Identification of molecular mechanisms used by *Finegoldia magna* to penetrate and colonize human skin

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Summary

*Finegoldia magna* is a Gram-positive anaerobic commensal of the human skin microbiota, but also known to act as an opportunistic pathogen. Two primary virulence factors of *F. magna* are the subtilisin-like extracellular serine protease SufA and the adhesive protein FAF. This study examines the molecular mechanisms *F. magna* uses when colonizing or establishing an infection in the skin. FAF was found to be essential in the initial adherence of *F. magna* to human skin biopsies. In the upper layers of the epidermis FAF mediates adhesion through binding to galectin-7 – a keratinocyte cell marker. Once the bacteria moved deeper into the skin to the basement membrane layer, SufA was found to degrade collagen IV which forms the backbone structure of the basement membrane. It also degraded collagen V, whereby *F. magna* could reach deeper dermal tissue sites. In the dermis, FAF interacts with collagen V and fibrillin, which presumably helps the bacteria to establish infection in this area. The findings of this study paint a clear picture of how *F. magna* interacts with human skin and explain how it is such a successful opportunistic pathogen in chronic wounds and ulcers.

Introduction

Skin is the largest organ in the human body and constitutes an important physical barrier to external stresses such as microorganisms, ultraviolet radiation, toxins, allergens and mechanical insults. It is composed of four structural layers: the epidermis, basement membrane, dermis and the subcutaneous layer (Koziel and Potempa, 2013). The skin is inhabited by a diverse array of bacteria, fungi and viruses, which vary between individuals and different sites on the skin (Schommer and Gallo, 2013). Colonization of the skin by commensal bacteria such as *Staphylococcus, Finegoldia, Micrococcus* and *Corynebacterium* sp., helps protect the host against colonization by more pathogenic microbes by depleting available nutrients and preventing their adherence and translocation across skin layers. However, when the host immune defence is compromised or the microbiota balance is disrupted, some of these commensals can act as opportunistic pathogens and cause infection (Nagy *et al.*, 2011).

The human skin inhabitant *Finegoldia magna* is a Gram positive anaerobic coccus (GPAC), whose first complete genome sequence of strain ATCC 29328 was published in 2008 (Goto *et al.*, 2008). Genomic analysis revealed that it can utilize fructose as an energy source, but also amino acids, due to its high number of amino peptidases and oligo-peptide transporters. It was found to harbour more aminopeptidase activities than other GPAC species, indicating a higher pathogenicity (Murphy and Frick, 2013). This is clearly seen in clinical infection, as *F. magna* is the most frequently isolated GPAC species in pure culture from various infection sites (Bourgault *et al.*, 1980; Murphy and Frick, 2013). It is typically isolated from infections such as wound infections, soft tissue abscesses and bone and prosthetic joint infections (Fitzgerald *et al.*, 1982; Davies *et al.*, 1988; Brook and Frazier, 2000; Brazier *et al.*, 2008; Brook, 2008; Levy *et al.*, 2009). Due to being a member of the skin microbiota, it was found to be one of the most common anaerobes isolated from skin specimens and also highly prevalent in chronic wounds, diabetic ulcers and pressure ulcers (Hansson *et al.*, 1995; Higaki and Morohashi, 2003; Stephens *et al.*, 2003; Dowd *et al.*, 2008a,b; Murphy and Frick, 2013).

The prominence of *F. magna* in GPAC infections could potentially be explained due to its expression of proteins that enhance virulence. The superantigen, protein L, is a surface protein with high affinity for immunoglobulin light chains and can induce the release of pro-inflammatory mediators (Björck, 1988; Genovese *et al.*, 2003). Protein...
L is expressed by approximately 10% of *F. magna* isolates and is known to be associated with bacterial vaginoses (Kastern *et al.*, 1990). Another surface protein that could promote virulence, is the albumin binding protein PAB, which was found to give the expressing strain a significant increase in growth rate (de Château and Björck, 1994; de Château *et al.*, 1996). Protein FAF (*F. magna* adhesion factor) is expressed by more than 90% of *F. magna* isolates. Its surface associated form causes bacterial clumping and helps bacterial adhesion to the basement membrane in skin by binding to BM-40 (Frick *et al.*, 2008). This form and a released extracellular form help neutralize the activity of the human antibacterial peptide LL-37, and human histones H2B and H4 which exhibit strong antibacterial activities (Frick *et al.*, 2008; Murphy *et al.*, 2014). Another important virulence factor of *F. magna* is the subtilase-like enzyme SufA, which protects the bacterium from antibacterial activities of LL-37, histones and MIG/CXCL9 by proteolytic degradation (Karlsson *et al.*, 2007; Murphy and Frick, 2013). *F. magna* also has the capability to produce a capsule and the enzymes collagenase and gelatinase, which could be other important pathogenicity factors (Brook, 1986; Krepel *et al.*, 1992).

In this study, the path of *F. magna* from commensal to opportunistic pathogen was studied by analysing binding and interaction of FAF and SufA, expressed by the majority of *F. magna* strains, to proteins in the epidermis and dermis of human skin. The epidermis is mainly composed of keratinocytes, which make up 90% of this layer (Tortora and Derrickson, 2009). Keratinocytes produce the structural protein keratin, which assembles into a web-like pattern of intracellular filaments bound together by the matrix protein filaggrin into tight bundles (Proksch *et al.*, 2008). It is in the epidermal layer that the skin microbiota reside, although a recent report suggests that some bacteria or their products exist below this, in the dermis and dermal adipose layers (Nakatsuji *et al.*, 2013). The basement membrane separates the epidermal and dermal layers and is made up of independent networks of collagen IV and laminin, which are linked by molecules such as nidogen and BM-40 (for a review, see Timpl, 1996). It has previously been shown that FAF binds the non-collagenous glycoprotein BM-40, allowing *F. magna* to reside at the basement membrane (Frick *et al.*, 2008). The dermis provides nutrients and physical support to the epidermis. It is composed of strong connective tissue containing collagen and elastic fibres that form a network providing strength and support (Pringle and Penzer, 2002).

In the present work, we discovered binding of protein FAF to galectin-7, collagen V and fibrillin, all of which are found in different layers of human skin. Furthermore, SufA was found to cleave collagen IV and V, which would help *F. magna* reach deeper tissue sites during infection.

**Results**

*F. magna* binds to galectin-7 in the epidermis of human skin

In a recent publication where the *F. magna* cell wall adhesion protein FAF was tested for an interaction with epidermal skin proteins, galectin-7 was identified as one of the ligands (Murphy *et al.*, 2014). Galectin-7 is a member of the galectin family of animal lectins that have an affinity for β-galactose containing oligosaccharides (Saussez and Kiss, 2006). In this current study, the interaction between FAF and Galectin-7 was further investigated. Binding of galectin-7 to protein FAF was examined in a slot binding assay together with proteins PAB and SufA, other potential virulence factors in *F. magna*, see Fig. 1A. This blot shows a concentration dependent binding of galectin-7 to FAF, but no interaction with SufA and protein PAB. Binding of FAF to galectin-7 was further confirmed through surface plasmon resonance analysis which showed a significant binding with a *K*<sub>d</sub> of 0.53 nM. In order to map the binding of galectin-7 to a particular region of protein FAF, various recombinantly expressed FAF fragments were applied onto a PVDF membrane and probed with galectin-7, see Fig. 1B. The fragments, which were constructed in another study, correspond to the N-terminal FAF I (amino acids 28–115, 10 kDa) and FAF II (amino acids 28–317, 32 kDa) and the C-terminal FAF III (amino acids 239–616, 42 kDa) (Frick *et al.*, 2008). The blot shows that FAF III, covering the C-terminal region of the molecule, is responsible for binding to galectin-7. There is no binding to FAF fragments I or II (Fig. 1B).

Next, FAF-expressing bacteria (strain ALB8) were incubated with galectin-7 and bound ligand was eluted by low pH buffer followed by Western blot analysis using a rabbit anti-galectin-7 antibody. Bacteria incubated with buffer only served as a negative control to ensure that any positive bands seen were not due to bacterial surface proteins cross reacting with the antibodies. As shown in Fig. 1C, lane 2, there is a strong binding by *F. magna* to galectin-7 in solution as judged by the immuno-reactive band corresponding to the molecular weight of 14 kDa for galectin-7. Galectin-7 is known to form dimers (Leonidas *et al.*, 1998), and this probably corresponds to the band close to the 35 kDa marker (Fig. 1C). There is no cleavage of galectin-7 by SufA present on the bacterial surface (Fig. 1C, lane 2) or when galectin-7 was incubated with purified SufA (data not shown).

Finally, binding of radiolabelled galectin-7 to different strains of *F. magna* was investigated, see Fig. 1D. The binding to FAF-expressing strains (ALB8, L3410, 1462 and 2133) varied between 20–30% of added radiolabelled galectin-7, while the non-FAF expressing strain 505 showed a significantly lower level of binding at 15% ± 3%.

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Taken together, the results of the binding experiments demonstrate that FAF interacts with galectin-7.

**SufA of *F. magna* degrades collagens found in the skin and its surface protein FAF binds to collagen V**

Collagens form a major part of the structural component of skin and thus the ability of *F. magna* to bind and degrade various collagens was investigated. Initially, the protease SufA was incubated with collagens I–V and cleavage was examined by SDS-PAGE, see Fig. 2A. The > 250 kDa band represents SufA and is indicated by a black arrow. This band is not visible in the analysis of collagen I and II cleavage due to the size of the collagen protein bands. The results clearly show that there is no breakdown of collagen I, II or III by SufA. However, collagen IV appears to be completely cleaved and collagen V is degraded into smaller fragments by SufA. Type IV collagen is the most important structural component of basement membranes separating the epidermis and dermis of skin (Gelse *et al.*, 2003), while type V collagen is located on or adjacent to the basement membrane (Birk *et al.*, 1988).

To investigate a possible interaction between collagens and protein FAF, collagen type I–V were separated by SDS-PAGE followed by Western blotting. The membrane was then incubated with protein FAF and bound protein was detected by antibodies against FAF. Only collagen V interacts with FAF as judged by the prominent band just above 130 kDa representing collagen V (Fig. 2B, lane 5).

In order to confirm this interaction using another method, recombinant collagen V was radiolabelled with iodine and its interaction with different strains of *F. magna* was examined, see Fig. 2C. Again, *F. magna* strains expressing FAF bound collagen V, while strain 505, which does not express FAF, bound almost no collagen V.

These results signify that *F. magna* can interact with collagens of the skin and the basement membrane. By breaking down collagens of the basement membrane, it could assist the bacteria to reach deeper tissue sites during infection.
F. magna binds to fibrillin through its surface adhesion protein FAF

Moving beyond the basement membrane into the dermis, fibrillin microfibrils are found which play vital roles in maintaining structural integrity and in regulating extracellular growth factors (Jensen et al., 2012). Fibrillin makes up 10–12 nm microfibrils, which form a scaffold for elastin deposition during elastogenesis allowing for elasticity and extensibility of connective tissues. As part of the path of F. magna into deeper dermal sites during infection, we investigated if it could bind and interact with fibrillin as a means of establishing infection in the dermis. Binding of 125I-labelled FAF to various fibrillin recombinant fragments was investigated in a slot binding assay, see Fig. 3A. This blot shows a concentration dependent binding of FAF to the N-terminal fragment of fibrillin-2 (rFBN2-N) and a slightly lower binding intensity to the N-terminal fragment of fibrillin-1 (rFBN1-N). There appears to be low/background binding of FAF to the C-terminal fragment of fibrillin-1 (rFBN1-C) and it does not appear to be concentration dependent. There is no binding at all to the C-terminal fragment of fibrillin-2 (rFBN2-C). Due to the strong interaction of FAF with the N-terminal of fibrillin-2, the interaction of FAF with the N-terminal of fibrillin-1 was also further investigated by radiolabelling both rFBN1-N and rFBN2-N with 125I and examining binding to different strains of F. magna, see Fig. 3B. Highest binding for both fragments

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Fig. 3. Examination of the interaction of FAF and SufA with fibrillin.
A. Indicated amounts of various recombinant fibrillin fragments and FAF (1 mg) were applied in slots to a PVDF membrane. The membrane was incubated with 125I-labelled FAF and bound FAF was determined using the Fuji Imaging system.
B. Percentage binding of 125I-labelled fibrillin fragments rFBN1-N and rFBN2-N to different strains of F. magna. The black bars represent binding to rFBN1-N and the grey bars represent binding to rFBN2-N. Bars represent mean ± S.E. of at least three experiments.
C. SufA cleavage of fibrillin was determined by co-incubation with the various fragments for 3 h at 37°C followed by analysis of cleavage on an 8% SDS-PAGE gel. Lanes containing fibrillin co-incubated with SufA are indicated with a + sign. As a control, fibrillin fragments were also incubated without SufA for the same length of time, indicated by a – sign. The black arrow indicates the 35 kDa fragment released by SufA from rFBN2-N. A protein band representing SufA is clearly seen above the N-terminal fibrillin fragments and is indicated by a star. The SufA band is hidden by the C-terminal fibrillin fragments due to their size.
D. FAF binds rFBN1-N and rFBN2-N in surface plasmon resonance experiments. FAF was immobilized on a sensor chip, and increasing concentrations of rFBN1-N/rFBN2-N were injected over the surface. RU, response units.

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was seen with *F. magna* strain ALB8 which displayed 24% binding to rFBN1-N and 17% binding to rFBN2-N. The pattern of binding for all strains was similar for both fragments, with a generally higher binding to rFBN1-N, than to rFBN2-N. Again, strain 505 showed the lowest level of binding, due to its lack of expression of protein FAF. This experiment confirms the results seen in the slot blot in Fig. 3A.

As SufA had previously been seen to degrade some collagen proteins, degradation of fibrillin components by SufA was also investigated, see Fig. 3C. No degradation of rFBN1-N, rFBN1-C or rFBN2-C was observed. However, SufA appears to release a 35 kDa fragment from rFBN2-N, as indicated by the black arrow. This 35 kDa fragment was cut out from the SDS-PAGE gel and analysed by tandem mass spectrometry (MS/MS), see Fig. S1. Results from this analysis show that the fragment is cleaved from the NH2-terminus of rFBN2-N, see Fig. S1. However, it is unknown if this cleavage is enough to allow *F. magna* to reach deeper tissue sites or if it interferes with tissue homeostasis.

Further investigations into the binding of FAF to fibrillin with surface plasmon resonance showed binding of FAF to both N-terminal fragments of fibrillin-1 and -2, with a slightly higher binding to rFBN2-N ($K_D$: 3.7 nM) than rFBN1-N ($K_D$: 22 nM), see Fig. 3D.

**Protein FAF binds fibrillin both at the bacterial surface and in solution**

Visual confirmation of *F. magna* binding to fibrillin via protein FAF was carried out using transmission electron microscopy. *F. magna* strains ALB8 and 505 were incubated with antibodies against FAF and recombinant fibrillin fragments labelled with colloidal gold, 5 and 20 nm respectively. Strain 505 was used as a negative control as it does not express FAF and therefore, no binding of FAF to fibrillin can be clearly seen on the bacterial surface. No binding of fibrillin fragments rFBN1-C and rFBN2-C to ALB8 bacteria could be observed (Fig. 4). As expected, no binding of FAF antibodies or gold labelled fibrillin fragments to the bacterial surface of strain 505 could be seen. Complexes formed, in solution, between FAF and fibrillin fragments were then analysed by electron microscopy following rotary shadowing, see Fig. 5. Fibrillin fragments are seen as long rod-like structures (black arrows) and FAF molecules are labelled with colloidal gold (black arrowheads). Fig. 5A and C show complexes formed between FAF and the N-terminal fibrillin fragments rFBN1-N and rFBN2-N, while no complex formation could be observed between FAF and the C-terminal fragments of fibrillin (Fig 5B and D). These results confirm that protein FAF mediates binding of fibrillin to *F. magna*, both at the bacterial surface and in solution.

*SufA is essential in the invasion of F. magna into deeper dermal skin layers*

In order to investigate *F. magna* interaction with human skin, and the effects of FAF and SufA expression, different strains of *F. magna* were incubated with human skin biopsies for 1 h. Following extensive washing to remove unbound bacteria the biopsies were prepared for scanning electron microscopy or incubated anaerobically for another 72 h, see Fig. 6. *F. magna* strain ALB8 expresses both FAF and SufA, strain 505 was used as it naturally does not express FAF and *F. magna* strain ΔSufA represents the ALB8 strain with SufA knocked out. Fig. 6, panel A, shows *F. magna* ALB8 bacteria bound to the skin epidermal surface at time point 0 h. This is in stark contrast to Panel B, which shows no binding of bacteria to the skin surface. This result underlines the importance of FAF in initial binding and adherence to the skin surface during colonization. Panel C shows that the absence of SufA from ALB8 has no effect on the adhesion of *F. magna* to host tissue as similar amounts of bound bacteria can be seen in Panel A and C. Here, the basement membrane is indicated by the letter B. Panels D to F represent the dermal layer, located below the epidermis, at time point 0 h. These micrographs demonstrate that the connective tissue, composed of collagen, fibrillin and elastic fibres, is intact. Also, no bacteria are present. Panels G to I represent the dermal layer after 72 h of incubation with the different strains of *F. magna*. Panel G shows that the ALB8 strain has caused significant destruction to the microfibrillar structure of the dermal layer. Also, strain 505, which expresses SufA, caused significant breakdown of the dermal fibres (Fig. 6H). Although no adherent 505 bacteria could initially be observed on the epidermal surface (Fig. 6B), some bacteria were present in the dermis after 72 h. Obviously low affinity adhesion of strain 505 is mediated by a mechanism different from FAF. Fig. 6I shows an entirely different picture, where the dermal structure is completely undamaged and intact. This image represents ALB8 ΔSufA and displays how SufA could be important in the infection process for *F. magna*. It can also be seen that there are far lower numbers of *F. magna* in panel I, when compared with panel G. Apparently, the absence of SufA has hindered the ability of *F. magna* to reach the deeper dermal layers. However, low amounts of *F. magna* can still reach the deeper skin layers through binding with FAF, but it cannot cause any tissue destruction or damage. This data has been evaluated in further detail by examining the bacterial structure...
cell density of 30 tissue profiles in electron microscopy, see Table 1. This data agrees with Fig. 6 and, significantly, shows that there is a 1000-fold lower density of *F. magna* strain 505 in the skin biopsies, compared to ALB8. Clearly, protein FAF is an important factor in the adhesion of *F. magna* to host tissue. Furthermore, the data in the table indicate, that after 72 h, most of the ALB8 strain have transitioned from the epidermis to the dermis. Whereas, both strain 505 and ΔSufA remain mostly at the epidermis and are hindered in delving into deeper tissue layers due to

**Fig. 4.** Transmission electron microscopy demonstrating binding of protein FAF on the ALB8 surface to the N-terminal region of fibrillin. *F. magna* strains ALB8 and 505 were incubated with anti-FAF antibodies and the various fibrillin fragments labelled with colloidal gold (anti-FAF antibody, 5 nm gold and fibrillin fragments, 20 nm gold). Samples were then prepared for transmission electron microscopy. Left panels: *F. magna* strain ALB8. Right panels: *F. magna* strain 505. The scale bar represents 50 nm.
Thus, the biopsies were incubated with antibodies against FAF, labelled with 5 nm colloidal gold, and antibodies against galectin-7, BM-40 and fibrillin, each labelled with 20 nm colloidal gold. In these images, colocalization between FAF and these proteins can clearly be seen. Following binding to the epidermal surface initially at 0 h, see Fig. 6A, F. magna enters the epidermal layer after 24 h facilitated by binding of FAF to galectin-7 on the keratinocyte cell surface (Fig. 7B). After 48 h, F. magna reaches the basement membrane, where it attaches through a FAF-BM-40 interaction (Fig. 7D). After 72 h, F. magna has already reached the dermal layer where colonization is promoted by attachment and binding to fibrillin microfibrils through FAF interaction (Fig. 7F). These images summarize our earlier experiments and visualize the path of F. magna through the skin in the establishment of infection.

**Discussion**

*F. magna* is a commensal commonly found on the skin, but under some circumstances, has been known to act as an opportunistic pathogen. It is probably one of the most pathogenic species of the GPAC and has been isolated in pure culture from a variety of clinical infection sites (Bourgault et al., 1980; Murphy and Frick, 2013). In recent years, two proteins with importance for virulence and colonization, were identified in *F. magna*—the surface adhesion protein FAF and the subtilisin-like serine protease SufA (Karlsson et al., 2007; Frick et al., 2008). In this study, we investigated the role these two proteins play in helping *F. magna* adhere to the skin and invade deeper tissue layers during the establishment of infection. Binding and interaction of FAF and SufA with proteins in the different layers of skin was studied, in order to identify the mechanisms by which *F. magna* reaches deeper tissue layers during infection.

Ligands for FAF in the epidermis was investigated using human skin epidermal extracts (Murphy et al., 2014). Galectin-7 was identified as one of the major binding partners for FAF in the epidermis. This lectin is thought to be a keratinocyte cell type marker and its expression is maintained in all living layers of the epidermis (Magnaldo et al., 1995; Saussez and Kiss, 2006). Galectin-7 can be found in the cytoplasm, in the nucleus or in the extracellular space and its function may vary, according to its cellular localization (Saussez and Kiss, 2006). It is especially found in the upper layers of the human epidermis in areas of cell-to-cell contact (Madsen et al., 1995). It is also thought to play a key role in the re-epithelialization process of epidermal wounds and is involved in epithelial cell migration (Saussez and Kiss, 2006). It modulates cell proliferation and cell interactions and plays an important role in the formation of stratified epithelia (Saussez and Kiss, 2006). As galectin-7 expression is highly concentrated in the

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**Fig. 5.** Electron micrographs after rotary shadowing showing complex formation between FAF and N-terminal fragments of fibrillin. The arrowheads point to FAF labelled with 10 nm colloidal gold and the arrows point to extended fibrillin molecules. The FAF molecules exhibit a globular appearance due to decoration with gold particles. A: Interaction of FAF with rFBN1-N; B: Interaction of FAF with rFBN1-C; C: Interaction of FAF with rFBN2-N; D: Interaction of FAF with rFBN2-C. The scale bar represents 100 nm.
upper levels of the epithelium, it may serve as an important first step in the binding of *F. magna* to the skin and binding interactions may help it pass to deeper levels of the epithelium. SufA releases a 53 kDa FAF fragment from the bacterial surface (Frick *et al.*, 2008). Binding of extracellular FAF to galectin-7 could possibly help affect and prevent re-epithelialization during wounding, thus maintaining an open chronic wound. However, this could not be conclusively proven (data not shown) and requires future studies.

Looking deeper into the basement membrane and dermis compartments of the skin, *F. magna* was found to have strong interactions with both collagen IV and V that have important structural roles in this area (Madri and Furthmayr, 1979; Gelse *et al.*, 2003). SufA was found to completely degrade collagen IV while it was capable of breaking down collagen V into smaller fragments. Collagen IV is the backbone for the basement membrane layer of skin and it is thought that collagen V acts as an intermedi-
The presence of extracellular and surface located FAF and SufA have proven to play an important role in assisting *F. magna* to colonize skin epidermal sites. As *F. magna* is, for the most part, a commensal bacterium, SufA would probably not interfere with the host and cause extracellular matrix destruction under normal conditions. SufA probably comes more into play after an initial external wounding event, allowing *F. magna* greater access to dermal tissue sites. Here, it could transform from a commensal to an opportunistic pathogen and use SufA to further destruct tissue, enabling it to initiate an infection deep in the anaerobic tissue.

Host–microbe relationships are based on a multitude of molecular interactions, and the present investigation revealed that *F. magna* uses a combination of proteolytic and adhesive mechanisms to penetrate and colonize human skin. *F. magna* is also part of the indigenous microbiota in the oral cavity and the gastrointestinal and genitourinary tracts, and future studies will show if *F. magna* has a similar strategy to colonize these sites.

**Experimental procedures**

*Bacteria and growth conditions*

*F. magna* strains ALB8, 505, L3410, 2133 and 1462 were isolated at the Department of Clinical Microbiology, Skåne University Hospital, Sweden and have been described earlier (de Château and Björck, 1994; Frick et al., 2008). The strains were isolated from various clinical infection sites: scrotal...

### Table 1. Evaluation in electron microscopy of percentage cell density of different *F. magna* strains after 0, 24 and 72 h from 30 skin biopsy tissue profiles.

| Strain | Cell density | Location | 0h | 24h | 72h |
|--------|--------------|----------|-----|-----|-----|
| ALB8   | 2.3 × 10⁷ mm⁻² | Epidermis | 98% | 67% | 36% |
|        |              | Dermis   | 2%  | 32% | 62% |
| 505    | 1.8 × 10⁷ mm⁻² | Epidermis | 99% | 88% | 81% |
|        |              | Dermis   | 1%  | 12% | 19% |
| ΔSufA  | 2.2 × 10⁷ mm⁻² | Epidermis | 100% | 97% | 94% |
|        |              | Dermis   | 0%  | 3%  | 6%  |

For a representative image, see Fig. 6.
abscess (ALB8), urethra (505), intra-abdominal abscess (L3410), foot abscess (2133) and foot wound (1462). For cultivation of *F. magna* ALB8 mutant ΔSufA which was produced in a previous study (Karlsson et al., 2009), 200 μg ml⁻¹ kanamycin was added to the culture medium. The bacteria were grown under strict anaerobic conditions in Todd-Hewitt broth (TH) (Difco) supplemented with 0.5% Tween-80 at 37°C.

**Proteins, plasma, antibodies and reagents**

Galectin-7 was purchased from Nordic BioSite. Horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG was purchased from Pierce. Collagen I, II and IV were purchased from Sigma and collagen III and V were purchased from Millipore. SufA was purified natively from *F. magna* ALB8 as previously...
described (Karlsson et al., 2007) and protein PAB was produced recombinantly as described (de Château and Björck, 1994). The purification of fibrillin recombinant fragments has been described previously (Jensen et al., 2001; Lin et al., 2002). Recombinant fragments included ref16 (N-terminal half of fibrillin-1, rFBN1-N), ref6H (C-terminal half of fibrillin-1, rFBN1-C), rFBN2-2 (N-terminal half of fibrillin-2, rFBN2-N) and rFBN2-1 (C-terminal half of fibrillin-2, rFBN2-C). Recombinantly expressed protein FAF [amino acid (AA) 28–616], and FAF fragments I (AA 28–115), II (AA 28–317) and III (AA 239–616), were obtained as fusion proteins with glutathione S-transferase (GST) as described (Frick et al., 2008). Prior to binding experiments, the GST tag was removed using PreScission Protease as described by the manufacturer (Amerham Biosciences). Anti-FAF antibodies were raised in rabbits as described (Frick et al., 2008) and rabbit anti-galectin-7 antibodies were purchased from AbD Serotec. The preparation of FAF-Au conjugates and gold-labelled anti-FAF antibodies was carried out as reviewed in (Baschong and Wrigley, 1990). Galectin-7, collagen V, rFBN1-N, rFBN2-N and FAF were radiolabelled with 125I using iodobeads (Pierce) as described by the manufacturer. Binding of radiolabelled proteins to bacteria was carried out as previously described (Björck and Kronvall, 1984).

Slot-binding, SDS-PAGE and Western blot analysis

SDS-PAGE was performed as described by Neville (1971). Samples were prepared for boiling in sample buffer containing 2% SDS and 5% β-mercaptoethanol for 5 min and 8% or 15% SDS-PAGE gels were used for sample separation. Separated proteins were visualized by Coomassie Blue staining. For Western Blot analysis, proteins were electrophoretically transferred to a polyvinylidene difluoride (PVDF) membrane (Amerham Biosciences). For slot-binding analysis, proteins were directly applied to PVDF membranes using a Milliblot-D system (Millipore). Membranes were blocked in a phosphate buffered saline-Tween [PBS containing 0.1% Tween 20 (PBS-T)] solution containing 5% (w/v) skim milk powder at 37°C for 30 min. The membranes were then incubated with galectin-7 (0.71 μM) or FAF (0.23 μM) for 1 h at 37°C in blocking buffer. Membranes were washed three times with PBS-T for 5 min followed by incubation with primary antibodies (rabbit anti-FAF 1:1000 dilution, rabbit anti-galectin-7 1:10 000 dilution) in blocking buffer at 37°C for 30 min. Membranes were washed three times with PBS-T for 5 min followed by incubation with HRP-conjugated goat anti-rabbit secondary antibody (1:3000 dilution) in blocking buffer at 37°C for 30 min. Following a repeat of the wash steps, bound antibodies were detected by chemiluminescence as described by Nesbitt and Horton (1992).

Binding of 125I-labelled FAF to fibrillin protein fragments

The fibrillin fragments were applied to a PVDF membrane using a Milliblot-D system (Millipore). The membrane was washed in PBS for 30 min at room temperature and then blocked with PBS + 3% BSA (Bovine serum albumin) for 1 h at room temperature. The membrane was incubated with radiolabelled FAF (0.2 × 10⁶ cpm ml⁻¹) in 10 ml PBS + 3% BSA for 3 h at room temperature, followed by washing twice for 30 min with PBS + 0.5% Tween-20. The membrane was dried, exposed with film and developed for radioactivity using a Bas 2000 radio imaging system (Fuji, Tokyo, Japan).

Cleavage of proteins by SufA

SufA (0.11 μM) was incubated with collagen I, II, III, IV and V fibrillin fragments rFBN1-N, rFBN1-C, rFBN2-N and rFBN2-C (3.5 μg of each protein) in 50 mM Tris-HCl pH 7.5. As a control, SufA was substituted with buffer. Following incubation for 3 h at 37°C, the samples were subjected to analysis by SDS-PAGE using a polyacrylamide concentration of 8%.

Binding of galectin-7 to whole bacteria in solution

A stationary culture of F. magna ALB8 was washed twice in PBS and bacteria were resuspended in a 2 × 10⁹ bacteria ml⁻¹ solution. One hundred microlitres of this bacterial solution was added to 7.2 μM galectin-7 in 100 μl PBS. As a control, the bacteria were added to PBS only. The solutions were incubated for 1 h at 37°C, end over end rotation. The bacteria were then washed four times in PBS to remove unbound protein, followed by incubation in 0.1 M glycine-HCl buffer, pH 2, for 30 min at room temperature to elute bound protein. The bacterial cells were removed by spinning at 6000 g for 5 min and the supernatant was transferred to a fresh tube where the pH was neutralized using 1 M Tris. Proteins were precipitated using 5% TCA and analysis of galectin-7 binding was carried out by SDS-PAGE and western blotting as described above.

Surface plasmon resonance assay (BIAcore)

FAF protein was immobilized on a CM5 sensor chip (GE Healthcare). Affinity measurements were monitored in a BIAcore 2000 instrument. Different concentrations of Galectin-7/rFBN1-N/rFBN2-N were injected over the coated surfaces. The association (kₐ) and dissociation (k₈) rate constants were determined simultaneously using the equation for 1:1 Langmuir binding in the BIA Evaluation 4.1 software (GE Healthcare). The binding curves were fitted locally and the equilibrium dissociation constants (Kₐ) were calculated from mean values of the obtained rate constants.

Rotary shadowing of FAF-fibrillin complexes

Fibrillin samples in complex with FAF-Au (10 nm Au) were dissolved in 0.2 M ammonium hydrogen carbonate at final concentrations of 10–50 μg ml⁻¹, mixed with an equal volume of 80% glycerol and then sprayed on to freshly cleaved mica pieces, followed by rotary shadowing with platinum/carbon at a 9° angle. Details of the electron microscopic procedures and methods of data evaluation have been described previously (Engel and Furthmayr, 1987).

Negative staining and transmission electron microscopy of fibrillin fragments and FAF on the F. magna ALB8 surface

F. magna ALB8 grown to stationary phase were washed with TBS (50 mM Tris-HCl, 150 mM NaCl, pH 7.5) and adjusted to 2 × 10⁹ bacteria ml⁻¹. One microlitre of 2 × 10⁹ bacteria ml⁻¹...
was incubated with 5 μl of 5 nm Au-labelled anti-FAF antibody and 5 μl of 20 nm Au-labelled fibrillin fragments for 30 min at 37°C. Following incubation, the bacteria were recovered by centrifugation at 3000 g and samples were prepared for negative staining by adsorption onto 400 mesh carbon-coated copper grids and staining with 0.75% (w/v) uranyl formate as described (Bober et al., 2010). Samples were observed in an FEI Tecnai Spirit BioTWIN transmission electron microscope (North America NanoPort, Hillsboro, OR) operating at an accelerating voltage of 60 kV. Images were recorded using an Eagle™ CCD camera.

Transmission and scanning electron microscopy of skin sections

For transmission electron microscopy of skin sections, a stationary phase culture of *F. magna* ALB8 was washed twice in PBS and the concentration adjusted to 2 × 10⁹ bacteria ml⁻¹ in PBS. Skin specimens were obtained as excess healthy tissue from skin surgery, under protocols approved by the Ethics Committee at Lund University (permit No. LU 762-02). A 4 cm² section of a skin specimen was washed 3 times in PBS. Skin specimens were then cut into 2.5 mm circular punches of epidermis were extracted. A measure of 2 × 10⁹ bacteria was incubated with each skin biopsy for 1 h at 37°C under anaerobic conditions. Following incubation, skin sections were removed to a 24-well plate, washed 3 times with PBS to remove unbound bacteria, followed by addition of 500 μl MEM (Life Technologies). Skin sections with bound bacteria were incubated for 24 h, 48 h or 72 h at 37°C under anaerobic conditions. The skin specimen was then added to 1 ml of 2.5% glutaraldehyde in cacodylate buffer overnight at 4°C.

For transmission electron microscopy, samples were washed with cacodylate buffer and post-fixed for 1 h at room temperature in 1% osmium tetroxide in cacodylate buffer, dehydrated in a graded series of ethanol, and then embedded in Epon 812 (SPI Supplies) using acetone as an intermediate solvent. Specimens were sectioned with a diamond knife into sections with a measure of 2 μm thick ultrathin sections on an LKB ultramicrotome. Immunolabelling was carried out with rabbit anti FAB antibody, titre 1:10, labelled with 5 nm colloidal gold and rabbit anti galectin-7 antibody (titre 1:100), rabbit anti BM-40 (titre 1:100) or rabbit anti-rFBN2-N (titre 1:10) all labelled with 20 nm colloidal gold. Analysis was carried out using a JEOL JEM 1230 transmission electron microscope (JEOL, Peabody, MA) as previously described (Svensson et al., 2011).

For scanning electron microscopy, specimens were fixed in 2.5% glutaraldehyde in 0.15 M sodium cacodylate, pH 7.4 for 2 h at room temperature, washed and stored in 0.15 M cacodylate buffer, pH 7.4. Fixed specimens were dehydrated for 10 min at each step of an ascending ethanol series and inserted into a Balzers critical point dryer using 100% ethanol as the intermediate solvent. The pressure chamber was then extensively flushed three times with carbon dioxide to remove all traces of residual ethanol. The samples were critical point dried, mounted on aluminium holders, palladium/gold sputtered and examined in a Jeol ST300 SEM.

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