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Fc-Mediated Non-specific Binding Between Fibronectin-Binding Protein I of *Streptococcus pyogenes* and Human Immunoglobulins

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Fibronectin-binding protein I (SfbI) from *Streptococcus pyogenes* plays a key role in bacterial adhesion to, and invasion of, eukaryotic cells. In addition, SfbI exhibits a considerable potential as mucosal adjuvant and can trigger polyclonal activation of B cells. Here, we report that SfbI is also capable of binding human IgG in a nonimmune fashion. SfbI was reactive with IgG1, IgG2, IgG3, and IgG4 isotypes (type IIo IgG-binding profile). The affinity constant ($K_a$) of the SfbI-IgG interaction was in the range of $1–2 \times 10^{-5}$ M. Further studies demonstrated that the SfbI binding was mediated by the Fc component of the IgG molecule. Experiments performed using purified recombinant proteins spanning different domains of SfbI showed that the IgG-binding activity was restricted to the fibronectin-binding domains, and in particular to the fibronectin-binding repeats. Finally, the presence of recombinant SfbI resulted in an impairment of both phagocytosis of IgG-coated RBCs and Ab-dependent cell cytotoxicity by macrophages. These results demonstrated for the first time that, in addition to its major role during the colonization process, SfbI may also favor bacterial immune evasion after the onset of the infection by interfering with host clearance mechanisms.


*S. pyogenes* is an important human pathogen able to cause either localized infections (e.g. pharyngitis and pyoderma) or highly invasive diseases (such as necrotizing fasciitis and toxic shock-like syndrome) (1). These infections can also lead to poststreptococcal diseases, such as glomerulonephritis and acute rheumatic fever, as a result of an immunological physiopathogenic process (2). Although streptococcal infections can be treated with antibiotics, an alarming increase in the incidence of streptococcal infections has been observed throughout the world (3, 4).

*S. pyogenes* produces different virulence factors that enable colonization and favor bacterial evasion of host clearance mechanisms. Among them should be mentioned the well-characterized M protein, whose antiphagocytic activity is based in the inhibition of complement deposition (5–7); fibronectin-binding protein I (SfbI)2 or its allelic variant, F protein, which plays a major role in bacterial adhesion (8–10) and internalization, being the first identified invasin of *S. pyogenes* (11, 12); C5a peptidase, which cleaves complement factor C5a, interfering with its activity as chemoattractant for neutrophils (13); and several molecules able to bind to Igs (14–19).

Although the exact role of Ig-binding proteins in the virulence process is unknown, it has been hypothesized that they may help bacteria to evade host defense mechanisms. In addition, their expression has been observed in skin isolates (20) being associated with a major invasiveness in a mouse skin infection model (21, 22). *S. pyogenes* also produces several extracellular proteins involved in virulence, such as proteinase, DNase, streptokinase, hyaluronidase, and hemolysins (23).

SfbI is expressed by ~75% of the clinical isolates from all over the world, independently of their serotypes (24), and the fibronectin-binding domains are highly conserved among the different isolates, indicating that SfbI might play an important role during bacterial infection. This was further supported by studies showing that SfbI-immunized mice were extremely resistant to bacterial challenge (24). The SfbI protein not only plays a key role during the initial stages of the infection process by enabling bacterial binding to cell-attached fibronectin (8, 10) and invasion of eukaryotic cells (11, 12), but it also seems to exert a wide range of activities at the level of the immune system. SfbI can specifically bind B cells, promoting their activation, and can also trigger up-regulation of MHC class II molecules in APCs (25). These properties of SfbI, which can be exploited to promote systemic and/or mucosal responses against coadministered Ags (26), might be also involved in the physiopathogenesis of poststreptococcal diseases. These observations highlight the importance of elucidating the different roles played by SfbI at specific stages of the infection process.

In this study we tried to obtain further knowledge about the biological activities of the SfbI protein on the components of the immune system. This might help to gain insights into the physiopathology of *S. pyogenes* infections and/or sequelae, facilitating the design of novel strategies to prevent streptococcal diseases or to exploit the potential of this protective Ag.

Materials and Methods

Production and purification of recombinant SfbI proteins

To generate recombinant proteins encompassing different domains of the SfbI protein, DNA fragments spanning different portions of the sfbI gene (9) were amplified by PCR and cloned into the BamHI/SalI sites of the pQE30 vector (Qiagen, Hilden, Germany). Plasmid pSTH2 contains the 1581-bp fragment encompassing positions 274 to 1854 of sfbI and codes

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2 Abbreviations used in this paper: SfbI, fibronectin-binding protein I; RU, resonance unit; ADCC, Ab-dependent cell-mediated cytotoxicity.

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for a His-tagged-SfbI fusion derivative lacking the NH₂-terminal signal peptide and the COOH-terminal wall- and membrane-anchor regions (H2 fragment). Plasmid pSTH10 harbors a 984-bp BamHI/SstI fragment encompassing positions 1247 to 2257 and encodes a His-tagged polypeptide that lacks the fibronectin-binding regions (H10 fragment). Plasmid pSTH12 contains a 615-bp fragment encompassing positions 1240 to 1854 and encodes a polypeptide (H12 fragment) that lacks the N-terminal domain of the SfbI molecule and the C-terminal wall- and membrane-anchor regions but retains the two fibronectin-binding domains (i.e., the fibronectin-binding repeats and the fibronectin-binding spacer regions). The plasmid pSTH4 harbors a 147-bp insert encompassing positions 1240 to 1386 of sfbI and codes for the fibronectin-binding spacer region (H4 fragment), whereas the 471-bp fragment contained in pSTH10 encompasses positions 1240 to 1386 and codes for the fibronectin-binding repeats region (H8 fragment). DNA manipulations were performed as described by Sambrook et al. (27), and restriction and modification enzymes were supplied by New England Biolabs (Beverly, MA). For overexpression of the recombinant proteins, E. coli HOFEN, Germany, SfbI protein, or truncated SfbI polypeptides in coating wells, and plates were further incubated for 2 h at 37°C. After four washes, appropriate amounts of either mouse anti-human IgA, IgG, or IgM (Sigma) or rabbit anti-SfbI Abs were added, and plates were incubated for 1 h at 37°C. After six washes, 100 μl of peroxidase-conjugated goat anti-mouse IgG (Dianova, Hamburg, Germany) or peroxidase-conjugated goat anti-rabbit IgG (Dianova) Abs were added to each well, and plates were incubated at room temperature for 1 h. After eight washes, reactions were developed using ABTS (2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) in 0.1 M citrate-phosphate buffer (pH 4.5) containing 0.05% surfactant P-20. Deacti-vation of unreacted 3-nitro-4-hydroxyanilide esters was performed by treatment with 1 M ethanolamine for 10 min. Binding was determined by measuring the increase of OD at 450 nm.

**BIAcore analysis**

A BIAcore biosensor 2000 (BIAcore, Freiburg, Germany) was used to analyze the interaction between SfbI and human IgG. Preceding the immobilization of SfbI or IgG on the biosensor chips, the carboxyl groups of the CM5 matrix were activated by derivatization with EDC/NHS (50 mM N-hydroxy-succinimid/200 mM N-ethyl-N’(dimethylamino)propyl)-carbodiimide) for 10 min. SfbI or IgG were immobilized on the CMS carboxymethylated dextran matrix via amina coupling. Immobilization was performed in 10 mM potassium acetate buffer (pH 4.5). For binding analysis, 1800 resonance units (RU) of SfbI or 2000 RU of IgG were immobilized, and binding interactions were determined by passing IgG or SfbI, respectively, over the immobilized protein in HBS buffer (10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, and 0.005% surfactant P-20). Deacti-vation of unreacted N-hydroxysuccinimide esters was performed by treatment with 1 M ethanolamine for 10 min. Binding was determined by measuring the increase of RU.

**Polyacrylamide gel electrophoresis and Western blotting analysis**

Human IgA and IgG and mouse, horse, and pig IgGs (3 μg/lane) were fractionated by discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with a 10% separating gel. Prestained m.w. standards (Bio-Rad, Munich, Germany) were included in each gel. Proteins were then transferred onto nitrocellulose using a semidyve device (Bio-Rad). Non-spe-cific binding sites were saturated with 10% (v/v) low fat milk (1.5%) in Tris buffer for 2 h at room temperature. Then, the membranes were incu-bated with the appropriate probe, washed between steps (four times, 10 min each time) with Tris buffer (TBS) containing 0.05% Tween 20 (TBST) at room temperature, and Ag-Ab complexes were visualized by chemilumi-nescence with an ECL kit (Amersham Life Science, Baunschweig, Ger-many) exposing Kodak (Rochester, NY) XAR-5 films. The ECL detection kit is based on the emission of light resulting from the dissipation of energy as a result of HRP/hydrogen peroxidase-catalyzed oxidation of luminol in alkaline conditions. Immediately following oxidation, the luminol is in an excited state, which then decays to basal state via a light-emitting pathway. The light emission is a wavelength of 428 nm, which can be detected by a short exposure to blue light-sensitive autoradiography film.

**Transmission electron microscopy**

Infected cells were fixed with 0.5% formaldehyde and 0.2% glutaraldehyde in PBS (pH 6.9) for 30 min at 4°C. After three washes with PBS containing 10 mM glycine for blocking free aldehyde groups, the cells were immo-bilized in 1.5% agar. The solidified agar was cut into small blocks, which were embedded by progressive lowering of temperature using the polar cryomicrotome. The sections were cut with a diamond knife, collected on formvar-coated nickel grids (300 mesh), and incubated for 12 h at 4°C with a protein A-purified rabbit polyclonal Ab against SfbI. After grids were washed with PBS, the bound Abs were labeled for 1 h at room temperature with protein A-gold complexes (gold-particle diameter 10 nm; concentration 10 μg/ml, 30 min; and incubation volume 10 μl). Grids were subsequently rinsed with PBS containing 0.01% Tween 20 and then with distilled water. After air drying, the sections were counterstained with 4% aqueous uranyl acetate (pH 4.5) for 5 min and examined with a Zeiss (Jena, Germany) electron microscope EM 910 at an acceleration voltage of 80 kV at calibrated magnifications.

**ELISA determination of nonimmune binding of soluble or matrix-bounded SfbI to human IgGs**

Nunc-Imuno MaxiSorp assay plates (Nunc) were coated overnight at 4°C with 50 μl/well of either purified human IgA, IgG, or IgM (Sigma, Deisen-hofen, Germany). SfbI protein, or truncated SfbI polypeptide in coating buffer (0.1 M Na₂HPO₄, pH 9.0). After four washes (0.05% Tween 20 in PBS), plates were blocked with 200 μl/well of 0.05% Tween 20, 1 mM EDTA, and 0.25% gelatin in PBS for 1 h at 37°C. Different concentrations of either human IgA, IgG, or IgM (Ig binding to matrix-bound SfbI) or SfbI polypeptides (SfbI binding to matrix-bound Ig) were then added to coated wells, and plates were further incubated for 2 h at 37°C. After four washes, appropriate amounts of either mouse anti-human IgA, IgG, or IgM (Sigma) or rabbit anti-SfbI Abs were added, and plates were incubated for 1 h at 37°C. After six washes, 100 μl of peroxidase-conjugated goat anti-mouse IgG (Dianova, Hamburg, Germany) or peroxidase-conjugated goat anti-rabbit IgG (Dianova) Abs were added to each well, and plates were incubated at room temperature for 1 h. After eight washes, reactions were developed using ABTS (2,2’-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) in 0.1 M citrate-phosphate buffer (pH 4.5) containing 0.05% surfactant P-20). Deacti-vation of unreacted N-hydroxysuccinimid esters was performed by treatment with 1 M ethanolamine for 10 min. Binding was determined by measuring the increase of OD at 450 nm.

**FIGURE 1.** Binding of SfbI to immobilized human IgA ( ), IgG ( ), and IgM ( ). Microtiter plates were coated with excess of the respective purified Ig and incubated with varying concentrations of SfbI (H2 fragment). The SfbI-IgG complexes were detected using rabbit anti-SfbI and peroxidase-conjugated goat anti-rabbit IgG as secondary Ab. The reported data are representative of three independent experiments. Results are ex-pressed as absorbances at 405 nm and represent the average of triplicate samples. SD were lower than 10%.

**Cells culture and invasion assay**

The human laryngeal epithelial cell line HEp-2 (American Type Culture Collection (ATCC), Manassas, VA; CCL23) was cultured in DMEM (Life Technologies, Karlsruhe, Germany) supplemented with 10% FCS, 5 mM l-glutamine at 37°C in an atmosphere containing 8% CO₂. Cells were seeded onto 12-mm-diameter glass coverslips (Nunc, Roskilde, Denmark) placed on the bottom of the tissue culture plates. Then, ~3 × 10⁶ bacterial cells were centrifuged, and the pellets were resuspended in HEPES-DMEM supplemented with 1% FCS. Bacterial suspensions were added to tissue culture dishes (bacterial infection rate 100:1), and plates were further in-cubated for 2 h. The wells were then washed twice with PBS, and extracellular bacteria were killed by adding fresh medium containing penicillin (5 μg/ml) and gentamicin (100 μg/ml).

**Polyacrylamide gel electrophoresis and Western blotting analysis**

Human IgA and IgG and mouse, horse, rabbit, and pig IgGs (3 μg/lane) were fractionated by discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with a 10% separating gel. Prestained reference standards (Bio-Rad, Munich, Germany) were included in each gel. Proteins were then transferred onto nitrocellulose using a semidyve device (Bio-Rad). Non-specific binding sites were saturated with 10% (v/v) low fat milk (1.5%) in Tris buffer for 2 h at room temperature. Then, the membranes were incu-bated with the appropriate probe, washed between steps (four times, 10 min each time) with Tris buffer (TBS) containing 0.05% Tween 20 (TBST) at room temperature, and Ag-Ab complexes were visualized by chemilumi-nescence with an ECL kit (Amersham Life Science, Baunschweig, Ger-many) exposing Kodak (Rochester, NY) XAR-5 films. The ECL detection kit is based on the emission of light resulting from the dissipation of energy as a result of HRP/hydrogen peroxidase-catalyzed oxidation of luminol in alkaline conditions. Immediately following oxidation, the luminol is in an excited state, which then decays to basal state via a light-emitting pathway. The light emission is a wavelength of 428 nm, which can be detected by a short exposure to blue light-sensitive autoradiography film.
Ab-dependent cell-mediated cytotoxicity (ADCC), consisting of the incubation of 100 nm urea. Hemoglobin was determined in each case by a colorimetric assay (Centeon, Liederbach, Germany). After three washes, 10^6 IgG-sensitized proteins were tested for binding against themselves. Binding activity is represented by increasing resonance units (RU) over time. Curves include the resonance signal obtained in the absence of protein attached to the sensor chips surface as well as the signal obtained when the proteins were tested for binding against themselves. Binding activity is represented by increasing resonance units (RU) over time.

Isolation and culture of peritoneal macrophages
Peritoneal exudate macrophages were harvested from BALB/c mice injected 4 days earlier with 2 ml of 2% thioglycolate broth (Life Technologies). Macrophages were resuspended in RPMI (Life Technologies) supplemented with 2 mM l-glutamine, 50 U/ml penicillin, 50 µg/ml streptomycin, 2 g/l of NaHCO₃, and 5% heat-inactivated FCS. Macrophages were seeded at a density of 2 × 10⁵ cells/well and allowed to adhere 4 days earlier with 2 ml of 2% thioglycolate broth (Life Technologies). After three washes, 10⁶ IgG-sensitized and nonsensitized erythrocytes were incubated with either H2, H10, or H4 polypeptides (20 µg/ml) in a final volume of 1.5 ml and added to adherent macrophages. Phagocytosis was allowed to proceed for 30, 60, 90, and 180 min at 37°C in a humidified atmosphere containing 5% CO₂. To measure Ab-dependent cell-mediated cytotoxicity (ADCC), 100 µl of supernatant was taken at each time point and the OD at 620 nm was determined. Phagocytosis and cytotoxicity assays were performed as previously described (29). In brief, a suspension of 4 × 10⁷ human RBCs (Rh0⁻) were incubated for 45 min with 2.5 µg/ml of human anti-D IgG (Rhesogam; Centeon, Liederbach, Germany). After three washes, 10⁶ IgG-sensitized and nonsensitized erythrocytes were incubated with either H2, H10, or H4 polypeptides (20 µg/ml) in a final volume of 1.5 ml and added to adherent macrophages. Phagocytosis was allowed to proceed for 30, 60, 90, and 180 min at 37°C in a humidified atmosphere containing 5% CO₂. To measure Ab-dependent cell-mediated cytotoxicity (ADCC), 100 µl of supernatant fluids was carefully taken when the incubation was finished, and then was centrifuged, and the level of extracellular lysis was determined. The nonphagocytosed erythrocytes were lysed with hypotonic solution (0.2% NaCl) for 3 min. After that, wells were washed three times with medium, and the remaining cells (macrophages containing phagocytosed erythrocytes) were resuspended in 100 µl of 0.2 M tris-HCl buffer containing 6 M urea. Hemoglobin was determined in each case by a colorimetric assay consisting of the incubation of 100 µl of supernatant or cell extract with 100 µl of DAF solution (1 ml of 2,7-diaminofluorene (Sigma), 0.1 ml of 30% hydrogen peroxide, and 10 ml of 0.2 M Tris-HCl buffer containing 6 M urea). The reaction mixture was incubated for 5 min, and the OD at 620 nm was determined.

RESULTS
SfbI binds to human IgG
First we investigated whether SfbI is able to bind human Ig. ELISA assays were performed coating microtiter plates with either purified human Ig (IgA, IgG, or IgM) or SfbI. Then, plates were incubated with either SfbI (Fig. 1) or human Ig (data not shown). Fig. 1 shows that SfbI binds to immobilized IgG but not to IgA or IgM. The binding of soluble IgG to immobilized SfbI was not detectable by ELISA assay. This can be due to alterations of the binding sites after immobilization of the SfbI protein or, alternatively, to the existence in the SfbI molecule of more than one binding site for IgG. In fact, the presence of multiple binding sites would allow SfbI to simultaneously bind two or more immobilized IgG molecules, resulting in a more stable association and detection by ELISA.

Therefore, additional studies were performed using the biosensor BIAcore. IgG was coupled to the sensor chip, and different concentrations of SfbI (200, 450, and 900 nM) were injected over the sensor surface (Fig. 2A). The obtained results confirmed the binding of SfbI to immobilized IgG and demonstrated that the binding activity was concentration dependent with an experimentally determined affinity constant ($K_d$) of 10 to 15 µM (1–2 × 10⁻⁵ M) according to the saturation response (Fig. 2A). Then, binding of soluble IgG to SfbI protein immobilized on the surface of the sensor chip was analyzed. The results displayed in Fig. 2B demonstrated that, by using a more sensitive technique, binding of soluble IgG to immobilized SfbI could also be detected, being the $K_d$ value ~1 µM. The sensograms also confirmed a tighter association...
between SfbI and immobilized IgG than between soluble IgG and immobilized SfbI protein. No significant binding activity was observed from sensor chip surfaces without immobilized proteins or when the proteins were tested for binding against themselves (Fig. 2B).

**SfbI binds to the Fc component of the IgG molecule**

The binding results were further confirmed by Western blot analysis, which also demonstrated that SfbI binds specifically to the H chain of the IgG molecule (Fig. 3).

We next investigated whether the IgG binding to SfbI was mediated by the Fab or Fc fragment. Purified IgG-Fab or IgG-Fc fragments were separated by SDS-PAGE, then transferred to a nitrocellulose membrane and incubated with SfbI. The results in Fig. 3B demonstrated that SfbI bind only to the Fc fragment of the IgG molecule. The binding properties of SfbI were further investigated by testing its ability to bind the different IgG isotypes including IgG1, IgG2, IgG3, and IgG4. As shown in Fig. 3C, SfbI bound to all IgG isotypes, being the binding to the IgG4 subclass the weakest. SfbI was also reactive with IgG from mouse, rabbit, pig, and horse (data not shown). No reactivity of the immunoblotted proteins with the secondary Abs was observed in control gels, in which Igs were directly exposed to the secondary reagent, excluding the incubation step with the SfbI protein.

**Localization of the IgG-binding domain within the SfbI protein**

The SfbI protein is involved in different biological activities, such as attachment, invasion, and activation of B lymphocytes (8, 10–12, 25, 26). Previous studies have enabled the identification of the protein domains involved in these process. To identify the region(s) of SfbI that exhibited IgG-binding activity, recombinant polypeptides spanning different segments of the SfbI protein were generated, purified, and tested for their ability to bind IgG by ELISA and Western blotting. Figs. 4 and 5 show that the polypeptide containing the fibronectin-binding domains (H12), but not that with the aromatic domain and proline repeats (H10), was able to bind to IgG. Similar results were obtained by Western blot analysis (data not shown). These data suggest that the interaction with IgG involves SfbI structures that are also required for protein binding to fibronectin. The SfbI protein can bind to fibronectin via the fibronectin-binding repeats or the fibronectin-binding spacer regions. To further define the part of the SfbI molecule responsible for the IgG binding activity, peptides encompassing the fibronectin-binding repeats (H8) and the fibronectin-binding spacer (H4) were compared for their efficiency to bind IgG. Only the fibronectin-binding repeats retained the capacity to bind IgG (Figs. 4 and 5).

**FIGURE 4.** Schematic representation of the SfbI protein and IgG-Fc-binding activity of its different domains.

**FIGURE 5.** Identification of the SfbI domain able to bind to the IgG-Fc fragment. Microtiter plates coated with IgG-Fc fragment were incubated with the recombinant polypeptides H10, H12, H4, and H8. Binding to the IgG-Fc fragment was revealed using rabbit anti-SfbI Abs. Results are expressed as absorbances at 405 nm and represent the average of three independent determinations. SD are indicated by vertical lines.
SfbI is released following S. pyogenes infection of eukaryotic cells

Although SfbI is a bacterial surface-attached protein, we found that SfbI was able to bind IgG both in soluble and matrix-attached forms (Fig. 2). Therefore, to assess whether SfbI can be found in a cell-free form during the infection process, HEp-2 cells were infected with S. pyogenes, and SfbI was detected by immunoelectron microscopy. As shown in Fig. 6, not only was SfbI detected attached to the bacterial surface, but also it was released within the phagosome. SfbI was also detected in the cytosol of HEp-2 cells (Fig. 6, circled areas), indicating the ability of the released protein to traffic out from bacteria-containing vacuoles, even reaching the cytoplasmic membrane (Fig. 6c).

Effect of SfbI on the phagocytic and ADCC activity of peritoneal macrophages

One of the most important functions of phagocytic cells is the ingestion of IgG-coated foreign particles. Receptors for the Fc component of IgG enable these cells to detect and destroy IgG-coated microorganisms, thereby contributing to the bacterial clearance during infections (30). To understand the potential role that the specific binding of SfbI to the IgG-Fc fragment might play during natural infections, we determined whether SfbI could interfere with Fc-dependent phagocytosis of IgG-sensitized human RBC by macrophages. IgG-sensitized human RBC were incubated, with or without SfbI (20 μg/ml), and then used in a phagocytosis assay. The obtained results demonstrated that the phagocytosis of IgG-sensitized RBC is strongly reduced (p < 0.05) in the presence of SfbI (Fig. 7A). No significant differences were observed in the presence or absence of SfbI when nonsensitized RBC were used or when IgG-sensitized RBC were incubated with either the H10 or H4 fragments of SfbI protein (data not shown).

Macrophages also contribute to the ADCC (31). Therefore, the Ab-dependent lysis of erythrocytes sensitized with IgG was measured in the presence and absence of SfbI. The results shown in Fig. 7B demonstrated that the lysis of extracellular RBC was significantly inhibited (p < 0.05) when sensitized RBC were preincubated with SfbI.

Discussion

Group A streptococci produce a wide range of virulent factors, which not only are essential for host colonization but also enable bacterial survival by favoring the escape from the specific and nonspecific host clearance mechanisms. Phagocytosis is an essential arm of the immune response, which is involved in the clearance and destruction of invading pathogens. In response to a bacterial infection, Abs are produced that coat the surface of the microorganisms, stimulating their ingestion following attachment...
to the Fc receptors present on the surface of macrophages (32). A major correlate of virulence of \textit{S. pyogenes} is its ability to resist phagocytosis in the nonimmune host (33). For many years, it was believed that the streptococcal M protein was the primary determinant of resistance to phagocytosis and virulence (34). However, it has been suggested in a recent report that, in addition to the M protein, other factors might contribute to the resistance to phagocytosis because mutants deficient in expression of M and M-like proteins were still resistant to phagocytosis, although to a lesser extent than the wild-type (35).

Here, we demonstrate that, in addition to its role as adhesion and invasin during bacterial infection and colonization of the upper respiratory tract (9–12), the SfbI protein can bind to the Fc component of human IgG. The SfbI-IgG interaction is mediated by the fibronectin-binding domains of SfbI and results in the impairment of Fc-mediated phagocytosis and ADCC by macrophages. The expression of SfbI by most streptococcal isolates (~75%) and the high conservation of the fibronectin-binding repeats further support its critical role during the infection process.

It can be hypothesized that \textit{S. pyogenes} use the surface-attached SfbI protein to achieve colonization during the initial steps of infection, via binding to the extracellular fibronectin, which, in turn, is attached to integrins located on the surface of the eukaryotic cells. At this stage, the surface-displayed molecules of SfbI that are not engaged in the interaction with fibronectin can bind to the Fc fragment of IgG molecules, thereby preventing bacterial uptake by phagocytic cells present at the site of infection. At a later stage, the SfbI released from the streptococcal surface during bacteria-host interaction can also bind IgG, thus interfering with the Ab-dependent host immune response against the pathogen. In this regard, we have shown that SfbI can be released from the streptococcal surface during the infection of eukaryotic cells, trafficking from phagosome to cytoplasm of infected cells. Then, SfbI easily reach the cellular surface and are released in the extracellular environment. A similar scenario, in which a protein can exhibit different functions depending on whether it is released or attached to the bacterial surface has been observed with the streptococcal protein \textit{M} and \textit{SfbI}, respectively. In this particular context, when the expression of \textit{M} protein is suboptimal, the up-regulation of a second antiphagocytic protein might protect bacteria against the phagocytic cells present at the specific niche.

All biological systems are ruled by the general principle of cost efficiency. This is particularly true for infectious microorganisms, in which protein expression is tightly regulated and the products essential for bacterial survival are produced only when they are required (39). The data reported here and functional studies have demonstrated that most isolates of \textit{S. pyogenes} are able to express more than one Ig-binding protein (17). Functional redundance is mainly observed when there is a differential chronology of activation during the infection process or when the critical nature of the specific function makes necessary the establishment of parallel back-up systems. The importance of Ig-binding proteins is highlighted by the fact that certain unrelated proteins of different bacterial species can interact with the Fc fragment of human IgG (40). This suggests that these proteins may represent a phenomenon of convergent evolution, because they provide an essential function that confers a selective advantage to the respective microorganism.

The expression of multifunctional proteins such as SfbI may endow \textit{S. pyogenes} with a selective advantage, enabling a rapid response to changing host environmental conditions without the need to synthesize additional products. In fact, SfbI not only is important for attachment to and invasion of eukaryotic cells but also might influence the course of the infection by promoting both modulation of the immune response elicited (25, 26) and impairment of bacterial clearance. The knowledge of the complex functions exhibited by streptococcal proteins such as SfbI may allow us to gain a better understanding of the intricate physiopathogenetic events that lead to disease and sequelae following \textit{S. pyogenes} infections.

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