Dihydrolipoamide Dehydrogenase of *Pseudomonas aeruginosa* Is a Surface-Exposed Immune Evasion Protein That Binds Three Members of the Factor H Family and Plasminogen

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The opportunistic human pathogen *Pseudomonas aeruginosa* causes a wide range of diseases. To cross host innate immune barriers, *P. aeruginosa* has developed efficient strategies to escape host complement attack. In this study, we identify the 57-kDa dihydrolipoamide dehydrogenase (Lpd) as a surface-exposed protein of *P. aeruginosa* that binds the four human plasma proteins, Factor H, Factor H-like protein-1 (FHL-1), complement Factor H-related protein 1 (CFHR1), and plasminogen. Factor H contacts Lpd via short consensus repeats 7 and 18–20. Factor H and FHL-1 displayed complement-regulatory activity, and bound plasminogen, when converted to the active protease plasmin, cleaved the chromogenic substrate S-2251 and the natural substrate fibrinogen. The Lpd of *P. aeruginosa* is a rather conserved gene; a total of 22 synonymous and 3 nonsynonymous mutations was identified in the lpd gene of the 5 laboratory strains and 13 clinical isolates. Lpd is surface exposed and contributes to survival of *P. aeruginosa* in human serum. Bacterial survival was reduced when Lpd was blocked on the surface prior to challenge with human serum. Similarly, bacterial survival was reduced up to 84% when the bacteria was challenged with complement active serum depleted of Factor H, FHL-1, and CFHR1, demonstrating a protective role of the attached human regulators from complement attack. In summary, Lpd is a novel surface-exposed virulence factor of *P. aeruginosa* that binds Factor H, FHL-1, CFHR1, and plasminogen, and the Lpd-attached regulators are relevant for innate immune escape and most likely contribute to tissue invasion. 


Upon infection, *P. aeruginosa* is immediately confronted and attacked by the complement system, which forms the first and central layer of host innate immune defense. Three major pathways activate the complement cascade, and each pathway initiates a series of tightly regulated reactions that all merge on the level of C3 and generate C3 convertases. Each C3 convertase cleaves C3 to the inflammatory anaphylatoxin and antimicrobial compound C3a and to the opsonin C3b (6). Further progression of the complement cascade generates a C5 convertase that cleaves C5 into the anaphylatoxins C5a and C5b. Surface-attached C5b initiates the terminal pathway and formation of the cell-damaging cytolytic terminal complement complex (TCC) (6). When complement activation is not properly controlled, the reaction is amplified, inflammatory and antimicrobial products are generated, and TCC is formed. These toxic molecules attack, damage, and ultimately allow removal of an infectious microbe. However, at the same time, host cells, tissues, and biomembranes can also be attacked, and therefore these self cells need to inhibit the damaging effects of complement. To this end, host cells and tissues use a series of complement inhibitors or regulators that are distributed both on membranes and as soluble molecules and that efficiently control and block complement activation.

Factor H and Factor H-like protein 1 (FHL-1) are two central fluid-phase regulators of the alternative complement pathway (7). Factor H is a 150-kDa plasma protein composed of 20 short consensus repeats (SCRs). FHL-1, which is derived from the *Factor H* gene by alternative splicing, is a 42-kDa plasma protein composed of 7 SCRs. The 7 SCRs of FHL-1 are identical to the N-terminal SCRs of Factor H, and, in addition, the FHL-1 protein has a C-terminal extension of four unique amino acids (7, 8). Factor H and FHL-1 regulate the alternative pathway of complement by binding C3b, by accelerating the decay of the alternative pathway C3-convertase (C3bBb), and by acting as a cofactor for
the Factor I-mediated cleavage of C3b (6–8). The complement factor H-related protein 1 (CFHR1) is a member of the factor H protein family, and the protein is transcribed from a separate gene. The corresponding plasma protein is composed of 5 SCR domains and appears as two isoforms with either two, four, 42-kDa (CFHR1β), or one, 37-kDa (CFHR1α) attached carbohydrate chains (9). CFHR1 inhibits complement at the level of the C5 convertases and blocks TCC assembly and formation (10).

To survive and establish an infection, any pathogenic microbe must control, inactivate, and evade the innate immune response of the host, including the complement attack. Acquisition of soluble host complement regulators is one common and important evasion strategy used by many, virtually all pathogenic microbes. *P. aeruginosa*, similar to other pathogenic bacteria, uses multiple strategies to actively evade and control host innate immunity and complement attack. *P. aeruginosa* binds the complement regulators factor H, FHL-1, and CFHR1, and the attached regulators inhibit complement activation at the bacterial surface, block the generation of the opsonin C3b, and increase serum resistance (11). Elongation factor Tuf, a 43-kDa surface protein, was identified as the first factor H, FHL-1, and CFHR1-binding protein of this Gram-negative pathogen (11). In addition, *P. aeruginosa* secretes the endogenous proteins elastase and alkaline protease, which degrade and inactivate human complement components, including C1q, C2, and C3b (12, 13). Degradation of the opsonin C3b reduces phagocytosis of the pathogen by human neutrophils, and degradation of C3 inhibits fibroblast cell growth (12, 14, 15). In addition, *P. aeruginosa* produces an alginate layer that forms a mechanical barrier and that reduces the accessibility and the action of host complement proteins and other plasma defense factors (16). During late infection, *P. aeruginosa* forms biofilms, which prevent complement-mediated attachment and phagocytosis (16, 17).

Following interaction with the complement system, many pathogens leave the site of infection and disseminate into deeper tissue layers. For this purpose, pathogenic microbes interact with and use either pathogen-encoded or host-derived proteolytic enzymes to degrade the extracellular matrix (ECM). The 92-kDa human serum protein plasminogen is a human proenzyme that is acquired by several pathogenic microbes (18). Plasminogen is composed of five consecutive kringle domains (K1–K5) that are linked to a protease domain (P) (19). When activated by endogenous activators like tissue-type plasminogen activator or urokinase-type plasminogen activator (uPA), the protease plasmin degrades ECM components like fibrinogen, fibronectin, vitronectin, and laminin and regulates cell migration, coagulation, fibrinolysis, inflammation, wound healing, and tissue remodeling (18, 19). Plasminogen is acquired by several pathogens, including *P. aeruginosa*, *Streptococcus pneumoniae*, *Borrelia burgdorferi*, *Staphylococcus aureus*, *Lactobacillus johnsonii*, and *Candida albicans* for ECM interaction, and activated plasmin is then used for destruction of host basement membranes and ECM (11, 20–23).

In the current study, we identify *P. aeruginosa* dihydrodipioamide dehydrogenase (Lpd) as a novel bacterial host regulator-binding protein, and thus as a multifunctional bacterial protein. Lpd is expressed at the bacterial surface and binds the human plasma proteins factor H, FHL-1, CFHR1, and also plasminogen. Bound to Lpd, the four human plasma proteins aid in complement evasion as well as ECM degradation. Taken together, Lpd is a novel virulence factor that mediates complement evasion and may facilitate tissue invasion of the Gram-negative bacterium *P. aeruginosa*. The identification of a new virulence factor and immune evasion protein of *P. aeruginosa* shows the complexity of the bacterial host immune crosstalk and offers new insights into the interaction of *P. aeruginosa* with the human host.

Materials and Methods

**Bacterial strains and culture conditions**

*P. aeruginosa* strains American Type Culture Collection (ATCC) 27853, National Collection of Type Cultures (NCTC) 10662, SG137, and PAO1; and the PAO1 derivative AH377 (24) were routinely cultivated in enriched nutrient broth (NB; Serva, Amstetten, Austria) at 37°C. Thirteen clinical isolates were derived from patients with different diseases. Cultures were grown to an OD_{600nm} = ~1.0. Transformed *Escherichia coli* M15-expressing Lpd were grown in Luria Bertani liquid broth supplemented with 25 μg/ml kanamycin and 10 μg/ml carbenicillin.

**Biotinylation and isolation of *P. aeruginosa* surface proteins**

Surface biotinylation of intact *P. aeruginosa* was performed, as described (25). Briefly, bacteria (2 × 10^{11}) were washed in buffer A (PBS, 1 mM CaCl_{2}, 0.5 mM MgCl_{2}) and resuspended in buffer B (buffer A supplemented with 1.6 mM D-biotin). Bacteria were pelleted, and surface proteins were labeled with biotin by incubation with 500 μl 400 μM EZLink Sulfo-NHS-LC-Biotin (Thermo Fisher Scientific, Bonn, Germany) for 30 min on ice. Cells were washed extensively in buffer C (50 mM Tris [pH 7.4], 100 mM NaCl, 27 mM KCl, 1 mM CaCl_{2}, 0.5 mM MgCl_{2}) and resuspended in buffer C supplemented with protease inhibitors (Complete; Roche, Mannheim, Germany). Bacteria were lysed by sonication, and biotinylated surface proteins were purified by affinity chromatography using Immunopure Immobilized Monomeric Avidin (Pierce). Proteins were eluted with D-biotin (2 mM in PBS), according to the manufacturer’s recommendations. The elute fractions were separated by SDS-PAGE and transferred to a membrane, and biotinylated proteins were identified by HRP-conjugated avidin (Roche).

**Isolation of Factor H ligands with magnetic beads**

Factor H SCR 8–20 (40 μg) was covalently coupled to magnetic beads (100 μl suspension), according to the manufacturer’s instructions (Invitrogen, Karlsruhe, Germany). Then the beads were incubated with a preparation of purified biotinylated *P. aeruginosa* surface proteins for 2 h at 37°C. After washing three times with 50 mM HEPES (pH 7.5), 1% Nonidet P-40, 1 mM dithioerythritol, 1 mM MgCl_{2}, and 1 mM CaCl_{2}, proteins were eluted with 40 μl 1 M NaCl for 10 min at 37°C, separated by SDS-PAGE, and visualized by silver staining. Individual bands were excised from the gel and analyzed by mass spectrometry.

**Protein identification by peptide mass fingerprinting**

Silver-stained bands were destained using the ProteoSilver Plus silver stain kit (Sigma-Aldrich, Steinheim, Germany), and Coomassie-stained bands were destained, according to the manufacturer’s protocol. Subsequently, proteins were reduced and alkylated using 10 mM DTT and 100 mM iodoacetamide in 25 mM NH_{4}HCO_{3}, washed with 25 mM NH_{4}HCO_{3} and dehydrated with acetonitrile. Digestion with trypsin, MALDI-TOF-mass spectrometry (MS), and protein identification by peptide mass fingerprinting were performed, as described (25).

Expression and purification of recombinant proteins

The *ldp* gene (GenBank accession no. Q913D1; BankIt15558120 Seq1 JX475907; BankIt15558120 Seq2 JX475908; BankIt15558120 Seq3 JX475909; BankIt1558120 Seq4 JX475910; BankIt1558120 Seq5 JX475911; BankIt1558120 Seq6 JX475912; BankIt1558120 Seq7 JX475913; BankIt1558120 Seq8 JX475914; BankIt1558120 Seq9 JX475915; BankIt1558120 Seq10 JX475916; BankIt1558120 Seq11 JX475917; BankIt1558120 Seq12 JX475918; BankIt1558120 Seq13 JX475919; BankIt1558120 Seq14 JX475920; BankIt1558120 Seq15 JX475921; BankIt1558120 Seq16 JX475922) was amplified from genomic DNA of *P. aeruginosa* strain PAO1. The amplicon was cloned into expression vector pQE (Qiagen, Hilden, Germany) and transformed into *E. coli* strain M15, and protein expression was induced by 1 mM isopropyl β-D-thiogalactoside. Recombinant Lpd was sequenced by MALDI-TOF-MS. The recombinant fragments of Factor H (SCRs 1–4, 1–5, 1–6, 8–11, 11–15, 15–18, 15–20, 18–20, and 19–20) and FHL-1 (SCRs 1–7) were expressed in the baculovirus system, as described (26). Recombinant CFHR1 fragments of CFHR1 (SCRs 1–2, 3–4) were expressed in *Pichia pastoris*, as described (27). The plasminogen gene (NM_000301) was amplified from human liver cDNA (Invitrogen) by PCR using Phusion High Fidelity DNA Polymerase (Finzymes, Espoo, Finland) or HotStarTaq DNA Polymerase (Qiagen) with specific primers (Table 1) introducing the restriction enzyme sites KpnI and Xhol. The sequence for the signal peptide was excluded. The PCR constructs were subcloned into *E. coli* Top10 cloning vectors pCR4Blunt-TOPO or pCR2.1Blunt-TOPO.
(Life Technologies, Darmstadt, Germany). The resulting fragments were cloned into the vector pPICZαA (Life Technologies) of *P. pastoris* strain X33. To produce recombinant proteins, *P. pastoris* was grown and induced with 1% methanol. After 2 days of expression, the culture supernatants were harvested and recombinant proteins were purified. All recombinant proteins were purified by nickel affinity chromatography using HisTrap chelating columns in a Äkta fast protein liquid chromatography system (GE Healthcare, Freiburg, Germany).

**Abs**

Polyclonal Lpd antisemur was raised by immunizing rabbits i.m. with 200 µg purified recombinant Lpd emulsified in CFA (Difco and BD Biosciences, Heidelberg, Germany) and boosted on days 18 and 36 with the same dose of protein in IFA. Blood was drawn 3 wk later. Generation of the human Factor H-specific mAbs, mAb E22 against SCR 3, and mAb C18 against SCR 20 has been described (28). Alexa fluo 488–conjugated polyclonal goat anti-rabbit was purchased from Molecular Probes, and the HRP-conjugated rabbit anti-goat, HRP-conjugated swine anti-rabbit, and HRP-conjugated rabbit anti-mouse were obtained from Dako (Glostrup, Denmark). A mouse His mAb was purchased from Qiagen. Polyclonal goat anti-Factor H and polyclonal goat anti-human C3 were obtained from Complement Technology (Tyler, TE), and polyclonal goat anti-plasminogen from Acris Antibodies (Herford, Germany). CFHR1 was detected with the mAb JDH10 (10) or rabbit CFHR1 antisemur (29). Fibrinogen degradation was assayed by rabbit anti-human fibrinogen (Calbiochem, Nottingham, U.K.).

**ELISA**

Microtiter plates (NUNC Maxisorb, Nunc-Immuno Module) were coated with Lpd (5 µg/ml) or gelatin (5 µg/ml) overnight at 4˚C or with live bacteria (0.5 × 10^9/well) for 1 h at 37˚C. The plates were washed four times with PBS containing 0.1% Tween 20 (PBS-Tw) and blocked for 1 h with PBS supplemented with 2% BSA (PBS-BSA) or blocking buffer I (Applichem, Darmstadt, Germany). After washing, the plates were incubated for 1 h at room temperature (RT) with Factor H (Complement Technology) (0.08–0.67 µM); FHL-1 (0.07–0.6 µM); equimolar amounts of Factor H, FHL-1, CFHR1, and plasminogen (0.13 µM); and equimolar amounts of the various Factor H constructs (0.13 µM), CFHR1 (0.07–0.58 µM), the two CFHR1 constructs (0.27 mM), plasminogen (Chromogenix, Milan, Italy) (0.001–0.54 µM), recombinant plasminogen fragments (10 µg/ml), or Lpd antisemur (1:10000). Thereafter, the wells were washed, and goat anti-human Factor H (1:500 to detect SCRs 8–11 and 1:2000 for the other deletion mutants), rabbit CFHR1 antisemur (1:1000), goat anti-plasminogen (1:1000 to detect K1-5-P and K1-5, 1:750 to detect K4-5-P, and 1:500 to detect K5), or mouse anti-CFHR1 mAb (JDH10) (1:500) (10) was added in PBS-BSA for 1 h at RT. After additional washings, HRP-conjugated rabbit anti-goat (1:2500), HRP-conjugated swine anti-rabbit (1:2500), or HRP-conjugated rabbit anti-mouse (1:2500) was added for 40 min at RT. The reaction was developed with 1,2-phenylenediamine dihydrochloride (Dako), and the absorbance was measured at 492 nm. In the competition assays, the effect of heparin (0.25–5 mg/ml) on Factor H (5 µg/ml), FHL-1 (5 µg/ml), or CFHR1 (5 µg/ml) binding; the effect of mAbs E22 (20 µg/ml) and C18 (20 µg/ml) on Factor H binding; or the effect of the lysine analog with 1,2-phenylenediamine dihydrochloride (Dako), and the absorbance was measured at 405 nm (SpectraMax 190; Molecular Devices).

**Fibrinogen degradation by Lpd-bound plasminogen**

Lpd (5 µg/ml) or BSA (5 µg/ml) was immobilized on microtiter plates overnight at 4˚C. The plates were washed with PBS-Tw and blocked for 6 h at 4˚C with PBS containing 0.2% gelatin. After washing, the immobilized proteins were incubated with plasminogen (20 µg/ml) overnight at 4˚C, washed, and incubated with uPA (160 ng/ml) together with fibrinogen (10 µg/ml) at 37˚C. The reaction was terminated at various time points (0.5–6 h) by addition of SDS-PAGE sample buffer (Rotiload1; Carl Roth). Samples were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and analyzed by Western blotting. Fibrinogen degradation was analysed using a rabbit anti-human fibrinogen (1:1000) that was added for 1 h, followed by HRP-conjugated swine anti-rabbit (1:2500) added for 40 min. Development was performed with ECL Western blotting detection reagents (AppliedChem).

**Flow cytometry**

The surface expression of Lpd on *P. aeruginosa* was analysed by flow cytometry. The *P. aeruginosa* strain SG137 was grown to an OD600 ~1.0 and washed once in PBS-BSA, and then bacteria (10^9) were incubated with rabbit Lpd antisemur (1:100) for 45 min at 4˚C. Bacteria were washed and incubated for 30 min with Alexa fluo 488–conjugated goat anti-rabbit (1:200), washed, and analyzed by flow cytometry (FACScan LRII; BD Biosciences, Mountain View, CA). All incubations were kept in PBS-BSA, and secondary pAb was added separately as a negative control.

**Transmission electron microscopy**

The expression of Lpd on the surface of *P. aeruginosa* and the binding of Factor H and CFHR1 were analysed by negative staining and electron microscopy, as described (31). Rabbit Lpd antisemur, plasma-purified human Factor H, or recombinant CFHR1 was labeled with 5 nm colloidal gold, 15 nm colloidal gold, or 45 nm colloidal gold, respectively. Bacteria (SG137) were mixed with the Abs or Factor H and/or CFHR1, and 5 µl aliquots were adsorbed onto carbon-coated grids for 1 min, washed with two drops of water, and stained on two drops of 0.75% uranyl formate. The grids were rendered hydrophilic by glow discharge at low pressure in air. Specimens were observed in a Jeol JEM 1230 electron microscope (JEOL, Tokyo, Japan) operated at 60 kV accelerating voltage. Images were recorded with a Multiscan 791 CCD camera (Gatan, Pleasanton, CA).

**Isolation of *P. aeruginosa* outer membrane proteins**

Bacteria grown to exponential phase were washed with 50 mM Tris-HCl (pH 8.0). The pellet was resuspended in 50 mM Tris-HCl (pH 8.0) containing 3% Empigen (Calbiochem) and protease inhibitors (Complete; Roche). Outer membrane proteins (OMPs) were extracted by rotating the mixture at 37˚C for 2 h. The bacterial cell walls, stripped of their outer membrane, were centrifuged, and the supernatants were collected. Thereafter, the supernatants were subjected to SDS-PAGE, transferred to a membrane, and analyzed by Western blotting using a rabbit Lpd antisemur (1:500) for 1 h, followed by HRP-conjugated swine anti-rabbit (1:2500) for 40 min. Development was performed with ECL Western blotting detection reagents (AppliedChem).

**Sequencing of the lpd gene**

The lpd gene of the 5 *P. aeruginosa* strains (SG137, ATCC 27853, NCTC 10662, PAO1, AH377) and of 13 clinical isolates was amplified by PCR

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Table I. Primer used for the plasminogen fragments

<table>
<thead>
<tr>
<th>Plasminogen Fragment</th>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1-K5-P</td>
<td>Forward</td>
<td>GGTTACCAACAGCTCCTGATGAC</td>
</tr>
<tr>
<td>K1-K5-P</td>
<td>Reverse</td>
<td>GCGGCCCACATATTCTCCTCAATTC</td>
</tr>
<tr>
<td>K4-K5-P</td>
<td>Forward</td>
<td>GGTTACCACTAGCACCCTCCAGCATCA</td>
</tr>
<tr>
<td>K4-K5-P</td>
<td>Reverse</td>
<td>GCGGCCCACATATTCTCCTCAATTC</td>
</tr>
<tr>
<td>K5-P</td>
<td>Forward</td>
<td>GGTTACCACTAGCACCCTCCAGCATCA</td>
</tr>
<tr>
<td>K5-P</td>
<td>Reverse</td>
<td>GCGGCCCACATATTCTCCTCAATTC</td>
</tr>
<tr>
<td>K1-K5</td>
<td>Forward</td>
<td>GGTTACCACTAGCACCCTCCAGCATCA</td>
</tr>
<tr>
<td>K1-K5</td>
<td>Reverse</td>
<td>GCGGCCCACATATTCTCCTCAATTC</td>
</tr>
</tbody>
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using HotStarTaq (Qiagen) using the primers 5′-TAGCGTTTCTCTTCACGCGC-3′ and 5′-CCGGTGGCTGTTGACGCCG-3′. The sequence of the amplified products was determined using an ABI 3100 sequence analyzer (Applied Biosystems). The sequences were compared with that of the lpd gene of P. aeruginosa PAO1 (GenBank accession no. Q913D1).

**Serum bactericidal assay**

The five P. aeruginosa strains (SG137, ATCC 27853, NCTC 10662, PAO1, AH377) were grown to an OD600∼1.0 and diluted in Mg-EGTA buffer. Bacteria (10⁶ CFU) were incubated in normal human serum (NHS), used at increasing concentrations (0–50%), NHS or complement active HS2FH/FHL-1/CFHR1 used at 10–50% depending on the strain (SG137, 10%; ATCC 27853, 20%; NCTC 10662, 15%; PAO1, 50%; AH377, 50%), or depleted serum reconstituted with purified Factor H (100 μg/ml) in a final volume of 100 μl at 37°C. After 0 and 60 min, 10 μl aliquots were removed and spread onto NB plates. After 18 h of incubation at 37°C, CFU was determined. In the inhibition experiments, bacteria (strain SG137) moved and spread onto NB plates. After 18 h of incubation at 37°C, CFU was determined. To confirm that Lpd of P. aeruginosa was preincubated with Lpd antiserum (1:20), Factor H deletion mutants were determined. In the inhibition experiments, bacteria (strain SG137) were challenged with 10% NHS in a final volume of 100 μl at 37°C. After 0 and 60 min, 10 μl aliquots were removed and spread onto NB plates. After 18 h of incubation at 37°C, CFU was determined. In the inhibition experiments, bacteria (strain SG137) moved and spread onto NB plates. After 18 h of incubation at 37°C, CFU was determined.

**Identification of a second Factor H-binding protein of P. aeruginosa**

To identify additional Factor H-binding proteins of P. aeruginosa, surface proteins of P. aeruginosa strain PAO1 were biotinylated, extracted, and absorbed to a Factor H/SCR 8–20 matrix. Bound proteins were eluted, separated by SDSPAGE, and visualized by silver staining. This approach identified two major bands with mobilities of ~57 and 43 kDa (data not shown). The 57-kDa band was identified as Lpd, and the 43 kDa band as Tuf, the known Factor H-binding protein (11). Lpd of P. aeruginosa is a 478-aa flavoprotein with a predicted molecular mass of 50.2 kDa (32).

**Recombinant expression of Lpd and generation of polyclonal Lpd antiserum**

P. aeruginosa lpd gene was amplified, the corresponding PCR product was cloned into expression vector pQE, recombinantly expressed in E. coli as a N-terminal His-tagged protein, and purified by nickel chelate chromatography. The bacterial lysate, flow-through (FT), wash (w), and elute (e) fractions were separated by SDS-PAGE, and proteins were visualized by silver staining. This approach identified the elute fraction two bands, one of 57 and one of 50 kDa (Fig. 1A, lane 4). In addition, a His antiserum identified the 57- and the 50-kDa band from the 57-kDa band (Fig. 1B, lane 1). Purified recombinant Lpd was used to immunize rabbits, and the corresponding polyclonal Lpd antiserum identified both the 57- and 50-kDa bands (Fig. 1B, lane 3). The two bands were excised from the gel and analyzed by mass spectrometry, and both the upper and the lower band were identified as Lpd. The reactivity with the His- and the Lpd-specific antiserum, together with the mass spectrometry data, suggests that the 57-kDa band represents the full-length His-tagged Lpd protein, and the 50-kDa band most likely is a degradation product that has the His tag cleaved off.

**Recombinant Lpd binds Factor H and FHL-1**

To confirm that Lpd of P. aeruginosa is a Factor H-binding protein, binding of Factor H to Lpd was assayed. Factor H bound to immobilized Lpd, and binding was dose dependent (Fig. 2A). In addition, FHL-1 also bound to immobilized Lpd, and again binding was dose dependent (Fig. 2B). Factor H contains two heparin binding domains located in SCR 7 and SCRs 19–20, and FHL-1 contains one heparin binding domain in SCR 7 (33). Therefore, we asked whether heparin affects the Lpd/Factor H or Lpd/FHL-1 interaction. Heparin inhibited binding of both human regulators, and the effect was dose dependent (Fig. 2C, 2D). Heparin used at 1 mg/ml inhibited Factor H binding to Lpd by 73% and FHL-1 binding by 66%.

To localize the region(s) of Factor H and FHL-1 that contacts Lpd, a series of Factor H deletion mutants were tested for binding to immobilized Lpd. The N-terminal SCRs 1–7 (FHL-1) and the C-terminal constructs SCRs 15–20 and SCRs 18–20 bound to Lpd (Fig. 2E). Deletion fragments SCRs 1–4, SCRs 1–5, SCRs 1–6, SCRs 8–11, SCRs 11–15, SCRs 15–18, and SCRs 19–20 bound either with reduced intensity or did not bind. Thus, the human complement regulator Factor H binds to Lpd via two interaction domains that are included within SCR 7 and SCRs 18–20, and FHL-1 binds via one domain, that is, SCR 7. To confirm that the C terminus of Factor H binds to bacterial Lpd, epitope-specific mAbs were used as blocking agents. A mAb that binds to SCR3 (E22) and a mAb that binds to SCR 20 (C18) of Factor H were used. The mAb C18, which binds to the C terminus, blocked Factor H binding to immobilized Lpd by >60% (Fig. 2F). In contrast, mAb E22, which reacts with the N terminus, did not affect Factor H binding to Lpd. Thus, this demonstrates that inhibition is not caused by steric hindrance and that the surface recognition region of Factor H (SCRs 18–20) is important for binding of Lpd.

**Factor H and FHL-1 bound to Lpd exhibit cofactor activity**

Factor H and FHL-1 control alternative pathway-mediated complement activation at the level of the C3 convertase by acting as cofactors for the serine protease Factor I. To demonstrate that the regulators bound to Lpd are functionally active, we assayed cofactor activity of Lpd-bound Factor H or FHL-1 for C3b degradation. C3b degradation was revealed as the appearance of the degradation fragments α′43 and α′41 kDa. Bound to Lpd, Factor H and FHL-1 were functionally active, acting as cofactors in the degradation of C3b (Fig. 3A, 3B, lanes 3). Thus, Factor H and FHL-1 bound to P. aeruginosa Lpd maintain their central regulatory functions and control complement activation at the level of the C3 convertase.
The C-terminal surface attachment region SCRs 18–20 of Factor H (SCRs 18–20) and CFHR1 (SCRs 3–5) (100, 100, and 97%) (10), we asked whether CFHR1 also binds to Lpd. CFHR1 bound to immobilized Lpd, and binding was dose dependent (Fig. 4A). CFHR1 is a heparin-binding protein, and, therefore, we also assayed the effect of heparin for the CFHR1/Lpd interaction (10). Heparin inhibits the binding of the complement regulator CFHR1 to P. aeruginosa Lpd.

In addition, the interacting domains of CFHR1 that are relevant for contact of Lpd were localized by assaying binding of equimolar amounts of CFHR1 deletion mutants. Both deletion mutants bound to immobilized Lpd, but CFHR1/SCRs 3–5 bound with ∼4 times higher intensity to Lpd compared with CFHR1/SCRs 1–2 (Fig. 4C). Thus, the C-terminal CFHR1/SCRs 3–5 represent the major binding region of Lpd.

**FIGURE 3.** Lpd-bound Factor H and FHL-1 are functionally active and maintain complement-regulatory activity. Factor H (A) and FHL-1 (B) when bound to immobilized Lpd displayed cofactor activity for Factor I in C3b inactivation. Factor H (A) or FHL-1 (B) was bound to Lpd and, after extensive washing, C3b and Factor I were added. After incubation, the supernatants were separated by SDS-PAGE, and C3b cleavage products were analyzed by Western blotting using goat anti-C3 and HRP-conjugated anti-goat. Cofactor activity of Factor H or FHL-1 bound to Lpd is visualized by the appearance of the cleavage fragments α'43 and α'41 (A, B, lane 3). The C3b cleavage pattern obtained with immobilized Factor H (A) or FHL-1 (B) incubated with C3b and Factor I was used as a positive control to identify the degradation products (A, B, lane 2). No cleavage occurred when HSA was used as a matrix (A, B, lane 4). A representative experiment of three is demonstrated.
Lpd binds human plasminogen

Several microbial Factor H-binding proteins also bind plasminogen (34–37). We therefore asked whether P. aeruginosa Lpd also binds plasminogen. Plasminogen bound to Lpd, and binding was dose dependent and saturated at 0.27 μM plasminogen (Fig. 5A). As lysine residues are often relevant for plasminogen binding, we assayed whether the lysine analog εACA influences the plasminogen/Lpd interaction. εACA used at 1.0 mM inhibited plasminogen binding to immobilized Lpd by 50%, and at 10 mM εACA blocked the interaction completely (Fig. 5B). Thus, lysine residues are relevant for the Lpd/plasminogen contact.

To localize the domain(s) of plasminogen that contacts Lpd, recombinant plasminogen and a series of recombinant plasminogen deletion mutants were generated, and these proteins were tested for Lpd binding. Recombinant full-length plasminogen (K1-5-P), the deletion mutant that has kringle domains 4 and 5 linked to the protease domains (i.e., K4-K5-P) and a fragment that includes all five kringle domains, but lacks the protease domain (K1-5), bound to immobilized Lpd (Fig. 5C). The fragment that includes kringle 5 and the protease domain bound to immobilized Lpd with a reduced intensity. Thus, this indicates that kringle domain 4 of plasminogen contains the major Lpd binding site.

Lpd-bound plasminogen is accessible for uPA and is proteolytically active

Plasminogen is a zymogen that can be converted to active plasmin (19). To determine whether Lpd-bound plasminogen is accessible for

**FIGURE 5.** Plasminogen binds to Lpd, and bound to Lpd it is functionally active. (A) Plasminogen binding to Lpd was assayed by ELISA. Plasminogen used at 0.001–0.54 μM bound to immobilized Lpd dose dependently. Bound plasminogen was detected with polyclonal plasminogen antiserum, followed by HRP-conjugated anti-goat. (B) Lysine residues are responsible for the interaction between Lpd and plasminogen. The effect of increasing concentrations (0.1–10 mM) of the lysine analog εACA on plasminogen binding to immobilized Lpd was assayed. (C) Localization of the Lpd binding site within plasminogen. The major Lpd binding site is located within kringle domain 4 of plasminogen. Recombinant deletion mutants of plasminogen, as indicated in the left part of the figure, were generated in P. pastoris. Binding of purified deletion mutants (10 μg/ml) to immobilized Lpd was assayed. Bound fragments were detected with polyclonal plasminogen antiserum and HRP-conjugated rabbit anti-goat. The mean values of three independent experiments and SD are presented. Statistical significance of differences was estimated using Student t test. *p < 0.05, **p < 0.01, ***p < 0.001. (D) Plasminogen bound to Lpd was treated with the activator uPA to generate plasmin, and degradation of the synthetic chromogenic substrate S-2251 was assayed photometrically. Lpd (2.5–20 μg/ml) was immobilized onto microtiter plates, and then plasminogen (8 μg/ml) was added. After extensive washing, the activator uPA was added together with the chromogenic substrate S-2251, and conversion of the chromogenic substrate was determined by measuring the absorbance at 405 nm. (E) Degradation of the natural substrate fibrinogen. The fibrinolytic activity of uPA-activated plasminogen bound to Lpd was evaluated. At the indicated time points (15, 30, 45, 60, and 120 min), the supernatants were separated by SDS-PAGE, proteins were transferred to a membrane, and degradation of fibrinogen was assayed by Western blotting using a rabbit fibrinogen antiserum and HRP-conjugated anti-rabbit IgG. When BSA, which does not bind plasminogen, was used as a matrix, fibrinogen remained intact and was not cleaved. Data shown are representative of three independent experiments.
and converted by the physiological activator uPA to plasmin, plasminogen bound to Lpd was treated with uPA, and the activity of plasmin was assayed. Lpd-bound plasminogen was converted to plasmin, which cleaved the chromogenic substrate S-2251 (Fig. 5D).

In addition, we tested whether plasmin bound to Lpd also cleaved the physiological substrate fibrinogen. Fibrinogen was degraded by newly formed plasmin, as revealed by the decrease in intensity of the two fibrinogen bands (64 and 58 kDa). Already after 30 min fibrinogen was degraded (Fig. 5E, lane 3, upper panel), and, after 1 h, fibrinogen was no longer detectable (Fig. 5E, lane 5, upper panel). Thus, Lpd-bound plasminogen is accessible for the activator uPA and is activated to plasmin, which cleaves both a synthetic and a natural substrate.

**Competition of CFHR1 and Factor H for binding to Lpd**

Both CFHR1 and Factor H bind Lpd via their conserved C terminus. We therefore asked whether the two proteins bind simultaneously to different sites of Lpd or whether they compete for binding. CFHR1 when used at increasing concentrations slightly reduced Factor H binding to Lpd at a molar ratio of 0.1:1–1:1 and was further reduced by 53% at a ratio of 4:1 (Fig. 6A). Similarly, in a reverse setting, Factor H slightly reduced CFHR1 binding to Lpd. At a 4-fold excess of Factor H, CFHR1 binding was reduced by 50% (Fig. 6B). The latter ratios exceed the physiological level of the two regulators in human plasma, where the molar ratio is 0.35:1 (CFHR1:Factor H). These results show that CFHR1 and Factor H bind simultaneously to bacterial Lpd at physiological plasma concentrations.

**Factor H and plasminogen bind simultaneously to Lpd**

As plasminogen and Factor H also bind to Lpd, we asked whether the two proteins bind simultaneously to Lpd and whether the two proteins compete for binding. Plasminogen bound dose dependently to immobilized Lpd and did not affect Factor H binding (Fig. 6C). Similarly, Factor H when used at increasing concentrations bound dose dependently to Lpd, and plasminogen binding was not affected (Fig. 6D). Thus, the two human plasma proteins plasminogen and Factor H bind independently and most likely to different regions of the Lpd protein. At physiological plasma levels (plasminogen:Factor H molar ratio of 1:1.5, indicated by the arrow), both Factor H and plasminogen bound to Lpd.

**Lpd binds equimolar amounts of Factor H, FHL-1, CFHR1, and plasminogen**

The four human regulators, Factor H, FHL-1, CFHR1, and plasminogen, bind to Lpd. To compare the intensity of binding, each human plasma protein was bound at equimolar amounts to immobilized Lpd. Factor H, FHL-1, and CFHR1 bound to Lpd with comparable intensity and plasminogen with a stronger intensity (Fig. 6D). As Factor H, FHL-1, and CFHR1 are detected with the same antiserum, it is concluded that the three human complement proteins bind with similar intensity to Lpd. In this setup, the intensity of plasminogen binding cannot be directly compared with the other plasma proteins as plasminogen was detected with a different antiserum.

**Lpd is a bacterial surface protein**

Surface biotinylation and also binding of human regulators suggested surface expression of *P. aeruginosa* Lpd. Therefore, we analyzed Lpd expression at the bacterial surface. First, Lpd was detected at the bacterial surface by flow cytometry (Fig. 7A). In addition, when Lpd was analyzed on the surface of intact bacteria by electron microscopy, the protein was evenly distributed over the surface (Fig. 7B). Next, expression levels of Lpd were compared for the...
Lpd is a surface-exposed protein of *P. aeruginosa* and contributes to bacterial serum resistance. (A) Surface expression of Lpd was identified by flow cytometry. Bacteria (*P. aeruginosa* strain SG137) were stained with Lpd antiserum, and bound Abs were detected with Alexa 488-labeled rabbit antiserum by flow cytometry (mean fluorescence intensity 1015 ± 330, compared with background mean fluorescence intensity 408 ± 82). (B) Lpd surface expression was confirmed by transmission electron microscopy. The *P. aeruginosa* strain SG137 was incubated with a gold-labeled Lpd antiserum and revealed an even distribution of Lpd on the bacterial surface. (C) Five laboratory strains of *P. aeruginosa* were analyzed for Lpd surface expression using a whole-cell ELISA. Intact bacteria were immobilized onto microtiter plates, and Lpd surface expression was detected with Lpd antiserum and HRP-conjugated goat anti-rabbit pAb. The mean values of three independent experiments and SD are presented. (D) Lpd is present in the outer membrane fractions of the five laboratory strains of *P. aeruginosa* as a 57-kDa band. A membrane fraction enriched for outer membrane proteins was prepared from the indicated *P. aeruginosa* strains, separated by SDS-PAGE, transferred to a membrane, and analyzed by Western blotting using the Lpd antiserum. A typical result of three independent experiments is shown. (E) Lpd contributes to survival of *P. aeruginosa*. Lpd on the surface of *P. aeruginosa* strain SG137 was blocked with Lpd antiserum. Then bacteria were challenged with NHS, and, after incubation, bacterial survival was assayed. Blockade of Lpd reduced the bacterial survival by 56% compared with the survival of SG137 in NHS (Fig. 7E). The effect was specific, as neither an unrelated antiserum nor purified rabbit IgGs affected bacterial survival. Thus, surface-exposed Lpd contributes to serum resistance of *P. aeruginosa*.

**Lpd sequence variation in clinical *P. aeruginosa* isolates**

To assay whether the sequence of the lpd gene in the different isolates is conserved or polymorphic, the lpd gene of all 13 clinical *P. aeruginosa* isolates and of the 5 laboratory strains was amplified and the sequence was determined. Sequence analysis revealed a total of 25 nucleotide exchanges among the 18 strains. Twenty-two nucleotide exchanges represented synonymous exchanges. The nonsynonymous exchange, which causes an exchange of Asn to Ser, was identified in the laboratory strain ATCC 27853 and in two clinical isolates (i.e., 9 and 11) (Fig. 8C). An Ala to Val exchange was identified in the laboratory strain SG137, and a His to Tyr in the clinical isolate 1 (Fig. 8C). Thus, the lpd gene of *P. aeruginosa* is rather conserved.

To assay whether the clinical isolates of *P. aeruginosa* express Lpd in the outer membrane, the 13 clinical *Pseudomonas* isolates were tested for the presence of Lpd in the OMP fractions. Lpd was detected in the OMP fraction of all 13 *P. aeruginosa* clinical isolates as a 57-kDa band (Fig. 8B). The secondary Ab alone did not crossreact with Lpd (Supplemental Fig. 1). However, when assaying Lpd levels by whole-cell ELISA, 11 clinical isolates expressed Lpd; however, the level of expression varied (Fig. 8C). Lpd expression was highest for isolate 1, followed by the clinical isolates 2, 5, and 4. Compared with isolate 1, Lpd levels among the 10 other clinical isolates ranged from 38 to 75%. Clinical isolates 10 and 13 did not express Lpd when analyzed by whole-cell ELISA. Thus, Lpd is expressed in the outer membrane of all tested *P. aeruginosa* strains, but surface expression varies between strains.

**Distribution of Factor H and CFHR1 on the bacterial surface**

Binding and surface distribution of the two human complement regulators Factor H and CFHR1 were evaluated by electron microscopy. Used as single proteins, Factor H (Fig. 9A1) and also CFHR1 (Fig. 9AII) bound to the surface of *P. aeruginosa*. Both proteins were distributed over the whole bacterial surface. When added together, the two proteins, labeled with either gold particles of 15 nm (Factor H) or 45 nm (CFHR1) bound in close vicinity to each other (Fig. 9AIII, black arrows), but also to distinct sites (Fig. 9AIII, white arrows). Thus, the two human proteins colocalize at the surface of *P. aeruginosa* and apparently bind to the same bacterial proteins.

**Bound to the surface of *P. aeruginosa*, the complement regulators Factor H, FHL-1, and CFHR1 protect against the complement attack**

We were interested to determine the contribution of all three human regulators for serum resistance. To assay whether the five laboratory *P. aeruginosa* strains varied in serum resistance and to determine the optimal serum concentration for bacterial survival in complement regulator-depleted serum, the five laboratory *P. aeruginosa*.
strains were challenged with increasing concentrations of NHS. The *P. aeruginosa* strains showed differences in serum resistance (Fig. 9B). Survival of the strains SG137, ATCC 27853, and NCTC 10662 was reduced dose dependently when the NHS concentration was increased. In contrast, PAO1 and AH377 survived in all tested NHS concentrations. Therefore, the various *P. aeruginosa* strains were cultivated in different concentrations of regulator-depleted human serum to obtain optimal results. To this end, all five laboratory strains were challenged with complement active human serum in which all three regulators were depleted, that is, HS\(_D\)FH/FHL-1/CFHR1 (Supplemental Fig. 2). After incubation, bacteria were recovered and survival was evaluated. In this case, survival of strain SG137 was reduced by 84% (Fig. 9C). Survival of the other strains was also reduced, but to a different extent. Survival of strain AH377 was reduced by 70%, strain ATCC 27853 by 62%, strain PAO1 by 37%, and strain NCTC 10662 by 34% (Fig. 9C). Addition of Factor H to the depleted serum reversed the effect, and survival of all three strains was increased (Fig. 9D). To confirm the protective effect of Factor H for the survival of *P. aeruginosa*, one of the Lpd binding sites within Factor H was blocked by preincubating strain SG137 with the C-terminal surface binding region SCRs 18–20 of Factor H. Then bacteria were challenged with NHS, and, after incubation, bacterial survival was assayed. Blockade of Factor H binding to *P. aeruginosa* by the C-terminal SCRs 18–20 reduced the bacterial survival by 62% (Fig. 9E). The effect was specific, as SCRs 8–11, which do not bind to *P. aeruginosa*, did not affect the bacterial survival. Thus, this demonstrates that the human complement regulators when bound to the bacterial surface protect the bacterium from the damaging effects of the activated complement system.

**Discussion**

In this study, we identify Lpd as a novel surface-exposed complement regulator-binding protein of the Gram-negative bacterium *P. aeruginosa*. Lpd binds Factor H and three additional human plasma proteins, that is, FHL-1, CFHR1, and plasminogen. The surface-bound complement inhibitors contribute to serum resistance, and bacterial survival was reduced when *P. aeruginosa* strain SG137 was challenged with complement active human serum depleted of Factor H/FHL-1/CFHR1. Lpd is a bacterial surface protein involved in serum resistance of *P. aeruginosa*. Thus, Lpd is a new virulence factor of *P. aeruginosa* that binds Factor H, FHL-1, CFHR1, and plasminogen.

Lpd is the second Factor H, FHL-1, CFHR1, and plasminogen-binding immune evasion protein identified from *P. aeruginosa*. Lpd, which has a predicted mass of 51.6 kDa, was initially identified as a cytoplasmic protein and as a component of the enzymatic pyruvate dehydrogenase complex, which catalyzes the electron transfer between pyridine nucleotides and disulfide components (32, 38, 39). Sequence analysis of the 5 laboratory strains and 13 clinical

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**FIGURE 8.** Clinical isolates of *P. aeruginosa* express Lpd. (A) Sequence variation of the lpd gene of *P. aeruginosa* was analyzed in the 5 *P. aeruginosa* strains and 13 clinical isolates. A total of 25 nucleotide exchanges was detected in the 1437-bp–long lpd gene. Twenty-two nucleotide exchanges and three nonsynonymous nucleotide exchanges were identified. The three nonsynonymous exchanges resulted in substitution of Asn\(^{97}\) to Ser, which was detected in the laboratory strain ATCC 27853 and in two clinical isolates (9 and 11); the Ala\(^{79}\) to Val exchange was identified in the laboratory strain SGI37; and the His\(^{106}\) to Tyr exchange in the clinical isolate 1. (B) Lpd is detected in the outer membrane fractions of all clinical isolates as a 57-kDa band. A fraction containing outer membrane proteins was prepared from the 13 clinical *P. aeruginosa* isolates, separated by SDS-PAGE, transferred to a membrane, and analyzed by Western blotting using the Lpd antiserum. A typical experiment of three is demonstrated. (C) Lpd expression was tested on the surface of 13 clinical *P. aeruginosa* isolates using a whole-cell ELISA. Intact bacteria were immobilized onto microtiter plates, and Lpd surface expression was detected with the Lpd antiserum and HRP-conjugated goat anti-rabbit pAb. The mean values of three independent experiments and SD are presented. Statistical significance of differences was determined by using Student t test. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.
were bound to and evaluated by electron microscopy. Factor H was directly labeled with gold particles of \( \Phi 15 \text{ nm} \), and CFHR1 with particles of \( \Phi 45 \text{ nm} \). The two proteins colocalize at the surface of \( \text{P. aeruginosa} \) strains SG137, ATCC 27853, and NCTC 10662 were incubated with HS \( \times 5 \) diluted in Mg-EGTA buffer. After incubation, the cells were plated on NB agar plates, and the number (CFU) of surviving bacteria was determined. Survival after 1 h is shown. (* \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \)).

The human complement regulator Factor H binds \textit{Pseudomonas} Lpd via two contact domains, that is, SCR 7 and SCRs 18–20, and FHL-1 binds via one region, that is, SCR 7. Consequently, when bound to Lpd, the complement-regulatory region (i.e., SCRs 1–4) of both human proteins is exposed to the outside and able to bind C3b and to regulate complement. Factor H and FHL-1 bound to Lpd maintained their complement-regulatory activity as cofactors for Factor I and contributed to C3b inactivation. This degradation of C3b can block C3b opsonization on the bacterial surface and consequently interfere with uptake and phagocytosis of the bacteria. In addition, Lpd-bound Factor H and FHL-1, by inactivating C3b, would impede formation of the C3 convertases, potentially blocking progression of the complement cascade and generation of the inflammatory anaphylatoxins C3a and C5a.

Acquisition of human regulators from serum is a common evasion strategy used by many pathogenic microbes. Factor H and FHL-1 are recruited by bacterial and fungal complement regulator-acquiring surface proteins and are attached via the same domains, that is, SCRs 6–7 and SCRs 18–20. Microbial proteins that bind Factor H and FHL-1 via these domains include Rck from \textit{Salmonella enterica} and Gpm1 and Pra1 from \textit{C. albicans} (23, 35, 44, 45). A second group of microbial surface proteins binds the two human regulators either via SCRs 6–7 (e.g., M proteins and Fba from \textit{Streptococcus pyogenes}, NspA from \textit{Neisseria meningitidis}, CRASP-1 from \textit{B. burgdorferi}) or via SCRs 18–20 (e.g., Sc11 from \textit{S. pyogenes} and Por1B from \textit{Neisseria gonorrhoeae}) of Factor H (30, 46–48). In addition, pathogenic microbes also bind the human terminal complement pathway regulator CFHR1. The emerging list of microbial CFHR1-binding proteins include CRASP-3, CRASP-4, CRASP-5 of \textit{B. burgdorferi}; Sbi of \textit{S. aureus}; and Sc11 of
S. pyogenes (30, 49, 50). In this study, we show that Lpd binds human CFHR1 and the major binding region is located within the C-terminal surface binding region SCRs 3–5. This region shows high level of sequence identity with SCRs 18–20 of Factor H (100, 101, and 97%) (10), thus explaining cooperative binding of the two human regulators to Lpd. Lpd is a multifunctional bacterial protein that, in addition to Factor H, FHL-1, and CFHR1, also binds plasminogen. Recruitment of plasminogen is used by many pathogens and is a strategy for tissue penetration. Several microbial proteins bind Factor H, FHL-1, and also plasminogen. These include Tuf from P. aeruginosa, CRASPs from B. burgdorferi, BpCA from Borrelia parkeri, and M protein from S. pyogenes (11, 22, 36, 51, 52). Plasminogen is composed of five kringle domains each of ~80 aa in size linked to a protease domain. This human plasma protein also binds fibrinogen and to other ECM proteins. Apparently, plasminogen is recruited by microbial proteins via different domains. Lpd attaches plasminogen via a major binding site localized in kringle domain 4. Streptococcal proteins bind Factor H, FHL-1, and CFHR1, also binds plasminogen. Recruitment of plasminogen to the bacterial surface alters the plasminogen/plasmin system by increasing its affinity to fibrin and degradation via a major binding site localized in kringle domain 4. Streptococcal surface protein PAM binds human plasminogen via kringle domain 2, and the Streptococcus canis protein SCM binds plasminogen via kringle domain 5 (53, 54). Thus, the three bacterial proteins bind to different kringle domains of plasminogen. Plasminogen bound to Lpd is accessible for the activator uPA, and, when converted to the active protease plasmin, cleaved the synthetic chromogenic substrate S-2251 and also the natural substrate fibrinogen. As plasmin degrades ECM components such as fibrinogen, laminin, fibronectin, and vitronectin (18), acquisition of plasminogen to the bacterial surface may facilitate tissue invasion and allow dissemination into deeper tissue layers. P. aeruginosa attaches the human regulators Factor H and CFHR1 simultaneously, and the two human proteins colocalize at the surface of intact bacteria. This binding profile suggests that, in addition to Lpd, P. aeruginosa uses additional surface proteins to bind Factor H and CFHR1. At present, the two Pseudomonas proteins Lpd and Tuf have been identified to bind Factor H, FHL-1, and CFHR1 (11). The relevance of surface-attached human complement regulators was determined by challenging intact bacteria with complement active, Factor H-, FHL-1-, and CFHR1-depleted human serum, and then survival of bacteria was evaluated. In this case, bacterial survival was reduced by up to 84%. Addition of Factor H to the depleted serum resulted in an increase in survival of P. aeruginosa. Thus, Factor H when attached to the surface protects the pathogenic bacterium from complement damage and contributes to serum resistance. The damaging effect of the depleted human serum was strain dependent and ranged from 84 to 34%. However, the survival rate did not directly correlate with Lpd surface levels. This suggests that P. aeruginosa uses additional bacterial complement regulator-binding proteins. Thus, Lpd of P. aeruginosa is a novel bacterial virulence factor expressed at the bacterial surface, which acquires human complement regulators and consequently protects the bacterium from complement attack. The elucidation of the mechanisms that P. aeruginosa uses to interact with the human host and identification of a novel P. aeruginosa virulence and immune evasion protein identify novel candidates for vaccine development.

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Disclosures

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