellularly and in a membrane-bound form, attached by an LP(X)SG motif cell wall anchor [2]. SOF is comprised of a C-terminal fibronectin-binding domain [3], and an N-terminal enzymatic domain responsible

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0531-5131/
doi:10.1016/j.ics.2005.11.029

for cleavage of apolipoprotein A1 resulting in the aggregation of high-density lipoprotein to give serum a cloudy, opacified appearance [4]. The SfbX protein possesses a C-terminal fibronectin-binding domain homologous to that of SOF, but the function of its N-terminal sequences have yet to be elucidated [2].

Previous work using SOF-coated latex beads has suggested that this protein could mediate adherence and invasion of epithelial cells [5]. In this study, we use isogenic SOF and SfbX mutants and heterologous gene expression to directly assess the contribution of each protein to GAS invasion of Hep-2 pharyngeal epithelial cells. Unexpectedly, our results uncovered a key contribution of the N-terminal SOF enzymatic domain to GAS cellular invasion independent of the C-terminal fibronectin-binding motifs.

2. Materials and methods

WT M49 GAS strain NZ131 was employed along with the precise, in-frame allelic replacement mutants NZ131Δ*sof* and NZ131Δ*sfbX* [2]. Sof and SfbX were expressed in WT M1 GAS strain 5448 and *Lactococcus lactis* strain NZ9000 by transformation with previously described expression vectors pAJ-*sof* and pAJ-*sfbX* [2]. Serum opacification was tested using 100 μl of stationary phase supernatant added to 1 ml of heat-inactivated horse serum and incubated overnight at 37 °C. Intracellular invasion of human pharyngeal epithelial cell line Hep-2 was assessed by an antibiotic protection assay using logarithmic phase GAS at MOI = 5 : 1, primary incubation time of 2 h, and penicillin/gentamicin treatment for 2 h prior for the disruption of the cell monolayer to enumerate intracellular cfu. All assays were performed in triplicate and repeated three times. The vector pSofΔFn was created from pAJ-*sof* by inverse PCR deleting the entire C-terminal fibronectin-binding region (P801 to R960) followed by blunt end religation.

3. Results

3.1. SOF and not SfbX contributes to epithelial cell invasion by M49 GAS

Allelic replacement mutants of M49 GAS lacking SOF or SfbX were compared to the WT parent strain for their ability to invade cultured monolayers of Hep-2 cells in vitro (Fig. 1). The isogenic SOF(−) mutant exhibited a 50% decrease in cellular invasion ($p = 0.0002$), which could be restored above WT levels by return of the single *sof* gene on a complementation plasmid ($p < 0.0001$). The isogenic SfbX mutant did not differ significantly from WT in Hep-2 invasiveness ($p = 0.255$). As SOF and SfbX share highly homologous C-terminal fibronectin-binding repeat domains, these results provided the first clue that N-terminal sequences in the “enzymatic” domain of SOF could possess unique properties facilitating cellular invasion.

3.2. Gain of function analysis by heterologous expression of SOF and SfbX

To determine if *sof* and *sfbX* are sufficient to confer increased invasion of epithelial cells, expression vectors for each GAS M49 gene were transformed into bacteria lacking this operon. Under the standard assay condition, overexpression of *sof49* in GAS M1 strain 5448 increased Hep-2 cell invasion 100-fold, while overexpression of *sfbX* produced a 10-fold gain in invasion frequency (Fig. 2A). When parallel studies of

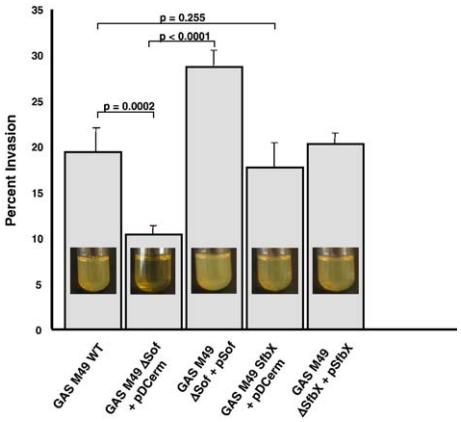

Fig. 1. Invasion of HEP-2 human pharyngeal epithelial cells by WT M49 GAS strain NZ131 and isogenic allelic replacement mutants lacking either SOF or SfbX.

heterologous expression were performed in *L. lactis*, which lacks significant fibronectin binding, *sof* conferred a nearly 1000-fold increase in Hep-2 cell invasion compared to a 10-fold increase for *sfbX* (Fig. 2B). These gains of function analyses corroborated the observed greater contribution of SOF to epithelial cell invasion when compared to SfbX.

3.3. The N-terminal enzymatic domain of SOF contributes to epithelial cell invasion independently of the C-terminal fibronectin-binding domain

We observed in our mutagenesis and heterologous expression studies that SOF contributed much more significantly than SfbX to epithelial cell invasion, although both proteins possess homologous C-terminal fibronectin-binding domains. We hypothesized that the N-terminal enzymatic domain of SOF must therefore play a direct role in promoting epithelial cell invasion. To test this hypothesis, we generated an in-frame deletion of the fibronectin-binding repeats of SOF within our expression plasmid to

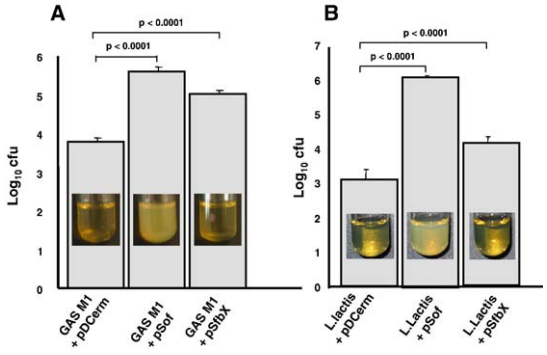

Fig. 2. Heterologous expression of the *sof* and *sfbX* genes from M49 GAS in (A) M1 GAS that normally lacks the corresponding operon and (B) *Lactococcus lactis* strain NZ9000.

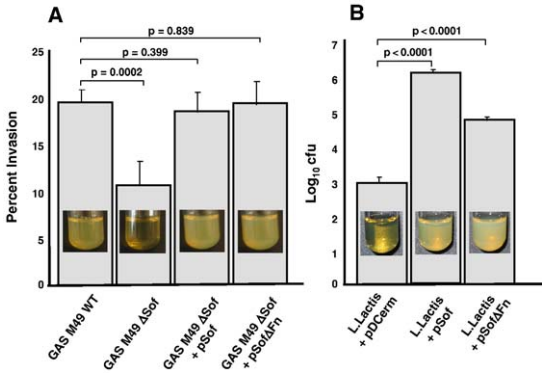

Fig. 3. Deletion of the fibronectin-binding domain of SOF protein does not eliminate the contribution of SOF to epithelial cell invasion as tested in (A) complementation studies using the M49 GAS ΔSOF mutant and (B) heterologous expression studies in *L. lactis*.

produce a truncated derivative of the protein that maintained opacification activity. The truncated SOFΔFn protein was observed to complement the invasion defect of the M49 NZ131Δ*sof* mutant to WT levels (Fig. 3A), and to confer a 100-fold increase in epithelial cell invasion when expressed heterologously in *L. lactis* (Fig. 3B). Taken together, these results indicate that the N-terminal enzymatic domain of SOF contributes to epithelial cell invasion independently of the C-terminal fibronectin-binding domain.

4. Discussion

SOF contributes to GAS epithelial cell adherence through its C-terminal fibronectin-binding domain. Here we have used isogenic mutants and heterologous expression to show for the first time that SOF promotes GAS invasion of human pharyngeal epithelial cells. We have further shown that the N-terminal enzymatic domain of SOF, in addition to its activity in serum opacification, possesses important pro-invasive properties independent of the C-terminal fibronectin-binding repeats. Ongoing investigations examine whether serum opacification per se contributes to GAS invasion and assess the overall importance of SOF-mediated epithelial cell invasion to virulence.

References

[1] B. Beall, et al., *emm* and *sof* gene sequence variation in relation to serological typing of opacity-factor-positive group A streptococci, Microbiology 146 (2000) 1195–1209.
[2] A. Jeng, et al., Molecular genetic analysis of a group A *Streptococcus* operon encoding serum opacity factor and a novel fibronectin-binding protein, SfbX, J. Bacteriol. 185 (2003) 1208–1217.
[3] H.S. Courtney, J.B. Dale, D.L. Hasty, Mapping the fibrinogen-binding domain of serum opacity factor of group a streptococci, Curr. Microbiol. 44 (2002) 236–240.
[4] G.A. Saravani, D.R. Martin, Opacity factor from group A streptococci is an apoproteinase, FEMS Microbiol. Lett. 56 (1990) 35–39.
[5] S. Oehmcke, A. Podbielski, B. Kreikemeyer, Function of the fibronectin-binding serum opacity factor of *Streptococcus pyogenes* in adherence to epithelial cells, Infect. Immun. 72 (2004) 4302–4308.