Flamingo Cadherin: A Putative Host Receptor for *Streptococcus pneumoniae*

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*Streptococcus pneumoniae* fructose bisphosphate aldolase (FBA) is a cell wall–localized lectin. We demonstrate that recombinant (r) FBA and anti-rFBA antibodies inhibit encapsulated and unencapsulated *S. pneumoniae* serotype 3 adherence to A549 type II lung carcinoma epithelial cells. A random combinatorial peptide library expressed by filamentous phage was screened with rFBA. Eleven of 30 rFBA-binding phages inhibited 90% of *S. pneumoniae* adhesion to A549 cells. The insert peptide sequence of 9 of these phages matched the Flamingo cadherin receptor (FCR) when aligned against the human genome. A peptide comprising a putative FBA-binding region of FCR (FCRP) inhibited 2 genetically and capsularly unrelated pairs of encapsulated and unencapsulated *S. pneumoniae* strains from binding to A549 cells. Moreover, FCRP inhibited *S. pneumoniae* nasopharyngeal and lung colonization and, possibly, pneumonia development in the mouse intranasal inoculation model system. These data indicate that FBA is an *S. pneumoniae* adhesin and that FCR is its host receptor.

*Streptococcus pneumoniae* carriage is common among children and adults [1, 2]. To identify the stages crucial to disease development, a better understanding is required of the sequential molecular steps involved in the interaction between *S. pneumoniae* and its host.

To date, several *S. pneumoniae* adhesins have been identified. One of these is Pav-A protein, which binds the extracellular matrix component fibronectin that, in turn, binds epithelial cells via their integrin receptors [3]. Another *S. pneumoniae* adhesin is the choline binding protein A (CbpA) [4], which is reported to bind to the polymeric immunoglobulin receptor [5, 6]. Pneumococcal surface adhesin A [7] is suspected to interact with E-cadherin [8] and Ami-AliA/AliB [9], the target molecule of which is unknown. Also, the bacterial molecule phosphorylcholine is involved in interacting with the host via the platelet-activating factor receptor [10, 11].

There have been several efforts to delineate specific roles for the various pneumococcal proteins in interacting with the host [12]. Deficiency in pneumococcal surface protein A increases nasopharyngeal colonization, whereas lack of CbpA, pneumolysin (Pln), pyrolyte oxidase (SpxB), autolysin (LytA), or neuraminidase (NanA) decreases nasopharyngeal colonization. In the intratracheal mouse inoculation model, bacteria deficient in Pln, SpxB, or LytA have a reduced capacity to replicate in the lungs, whereas deficiency in CbpA or NanA does not affect replication in the lungs.

A role for *S. pneumoniae* lectins in adhering to host mucosal surfaces has been suggested [13, 14]. Previously, we demonstrated that the adhesion of *S. pneu-
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**MATERIALS AND METHODS**

**Reagents.** Unless otherwise stated, all chemicals and biochemicals were purchased from Sigma-Aldrich.

**Bacterial strains, growth conditions, and growth medium.** The strains used included *S. pneumoniae* serotype 3 strain WU2 [18, 19], its unencapsulated mutant strain 3.8DW [20], serotype 2 Avery strain D39 [21], and its unencapsulated derivative strain R6. Pneumococci were grown in Todd-Hewitt broth supplemented with yeast extract (Difco Laboratories). *Escherichia coli* DH5α and BL-21 were grown in Luria-Bertani broth.

**Recombinant protein production.** Recombinant FBA (NP_345117) was expressed as described elsewhere [17]. Pneumococcal immunogenic protein D (PsipD NP_346607) was expressed similarly using the forward primer 5'-GGATCCTTGAAAAAGAAGGAACTATC-3' and the reverse primer 5'-GAAT-TCCAATTCTTTCCTTGTAGCGT-3'.

**Phage display library.** The present library represents a mosaic of wild-type and recombinant PVIII envelope protein and, thus, can tolerate peptide inserts of up to 12 aa (fth1) [22]. Cysteine was added at the beginning and end of each peptide sequence to ensure a 3-dimensional configuration [23]. Recombinant (r) FBA–binding phages were selected by biopanning, as described elsewhere [23]. Phages were grown in *E. coli* strain DH5α and were selected by monitoring the tetracycline resistance of bacterial colonies. Phages were transferred to a nitrocellulose membrane and treated sequentially with rFBA, mouse anti-rFBA antibodies (1:5000), and a goat anti–mouse IgG antibody (peroxidase conjugated; Jackson Laboratories) and were detected by exposing the membranes to film (Agfa-Gevaert). The inserts in positive phages were sequenced (Daniel Biotec) using the primer 5'-TTTACGTTGAAAATCTCC-3'.

**Immunization of mice with rFBA and PsipD.** Seven-week-old BALB/cOlaHsd (BALB/c) female mice (Harlan Laboratories) were immunized intraperitoneally with 25 μg of rFBA [17], 25 μg of PsipD, or 25 μg of Flamingo cadherin receptor (FCR)–derived synthetic peptide (FCRP) fused to keyhole limpet hemocyanin (KLH; Sigma-Aldrich) with Inject Alum adjuvant (Pierce Biotechnology) at days 0 and 21. Blood samples were collected before and 1 week after booster immunization. All animal experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Ben Gurion University of the Negev, Beer Sheva, Israel.

**Immunization of rabbits.** Three-month-old albino rabbits were immunized with 200 μg of rFBA or 200 μg of FCRP-KLH fusion protein in complete Freund’s adjuvant subcutaneously...
and intramuscularly. Booster immunizations were performed with incomplete Freund’s adjuvant.

**Inhibition of S. pneumoniae adhesion to A549 cells.** A549 cells (type II epithelial lung carcinoma cells; American Type Culture Collection) were grown to confluence (on average, 5 × 10⁴ cells/well) in Dulbecco’s modified Eagle medium supplemented with 10% fetal calf serum on fibronectin-coated plates at 37°C in a humidified incubator. rFBA was added at 5–30 μg/mL for a 1-h incubation. After PBS washings (5×), S. pneumoniae (5 × 10⁶ cfu/mL) were added for a 1-h incubation; then, cells were washed 5 times, liberated (using 0.25% trypsin-EDTA), and plated onto blood agar plates. The number of bacteria adhering to fibronectin-coated plates (on average, 8000 ± 100 cfu/mL) was subtracted. The experiments were performed in triplicate and were repeated 3 times on different occasions.

To analyze the ability of anti-rFBA antibodies, rFBA-binding phages, and the synthetic peptide to inhibit bacterial adhesion to A549 cells, bacteria were treated for 30 min with increasing dilutions of the relevant material and centrifuged at 1100 g before they were added to the cultured A549 cells for a 60-min incubation. The subsequent experimental procedure was as described above.

**Flow cytometry and antibodies.** A549 cells were incubated with mouse anti–FCRP-KLH serum, mouse anti-PsipD serum, or control mouse serum and were washed and stained with fluorescein isothiocyanate (FITC)–conjugated F(ab′)₂ goat–anti–mouse IgG+IgM (Jackson ImmunoResearch). Flow cytometry was performed using a FACSCalibur flow cytometer (Becton Dickinson). Data files were acquired and analyzed using CellQuest software (version 3.3; BD Biosciences). Monoclonal anti-KLH antibodies were a donation from Prof. Z. Eshhar (The Weizmann Institute, Rehovot, Israel).

**Western blots.** rFBA and FCRP were separated by 1-dimensional SDS-PAGE, transferred to nitrocellulose, and probed with FCRP or rFBA, respectively. Rabbit anti–FCRP-KLH serum or rabbit anti-rFBA serum was added, respectively. In both cases, anti–rabbit IgG (Jackson ImmunoResearch) was used for final identification.

**Inhibition of S. pneumoniae nasal and lung colonization.** Seven-week-old BALB/c female mice (n = 120) were anesthetized with isoflurane (R_XElite) and inoculated intranasally with S. pneumoniae serotype 3 strain WU2 (5 × 10⁶ or 1 × 10⁶ bacteria in 25 μL of PBS) before (n = 40) and after treatment with 20 μg (n = 40) or 40 μg (n = 40) of FCRP. In a control experiment, bacteria were treated with PsipD (40 μg; n = 20) before mice were inoculated. At 3, 6, 24, and 48 h after inoculation, the nasopharynx and lungs were excised, homogenized, and plated onto blood agar plates. The experiments were repeated on 3 different occasions.

**Bioinformatic analysis.** The nucleic acid sequences of the inserted peptides in the positive phages were aligned against all the human proteins in the gene bank at the National Center for Biotechnology Information (NCBI) database using Blast software (version 2.2.10; NCBI) and substitution matrix PAM30. Prediction of membrane-spanning regions and their orientation was done using the TMHMM server (version 2.0; available at: http://www.cbs.dtu.dk/services/TMHMM-2.0). Alignment of all the sequences obtained from positive phages against FCR
Figure 3. Inhibition of *Streptococcus pneumoniae* adherence to A549 epithelial cells by recombinant fructose bisphosphate aldolase binding phages. A–K, Significant inhibition, in a concentration-dependent manner, of 86%-94% of *S. pneumoniae* adhesion to A549 cells by positive phages. Spearman regression analysis for these phages, analyzed individually, did not exceed $r = -0.778$ and $P < .002$. L, Phage without a peptide insert inhibiting 31% of *S. pneumoniae* adhesion to A549 cells ($r = -0.551$, $P = .005$). The inhibition of *S. pneumoniae* adhesion by the positive phages differs significantly from the inhibition by the phage without insert ($P < .001$). M, Inhibitory activity of negative phages only in the presence of high phage concentration ($r = -0.843$ [$P < .005$]) (exemplified with phage 15). The inhibition of *S. pneumoniae* adhesion by the positive phages differed significantly from the inhibition by the negative phages ($P < .001$).
way analysis of variance. was regarded as significant.

Involvement of FBA in S. pneumoniae adhesion to A549 lung carcinoma cells. To study the involvement of FBA in bacterial adhesion, cultured A549 cells were incubated with or without rFBA before the addition of S. pneumoniae. The adhesion to A549 cells of both the encapsulated S. pneumoniae serotype 3 strain WU2 and its unencapsulated derivative, mutant strain 3.8DW, was inhibited significantly in a concentration-dependent manner by rFBA \( (r = -0.712 \ [P = .001] \) and \( r = -0.646 \ [P < .001] \), respectively). The IC\(_{50}\) of rFBA was 0.8 \( \mu \)g/mL for S. pneumoniae strain WU2 and 1.1 \( \mu \)g/mL for S. pneumoniae strain 3.8DW (figure 1A and 1B, respectively). Inhibition of encapsulated serotype 3 strain WU2 adhesion to A549 cells was obtained only at 40 \( \mu \)g/mL of PsipD (an unrelated bacterial protein), with a low correlation \( (r = -0.419 \ [P < .001]) \) (figure 1C). These results differed significantly from the inhibition the bacteria adhesion with rFBA \( (P < .001) \).

To further assess the involvement of FBA in bacterial adhesion, S. pneumoniae serotype 3 strains WU2 and 3.8DW were treated with serum obtained from a rabbit immunized with rFBA. Preimmune serum had no effect on bacterial adhesion (data not shown). Anti-rFBA serum inhibited S. pneumoniae strains WU2 and 3.8DW adherence to A549 cells significantly, in a concentration-dependent manner \( (r = -0.884 \ [P < .001]) \) and \( r = -0.841 \ [P < .001] \), respectively) (figure 2A and 2B). Anti-PsipD antibodies did not affect bacterial adhesion to A549 cells significantly \( (r = 0.159 \ [P < .001]) \) (figure 2C). Notably, none of the sera influenced bacterial viability (no serum, 6.8 \( \times 10^4 \) \( \pm \) 2900 cfu; preimmune serum, 6.9 \( \times 10^4 \) \pm 3300; immune serum, 7.1 \( \times 10^4 \) \pm 3500 cfu) after incubation with the bacteria for 90 min.

Identification of biologically active rFBA-binding peptides. To identify rFBA-binding peptides, a random combinatorial peptide library expressed in the filamentous phage fd was screened by rFBA. The 30 phages binding to rFBA with the highest affinity, as determined using a Western blot–like assay, were selected for further study (data not shown). These 30 phages and a phage without insert were amplified in E. coli strain DH\( _{\alpha}\) and tested for their ability to interfere with bacterial adhesion. S. pneumoniae serotype 3 strain 3.8DW was incubated with the selected phages before it was added to A549 cells. Eleven of 30 phages inhibited S. pneumoniae adhesion to cultured A549 cells in a dose-dependent manner—significantly at 1 \( \times 10^4 \) phages/mL \( (P < .001) \), but 86%–94% inhibition was reached in the presence of 1 \( \times 10^3 \) phages/mL (the highest \( r \) value was \( r = -0.778 \), and the highest \( P \) value was \( P < .002 \)) (figure 3A–3K). By contrast, the phage lacking a peptide insert inhibited S. pneumoniae adhesion to A549 cells by only 31% \( (r = -0.551 \ [P = .005]) \) (figure 3L). Although significant \( (P < .001) \), the inhibition of bacterial adhesion was significantly lower \( (P < .001) \) than that exhibited by the 11 positive phages. The inhibitory activity of the other 19 phages was also examined, and, although it was significant in the presence of high numbers of phages \( (1 \times 10^4; \ r = -0.843 \ [P = .05]) \) (exemplified by phage 15 in figure 3M), it was significantly less than the inhibitory activity of the “positive” phages \( (P < .001) \). The inhibitory activity of these phages did not differ significantly from each other and from that observed with the phage without insert.

Identification of rFBA target protein. To identify the target protein of rFBA, the peptide inserts in the positive phages were amplified, sequenced, and the nucleic acid sequence translated to a polypeptide sequence. Then these polypeptide sequences were aligned against all the human proteins in the gene bank at the NCBI database using Blast software (version 2.2.10) and substitution matrix PAM30. The insert of phase 7 (DSSDCD-
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Figure 4. Localization of Flamingo cadherin receptor (FCR) on A549 cells and binding of the fructose bisphosphate aldolase (FBA)–binding region of FCR (FCRP) to recombinant (r) FBA. A, Flow cytometry results of A549 cells and mouse anti–FCRP–keyhole limpet hemocyanin (KLH) serum (1:15; solid line), anti–pneumococcal immunogenic protein D (PsipD; broken line), or control mouse serum (dotted line). The majority of A549 cells (92%) were positively stained with the anti–FCRP-KLH serum. B, Flow cytometry results of A549 cells and anti-KLH antibodies (1:5). No positive staining of A549 cells was observed when this antibody was used. C, rFBA, separated by 1-dimensional SDS-PAGE and probed sequentially with FCRP, anti–FCRP–KLH antiserum, and anti–rabbit IgG. In a control experiment, the FCRP probing stage was omitted, and no bands were detected. D, FCRP was separated by 1-dimensional SDS-PAGE and probed sequentially with rFBA, rabbit anti-rFBA antiserum, and anti–rabbit IgG. In a control experiment, the rFBA probing stage was omitted, and no bands were detected.

Localization of FCR on the surface of A549 cells was verified by flow cytometry using mouse anti–FCRP-KLH serum followed by goat anti–mouse IgM+IgG FITC. The majority (92%) of A549 cells stained positively by anti–FCRP-KLH serum, whereas the cells were not stained by preimmune serum, another control serum (anti–PsipD serum; figure 4A), or anti–KLH antibodies (figure 4B).

To determine more directly whether FCRP binds to rFBA, Western blot–like assays were performed in which rFBA was separated using 1-dimensional SDS-PAGE, transferred to a nitrocellulose membrane, and probed with FCRP. Binding of FCRP was detected by incubating the membrane sequentially with rabbit anti–FCRP-KLH serum and anti–rabbit IgG. When this protocol was used, a band of 33.5 kDa, corresponding to the molecular weight of the rFBA–histidine affinity tag fusion protein, was shown to bind FCRP (figure 4C). In the converse experiment, when FCRP was separated on SDS-PAGE, a band of ~1.5 kDa corresponding to the molecular weight of FCRP was bound by rFBA (figure 4D).

Inhibition of S. pneumoniae adhesion to A549 epithelial cells by FCRP. Having confirmed that FCRP binds rFBA, it
was tested whether FCRP was able to inhibit 2 encapsulated \textit{S. pneumoniae} strains and their respective unencapsulated derivatives from adhering to A549 cells. FCRP significantly inhibited, in a dose-dependent manner, \textit{S. pneumoniae} serotype 3 strains WU2 and 3.8DW from adhering to A549 cells (\( r = -0.844 \) [\( P < .001 \)] and \( r = -0.551 \) [\( P = .018 \)], respectively) (figure 5A and 5B). The IC\( _{50} \)s of FCRP for strain WU2 and 3.8DW were 12 and 20 \( \mu \)g/mL, respectively. FCRP also significantly inhibited, in a concentration-dependent manner, \textit{S. pneumoniae} serotype 2 strain D39 and strain R6 from adhering to A549 cells (\( r = -0.768 \) [\( P < .001 \)] and \( r = -0.833 \) [\( P = .001 \)], respectively) (figure 5C and 5D). The FCRP IC\( _{50} \)s for the D39 and the R6 strains were 6 and 3.5 \( \mu \)g/mL, respectively. It was confirmed that incubation of FCRP with \textit{S. pneumoniae} for the duration of the experiments described above had no effect on bacterial viability (up to 4 \( \mu \)g/mL FCRP; data not shown).

\textbf{Inhibition of \textit{S. pneumoniae} nasopharyngeal colonization and pneumonia development by FCRP.} To discover whether FCRP is capable of inhibiting \textit{S. pneumoniae} colonization and disease development, mice were inoculated intranasally with \textit{S. pneumoniae} serotype 3 strain WU2 before and after incubation with FCRP. Inoculation of mice with \( 5 \times 10^5 \) \textit{S. pneumoniae} preincubated with 20 \( \mu \)g/mL FCRP resulted in significantly reduced nasopharyngeal colonization at 3, 6, 24, and 48 h after inoculation (\( P \leq .006 \)) (figure 6A). Preincubation of the \( 5 \times 10^5 \) \textit{S. pneumoniae} with 40 \( \mu \)g/mL FCRP almost completely prevented \textit{S. pneumoniae} colonization of the nasopharynx at all time points tested (\( P \leq .001 \)) (figure 6A). Furthermore, treatment of \( 5 \times 10^5 \) \textit{S. pneumoniae} with 20 or 40 \( \mu \)g/mL FCRP significantly inhibited the number of bacteria detected in the lungs at 3 and 6 h after inoculation (\( P \leq .001 \)) (figure 6B). When this size of inoculum was used, bacteria were generally cleared from the lungs by 24 h after inoculation.

Nasopharyngeal colonization of mice at 3, 6, 24, and 48 h after inoculation with \( 1 \times 10^5 \) \textit{S. pneumoniae} serotype 3 strain WU2 that had been preincubated with 20 \( \mu \)g/mL FCRP was observed to be inhibited significantly (\( P \approx 0.036 \)) (figure 6C). Preincubation of \( 1 \times 10^5 \) \textit{S. pneumoniae} serotype 3 strain WU2 with 40 \( \mu \)g/mL FCRP resulted in a significantly greater inhibition of \textit{S. pneumoniae} nasopharyngeal colonization (\( P \leq .001 \)) (figure 6C). Moreover, preincubation of \( 1 \times 10^5 \) \textit{S. pneumoniae} serotype 3 strain WU2 with 40 \( \mu \)g/mL FCRP significantly reduced the number of bacteria in the lungs at 3 and 6 h after inoculation (\( P = .016 \)) (figure 6D). After 48 h, almost no bacteria were found in the lungs of mice inoculated with bacteria that had been preincubated with either 20 or 40 \( \mu \)g/mL FCRP. 

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure5.png}
\caption{Inhibition of \textit{Streptococcus pneumoniae} adherence to A549 epithelial cells by Flamingo cadherin receptor (FCR)–derived synthetic peptide. The fructose bisphosphate aldolase–binding region of FCR (FCR) was preincubated with \textit{S. pneumoniae} (\( 1 \times 10^5 \) cfu), then bacteria were added to A549 cells. After the removal of excess bacteria, the cells were detached and plated onto blood agar plates. \textbf{A}, Significant inhibition of encapsulated serotype 3 strain WU2 adherence to cells by FCRP (\( r = -0.844 \) [\( P < .001 \)]). \textbf{B}, Significant inhibition of unencapsulated serotype 3 strain 3.8DW adherence to cells by FCRP (\( r = -0.551 \) [\( P = .018 \)]). \textbf{C}, Significant inhibition of encapsulated serotype 2 strain D39 adherence to A549 cells by FCRP (\( r = -0.768 \) [\( P < .001 \)]). \textbf{D}, Significant inhibition of unencapsulated serotype 2 strain R6 adherence to A549 cells by FCRP (\( r = -0.833 \) [\( P = .001 \)]).}
\end{figure}
Figure 6. Inhibition of *Streptococcus pneumoniae* nasopharyngeal colonization and pneumonia development by the fructose bisphosphate aldolase–binding region of the Flamingo cadherin receptor (FCRP). BALB/c mice were inoculated nasopharyngeally with *S. pneumoniae* that had been sham treated or treated with 20 or 40 μg/mL of FCRP. A, Significant inhibition of nasopharyngeal colonization in the presence of FCRP when an inoculum of 5 × 10^6 cfu was used (P < .006 and P < .001, respectively). B, Significant inhibition of lung colonization in the presence of FCRP when an inoculum of 5 × 10^6 cfu was used (P < .001). C, Significant inhibition of nasopharyngeal colonization in the presence of FCRP when an inoculum of 1 × 10^6 cfu was used (P < .036 and P < .001, respectively). D, Significant inhibition of lung colonization in the presence FCRP when an inoculum of 1 × 10^6 cfu was used (P < .016). E, Significant inhibition bacterial adhesion to the nasopharynx by FCRP, compared with pneumococcal immunogenic protein D (PsipD), when an inoculum of 1 × 10^6 cfu was used (P < .05). F, Significant inhibition of bacterial adhesion to the lungs by FCRP, compared with PsipD, when an inoculum of 1 × 10^6 cfu was used (P < .05).

DISCUSSION

The importance of FBA in *S. pneumoniae* pathogenesis was indicated initially by lectin characteristics, the increase in antibodies in children against it with age, and its ability to induce a partially protective immune response from lethal *S. pneumoniae* intranasal challenge in mice [17]. In the present study, it has been demonstrated that FBA functions as an adhesin. Furthermore, using a random combinatorial peptide library expressed in a filamentous phage, the FCR has been identified to be FBA’s putative cognate receptor.

The ability of rFBA and anti-rFBA antisera to interfere in vitro with *S. pneumoniae* adhesion to A549 cells supports the premise that FBA functions as an adhesin. It might be argued that, because FBA is localized in the cell wall, it cannot be an adhesin as it is masked by the capsule. However, a recent study reported that the polysaccharide capsule is shed when *S. pneumoniae* interacts with host cells [24]; thus, cell wall–associated proteins may in fact be exposed [25]. Of note, the shedding of polysaccharides is incomplete, and this likely explains the different adhesion efficiency to cultured cells of encapsulated and unencapsulated *S. pneumoniae* and the differences observed in
the inhibitory activity of rFBA and anti-rFBA serum. FBA may belong to a group of surface adhesins and invasins discovered in various gram-positive bacteria that lack a signal peptide and recognizable anchor sequences. Moreover, as appears to be the case for FBA, some of these proteins have dual intracellular and extracellular functions, the latter in pathogenesis [26].

The human lung carcinoma cell line (A549) was chosen for these studies because it is used widely as an in vitro model for studying S. pneumoniae interactions with human cells [24, 27]. These cells retain many characteristics of type II lung epithelial cells [28–30]. The adhesion of S. pneumoniae to these cells was found to be similar to the adhesion of S. pneumoniae to primary nasal epithelial cells. Furthermore, S. pneumoniae invasion to A549 cells did not exceed 5% of the total number of adhered bacteria (Y.M.N., unpublished data).

To identify host target proteins to FBA, a random combinatorial peptide library expressed in the envelope protein of a filamentous phage was used. Eleven of 30 rFBA-binding phages inhibited 80%–90% of S. pneumoniae adhesion to cultured A549 cells. The peptide insert from phage 7 aligns with 3 proteins in the human genome: FCR, opioid growth factor receptor, and integrin-β. FCR was selected for further study for 2 reasons. First, the FCR region homologous to the inhibitory peptide most likely resides in its extracellular domain; second, several peptide-inserts from the inhibitory phages aligned to FCR.

The 3 human and 3 mouse FCR genes (Celsr1–3 and celsr1–3, respectively) are orthologs of Drosophila flamingo/starry night (fmi/stan); each encodes a large 7-transmembrane receptor that binds to the cadherin superfamily [31, 32], which constitutes a group of nonclassical cadherin receptors [33]. Fmi plays a pleotropic role in controlling epithelial and neuronal morphogenesis [34].

The cadherins belong to a category of mammalian surface proteins known as cell-adhesion molecules (CAMs), among which are integrins, selectins, and carinoembryonic CAM. These proteins establish contact between a cell and its surrounding and are key players in cell migration, in particular the extravasation of leukocytes from blood vessels [35]. Many microbial pathogens bind to CAMs, using them as their port of entry when invading the host—for example, Yersinia enterolitica [36, 37], Neisseria gonorrhoeae [38, 39], and Listeria monocytogenes [40]. Mice deficient in E- and P-selectins showed a marked increase in susceptibility to S. pneumoniae infection [41], which is probably a result of an impaired immune response. It is notable that CAMs mediate extravasation of leukocytes through the cellular and paracellular routes; thus, bacteria can use the same target CAM molecule for host invasion through the cellular and the paracellular routes [42], reversing the process of extravasation of leukocytes from blood vessels.

Pneumococcal lectins have been demonstrated in vitro to be involved in bacterial interaction with host epithelial cells [10, 16–19, 43–45]. There are 13 glycosylation sites on FCR. Thus, given that it is a lectin [16], one possibility is that FBA adheres to FCR via protein-carbohydrate recognition.

A synthetic peptide encoding the FCR region homologous to the insert sequence in phage 7 was verified to bind rFBA in vitro. Importantly, this FCR region is localized on the cell surface of A549 cells. Moreover, FCRP inhibits 2 genetically and capsularly unrelated S. pneumoniae strains from adhering to A549 cells. In the mouse model, FCRP inhibits S. pneumoniae nasopharyngeal and lung colonization, possibly by preventing FBA binding to FCR or other proteins that include sequences similar to FCRP. As a consequence, the reduced bacterial adhesion decreases bacterial growth in the lungs and, possibly, the development of pneumonia. Thus, it appears likely that FCR is indeed the target protein for FBA and that it mediates adhesion of S. pneumoniae to host cells. More research is required to evaluate whether the FCRP described in the present study might be used in preventive or therapeutic treatments for S. pneumoniae infection.

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References


