Human Complement Factor H Binds to Outer Membrane Protein Rck of Salmonella

Derek K. Ho, Hanna Jarva and Seppo Meri

J Immunol published online 9 July 2010
http://www.jimmunol.org/content/early/2010/07/09/jimmunol.1001244
Human Complement Factor H Binds to Outer Membrane Protein Rck of Salmonella

Derek K. Ho,* Hanna Jarva,*† and Seppo Meri*†

Serum resistance, or resistance to complement-mediated killing, is a key virulence property of microbial pathogens. Rck is a 17-kDa outer membrane protein encoded on the plasmid of Salmonella enterica serovars Typhimurium and Enteritidis. When expressed in either Escherichia coli or S. enterica Typhimurium, Rck confers serum resistance independent of LPS length. Recently, the Rck homolog from Yersinia enterocolitica, All, has been shown to bind the complement regulatory protein factor H (fH). Based on these observations, we hypothesized that Rck may also possess this ability. Using both flow cytometry and direct binding analysis, we demonstrate that Rck expressed in E. coli binds fH. We observed fH binding to Rck from human serum and also using the purified protein. Expression of Rck protected bacteria from alternative pathway-mediated killing and was associated with a reduction in C3b, Bb, and membrane attack complex deposition. fH bound to Rck promoted C3b cleavage in the presence of factor I. Binding was specific and mediated by two regions in fH, the short consensus repeats 5–7 and 19 to 20. These results suggest that fH recruitment by Rck is functional and can protect a normally serum-sensitive heterologous host against complement attack. Binding and exploitation of fH may thus contribute to Rck-mediated serum resistance. The Journal of Immunology, 2010, 185: 000–000.

T
o successfully infect the host, a pathogen must possess effective mechanisms for evading, resisting, or overcoming innate immune responses. A key effector arm of innate immune defense is complement. The complement system consists of >35 proteins found in serum and on cell surfaces. It plays a crucial role in immune defense against microorganisms as well as important roles in inflammatory processes, disposal of apoptotic cells and immune complexes, and enhancing adaptive immune responses (1). Upon recognition of a pathogen, complement is immediately activated by one or several routes: the classical pathway (CP), lectin pathway, or the alternative pathway (AP). All three pathways converge at the step of C3, and successful complement activation on the pathogen surface results in opsonization with C3b and its cleavage product iC3b. In the case of Gram-negative bacteria, direct lysis of the cell can occur via the membrane attack complex (MAC). Not surprisingly, resistance to complement-mediated killing and/or phagocytosis is a commonly recognized virulence trait of microbial pathogens (2).

Several serum resistance mechanisms have been described in the Gram-negative bacterial pathogen Salmonella. Long-chain LPS has been shown to confer resistance by promoting the deposition of complement components at a distance from the outer membrane, thus allowing the MAC to be shed from the bacterial surface without disrupting membrane integrity (3, 4). The PhoP-PhoQ-regulated gene pagC has been shown to confer resistance when expressed in both E. coli and S. enterica Choleraeaus by a currently uncharacterized mechanism (5). The surface protease PgtE, also expressed under PhoP-PhoQ control, mediates serum resistance presumably via its ability to cleave the key complement components C3, C4, and C5 (6). Two outer membrane proteins found on the 94-kb virulence plasmid of S. enterica Typhimurium and ENTERITIS are also associated with serum resistance. TraT, an outer membrane protein similar to those encoded by F-like plasmid conjugation systems, has been described to inhibit complement at the C6 step (7). Rck is a 17-kDa protein structurally related to PagC and has been shown to inhibit MAC function (8).

Rck was first described by Hackett and coworkers (9), who identified a 2.4-kb Cl–PstI fragment from a cosmid DNA library of a virulent strain of S. enterica Typhimurium. This fragment conferred high-level serum resistance when expressed in serum-sensitive E. coli K12 or a rough, virulence plasmid-cured strain of S. enterica Typhimurium. Serum resistance was associated with the production of a 17 kDa outer membrane protein (9). Heffernan and coworkers (10) have demonstrated that Rck appears to affect the formation and interaction of the MAC on the bacterial surface, as Rck-expressing cells are more sensitive to trypsin release of C9 and display fewer SDS-resistant C5b-9 complexes, suggesting disruption of MAC function (8). In addition to serum resistance, Rck expression in E. coli mediates adherence and invasion to cultured eukaryotic cell lines (10) as well as binding to laminin and fibronectin (11).

Rck belongs to a family of highly conserved 17–19-kDa outer membrane proteins including PagC, OmpX (E. coli and Enterobacter cloacae), and Ail (Yersinia) (12). All of these proteins have been shown to possess virulence-associated phenotypes. Based on structural models and the solved crystal structure of OmpX (13), these proteins are predicted to exhibit a common topology consisting of eight transmembrane amphipathic β-strands and four surface-exposed loops. Although significant homology has been observed between these proteins, the greatest sequence diversity is found in

*Infection Biology Program, Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki; and †HUSLAB, Helsinki University Central Hospital, Helsinki, Finland

Received for publication April 16, 2010. Accepted for publication June 2, 2010.

This work was supported by the Academy of Finland, the Sigrid Jusélius Foundation, Helsinki University Central Hospital Funds, and a U.S. Student Fulbright grant.

Address correspondence and reprint requests to Dr. Seppo Meri, Department of Bacteriology and Immunology, Haartman Institute, P.O. Box 21, Helsinki 00014, Finland. E-mail address: seppo.meri@helsinki.fi

Abbreviations used in this paper: ACL, acyl homoserine lactone; AP, alternative pathway; C3b, C3b-binding protein; CP, classical pathway; DPBS, Dulbecco’s PBS; fH, factor H; fI, factor I; GVB, Veronal-buffered saline supplemented with 0.1% gelatin; HIS, heat-inactivated serum; LB, Luria-Bertani; MAC, membrane attack complex; MFI, mean fluorescence intensity; NHS, normal human serum; SCR, short consensus repeat.

Copyright © 2010 by The American Association of Immunologists, Inc. 0022-1767/10S16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1001244
the surface exposed loops, which presumably mediate interactions with the host. This observation suggests that these proteins are functionally dissimilar. Nevertheless, Rck, Ail (14), and PagC (5) have been shown to confer resistance to complement-mediated killing, although it is not currently clear if these proteins do so by the same mechanism.

As complement activation can pose a significant risk to bystander host cells, it is necessary to maintain tight control over this system so that activation is restricted to the appropriate targets. Accordingly, the complement system contains numerous cell surface-bound and fluid-phase regulatory proteins that restrict complement at both the levels of activation and membrane attack (15). Not surprisingly, pathogenic microbes have evolved the ability to recruit and exploit fluid-phase complement regulatory proteins such as factor H (fH), C4b-binding protein (C4bp), and vitronectin (2, 16–18). The Rck homolog in Yersinia enterocolitica, Ail, has been shown to bind the key fluid-phase regulator of the AP, fH. Based on these observations, we tested the possibility that Rck also possesses fH-binding activity. We demonstrate in this study that Rck expressed in E. coli BL21 (DE3) can bind fH. This binding is associated with resistance to AP-mediated killing and reduced deposition of C3b, Bb, and MAC. These results show that Rck expression can confer AP resistance to a normally serum-sensitive heterologous host. Recruitment of fH may be an important mechanism of complement inhibition by Rck.

Materials and Methods
Bacterial plasmids, strains, and growth

Serum-sensitive E. coli strain BL21 (DE3) (Invitrogen, Carlsbad, CA) was used for all experiments. Bacteria were grown in Luria-Bertani (LB) broth cultures with shaking or on solid LB media at 37°C in room air. Plasmid pRck was used to express Rck (accession number NP_490501.1) in E. coli BL21 (DE3). This plasmid contains the rck gene PCR amplified from the virulence plasmid of S. enterica Typhimurium strain SL1344 and cloned into plasmid pBR322. Expression of Rck from this plasmid was achieved by culturing bacteria in the presence of ampicillin (100 μg/ml). pRck was a kind gift from Dr. Nobuhiko Okada (Kitasato University, Tokyo, Japan) and has been described previously (5).

Sera, proteins, and Abs

Normal human serum (NHS) was produced from blood collected from 7–10 healthy adult laboratory personnel with written informed consent and used anonymously. The study protocol has been approved by the Helsinki University Central Hospital Laboratory (TYH7214) (Helsinki, Finland). The blood was then allowed to clot, and the serum was subsequently harvested, pooled, and stored at −70°C until used. Heat-inactivated serum (HIS) was generated by incubating NHS for 1 h at 56°C. To generate NHS functional only in the AP, Mg2+ and EGTA were added to NHS at final concentrations of 9 mM and 5 mM, respectively. Purified human C3b, fH, and factor I (fI) were purchased from Calbiochem (San Diego, CA). BSA and heparin were purchased from Sigma-Aldrich (St. Louis, MO). Polyclonal rabbit anti-human C3c (Dako, Glostrup, Denmark), polyclonal goat anti-human factor B (Calbiochem), monomolecular mouse anti-human fH 196X (19), and monomolecular mouse anti-human SC5b-9 (Quidel, San Diego, CA) were used as primary and secondary Abs in flow cytometry experiments. The appropriate Alexa Fluor-labeled secondary Ab was purchased from Invitrogen. Polyclonal goat anti-human fH (Calbiochem) was used in Western blotting. HRP-conjugated secondary Abs were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

Outer membrane preparations

Outer membranes of overnight bacterial cultures were prepared as described by Cirillo et al. (20). Extracts were resolved by SDS-PAGE in 12% Tris-glycine gels under reducing conditions. Bands were subsequently visualized by staining with the GelCode Blue Safe Stain (Thermo Fisher Scientific, Rockford, IL).

Direct [125I]-fH binding assays

Bacteria were grown to stationary phase in 5 ml broth cultures overnight using disposable 16 × 125 mm tubes (BD Biosciences, San Jose, CA). Thereafter, they were washed and resuspended in Veronal-buffered saline supplemented with 0.1% gelatin (GVB) to a final concentration of 1 × 108 CFU/ml. A total of 20 μl of this solution was then incubated with 20 μl [125I]-fH (~2,000 cpm/sample) for 30 min at 37°C with agitation. Post-incubation, the samples were centrifuged through 250 μl 20% sucrose/GVB at 10,000 × g to separate free protein from protein bound to the bacteria. The supernatants and pellets were separated, and radioactivities were measured in a γ-counter. The ratio of bound to total radioactivity was then determined. Competition assays were performed by determining the relative binding of [125I]-fH in the presence of increasing amounts of unlabeled fH, heparin, BSA, or NaCl.

Flow cytometry

Bacteria grown as described above were centrifuged at 10,000 × g for 3 min and resuspended in Dubecco’s PBS (DPBS) to a final OD600 of 0.4 (4 × 109 CFU/ml). A total of 25 μl bacterial suspension was added to HIS or Mg2+-EGTA-NHS (final concentration specified for each experiment) or mixed with purified human fH in DPBS (final concentration 50 μg/ml) to a final volume of 50 μl. Samples were incubated at 37°C for the indicated times. The samples were removed, centrifuged, and washed three times in 50 μl DPBS supplemented with 1% BSA. After the final wash, bacteria were resuspended in 50 μl DPBS/1% BSA. A total of 20 μl 1:50 dilution of monoclonal mouse anti-human fH 196X or a 1:100 dilution of anti-factor B, anti-factor I, anti-human SC5b-9 (diluted in DPBS) was added to the bacteria and incubated at room temperature for 20 min. After washing in DPBS, bacteria were resuspended in 50 μl DPBS to which 20 μl of 1:100 dilution of the appropriate Alexa Fluor 488-conjugated secondary Ab was added, followed by incubation at room temperature in the dark for 20 min. The cells were washed twice as above and resuspended in 0.5 ml filtered DPBS containing 1% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA). Flow cytometric analysis was performed on an FACscan cytometer (BD Biosciences).

Serum bactericidal assay

Bacteria grown as described above were washed and resuspended in DPBS to a final concentration of 2 × 108 CFU/ml; 50 μl portions of the bacterial suspensions were added to 50 μl NHS, HIS, or Mg2+-EGTA-NHS and incubated for 30 min at 37°C. Postincubation, the samples were placed on ice to stop further bacteriolysis. Serial dilutions of the samples in DPBS were plated on LB agar plates and incubated overnight at 37°C in room air. Survival was determined by counting bacterial colonies the following day.

Cofactor activity for C3b cleavage

Bacteria grown as described above were resuspended to a final OD600 of 0.1 in DPBS (1 × 108 CFU/ml). The bacteria were then incubated with purified human fH at a final concentration of 0.1 μg/ml. Following a 30 min incubation at 37°C, the bacteria were washed five times in DPBS. After the last wash, bacteria were resuspended in DPBS and incubated with ~75,000 cpm of [125I]-C3b and fI (15 μg/ml). After a 1-h incubation at 37°C, the samples were centrifuged and the supernatants analyzed by SDS-PAGE under reducing conditions. The gels were subsequently dried and the results visualized by autoradiography.

Binding and elution of fH fragments from bacteria

Recombinant fH constructs containing short consensus repeats (SCRs) 1–5, 1–7, 5–7, 8–11, 11–15, 8–20, 15–20, and 19 to 20 were produced as described previously (21–23). Recombinant constructs 5–7 and 19 to 20 were kind gifts from Dr. Sakari Jokiranta, Markus Lehtinen, and Karita Haapasalo-Tuomainen (University of Helsinki, Helsinki, Finland). Bacteria grown as described above were resuspended to a final OD600 of 0.2 in DPBS (2.5 × 109 CFU/ml). The samples were incubated for 30 min at 37°C with agitation and washed three times with DPBS. Surface-bound proteins were eluted by resuspending the pellet in 50 μl 0.1 M glycine-HCl (pH 2.5) followed by incubation at 37°C for 20 min. Postcentrifugation, the supernatants were saved and neutralized by the addition of 1 ml 1 M Tris-HCl (pH 9.5). The neutralized supernatants were run on SDS-PAGE followed by Western blotting using a polyclonal goat anti-human fH Ab, which detects each fH construct.

Results

Rck is an fH-binding protein

Among Gram-negative pathogens, the recruitment of fH as a mechanism of defense against complement attack has been demonstrated for the pathogenic species of Neisseria (24), Haemophilus Salmonella Rck BINDS FACTOR H

Downloaded from http://www.jimmunol.org/ by guest on April 20, 2017
influenzae (25), Pseudomonas aeruginosa (26), Actinobacillus actinomycetemcomitans (27), and Yersinia enterocolitica (28). In the case of Y. enterocolitica, both the YadA and Ail proteins were shown to be receptors for fH (28). As Ail is homologous to Rck, we considered the possibility that Rck also possesses fH-binding ability. E. coli BL21 (DE3)-expressing Rck (Fig. 1A) was tested for fH binding in a direct binding assay using [125I] radiolabeled purified human fH. As shown in Fig. 1B, E. coli BL21 (DE3) expressing Rck bound fH, compared with the background strain, which did not exhibit binding. These results were confirmed using flow cytometry. As shown in Fig. 1C, E. coli BL21 (DE3) expressing Rck bound to both purified fH and fH from HIS. No binding was observed in bacteria expressing the empty vector pBR322 (data not shown). Taken together, these results demonstrate that Rck possesses fH-binding ability.

Rck mediates resistance to AP-mediated killing

Rck expression in both Salmonella and E. coli has been previously shown to mediate complete resistance to NHS (8). In these instances, all pathways of complement activation remained intact. As fH is the primary fluid-phase regulator of the AP, we decided to test whether fH recruitment by Rck could protect the bacteria specifically against AP attack. The presence of Ca2+ and Mg2+ are required for proper function of the CP/lectin pathway, whereas Mg2+ is required for the AP. Treatment of NHS with EGTA completely abolished fH binding. These results were confirmed using flow cytometry analysis on bacteria incubated with HIS or NHS and EGTA (final serum concentration 50%). Bacterial survival was enumerated by plating and counting colonies the following day. Data of an experiment performed at least three times are shown and expressed as means ± SD. In the absence of Rck, E. coli BL21 (DE3) is killed in 50% NHS and, to a lesser extent, in serum treated with Mg2+ EGTA. Bacteria expressing the empty vector pBR322 were killed to a similar extent (data not shown). In contrast, bacteria expressing Rck were able to survive in both NHS and Mg2+ EGTA serum, although in the former case to a lesser extent. These data indicate that Rck is able to confer protection against AP-mediated killing.

Rck inhibits AP-mediated C3b and MAC deposition

fH inhibits the AP by dissociating the catalytic subunit Bb from the C3 convertase by binding C3b, thus preventing interaction with factor B, and by serving as a cofactor for fI-mediated degradation of C3b (15). The ultimate effect of these regulatory functions is reduced C3b deposition on the target surface. Having shown that Rck confers resistance to AP-mediated killing, we next wanted to determine if fH recruitment by Rck resulted in reduced C3b deposition and subsequent inhibition of MAC. To test this possibility, we performed flow cytometry analysis on bacteria incubated with Mg2+EGTA-treated human serum and determined C3b and MAC deposition using anti-C3c and anti–SC5b-9 Abs, respectively. The C3c Ab recognizes both intact C3b and its degradation product, iC3b, whereas the SC5b-9 Ab recognizes a neoepitope generated in C9 during the formation of MAC. As shown in Fig. 3, less C3b (upper panels) and MAC (lower panels) deposition was observed on E. coli BL21 (DE3) expressing Rck when compared with the control lacking Rck. As we consistently observed increased background binding from HIS in the presence of Rck expression using the anti-C3c Ab (perhaps due to Ab cross-reactivity to Rck- or fH-mediated recruitment of C3b/C3b from HIS), we presented the data both as absolute mean fluorescence intensity (MFI) and as foldover background, which is the value obtained when the MFI from Mg2+EGTA-treated serum is divided by the corresponding value from HIS. In both cases, the data suggest that Rck expression results in reduced AP-mediated C3b deposition. Furthermore, our observation that MAC deposition is decreased in the presence of Rck is consistent with the observed increase in survival (Fig. 2) in Mg2+EGTA-treated human serum.

**Bb binding and cofactor activity**

To test if fH bound by Rck is in a functionally active form exposing C3b-binding sites, we measured both its effects on Bb deposition and cofactor activity for C3b cleavage. As shown in Fig. 4A, less Bb deposition was observed on the bacterial surface in the presence of Rck postincubation in Mg2+EGTA-treated serum, suggesting that fH bound to Rck can accelerate the decay of the AP C3

![FIGURE 1](http://www.jimmunol.org/)  
**FIGURE 1.** Rck is an fH-binding protein. A, Expression of Rck. Outer membrane preparations of BL21 (DE3) were resolved by SDS-PAGE and visualized by Coomassie staining. The Rck band is indicated by the arrowhead. Molecular weight markers (in kDa) are shown. B, Direct binding of [125I]-labeled fH to Rck. A total of 1 × 10^6 CFU/ml BL21 (DE3) and BL21 (DE3) pRck in GVB were incubated with [125I]-fH (~20,000 cpm/sample). The ratio of bound to total radioactivity was then determined (% bound). Data are expressed as means of three independent experiments ± SD. C, FACs analysis of fH binding. A total of 2 × 10^6 CFU/ml BL21 (DE3) and BL21 (DE3) pRck in DPBS were incubated with either 50% HIS (10 min) or 50 μg/ml purified fH (30 min). fH binding was measured by FACs using the anti-human fH mAb 196X. Representative data are shown.
convertase, and/or successfully compete with fB binding to C3b.

We next tested the ability of bound fH to act as a cofactor for fI-mediated cleavage of C3b. As shown in Fig. 4B, the incubation of purified C3b with both fH and fI led to the generation of three α-chain cleavage fragments of 67, 43, and 41 kDa. We observed these same cleavage products with *E. coli* BL21 (DE3)-expressing Rck after preincubation with fH, washing, and further treatment with fI. No cleavage was observed in the absence of Rck after a similar treatment. Omission of fH prevented the cleavage of C3b, suggesting that the bacteria do not possess intrinsic cofactor activity. Taken together, these results suggest that fH bound to Rck is functional in regulating the AP.

*fH*-binding competition assays

We further characterized the Rck–fH interaction by determining its sensitivity to competition by unlabeled fH, salt, heparin, and BSA. To determine the specificity of the interaction, we used either unlabeled fH or BSA as competitors to binding of radiolabeled fH. As shown in Fig. 5, unlabeled fH is able to compete with the radiolabeled protein in binding to Rck. No effect was observed using BSA. As full-length fH contains several heparin-binding sites (15), heparin was used to compete against fH binding to Rck. We observed that a high concentration of heparin (1 mg/ml) leads to a modest but statistically significant reduction in fH binding. No effect was observed using increasing NaCl concentrations, suggesting nonionic interactions between Rck and fH.

Mapping of Rck binding domains in fH

Recombinant constructs of fH were employed to determine which region(s) of fH were responsible for the interaction with Rck. Equimolar concentrations (0.33 μM) of fH constructs spanning SCRs 1–5, 1–7, 5–7, 8–20, 8–11, 11–15, 15–20, and 19 to 20 were incubated with *E. coli* BL21 (DE3)-expressing Rck followed by elution at low pH. The eluates were neutralized and analyzed by SDS-PAGE followed by Western blotting. As shown in Fig. 6, we observed binding between Rck and SCRs 1–7, 8–20, and 19 to 20, suggesting that fH contains a Rck binding site in both the N-terminal and C-terminal regions. Further investigation with smaller recombinant constructs indicates that the interaction between Rck and fH appears to be mediated primarily by SCRs 5–7 and 19 to 20. Relatively weaker binding was observed with SCRs 1–5 and 8–11.
The complement system provides a formidable first line of defense encountered by invading microbes. Accordingly, it is not surprising that a diverse range of pathogenic microbes possess the ability to acquire and exploit soluble complement regulatory proteins as a means of protection against complement attack. In this study, we present evidence that Rck of Salmonella Typhimurium and Salmonella Enteritidis, when expressed in mice preinfected with AHL-producing Y. enterocolitica. These investigators then constructed a S. enterica Typhimurium strain that constitutively expresses AHLs from Y. enterocolitica. In this background, they observed in a mouse coinfection that sdiA+ bacteria could rapidly outcompete sdiA− bacteria (36). Deletion of rck eliminated this competitive advantage, suggesting that Rck may be required for full virulence in vivo. However, as Rck has been shown to mediate other phenotypes such as adhesion and invasion (10), further investigation is required to determine if the rck deletion phenotype described above is due to loss of serum resistance.

We observed that bacteria expressing Rck appeared to proliferate during the course of the serum bactericidal assays in Mg2+-EGTA-treated serum, whereas incubation in NHS resulted in a modest loss in survival. In the absence of Rck expression, complete killing was observed in NHS, whereas some bacterial survival was observed in Mg2+-EGTA-treated serum (Fig. 3). If the AP alone were responsible for killing of bacteria, we would have expected to observe no difference in killing between Mg2+-EGTA-treated serum and NHS conditions. This suggests that under NHS conditions, the CP is activated on the bacterial surface and that Rck expression also protects against CP-mediated killing. In the context of CP activation, it is possible that Rck bound to Rck can attenuate AP amplification of the CP by preventing the ability of the AP to generate convertases from C3b deposited by the CP. Alternatively, resistance to CP-mediated killing may be due to the previously proposed ability of Rck to inhibit MAC (8), or Rck may possess the ability to bind other regulator proteins in addition to fH (see below). Equally plausible is the possibility that one or more of the mechanisms described above act synergistically.

The ability to bind several complement regulatory proteins has been reported in other Gram-negative pathogens. PorA (37) and GNA1870 (38) (or fH-binding protein) have been shown to be receptors for C4bp and fH in Neisseria meningitidis, respectively. In the case of C4bp, however, optimal binding was observed under hypotonic conditions (37). C4bp binding to both PorA and PorB (39) and fH binding to PorA (40) has also been observed in Neisseria gonorrhoeae. Binding of fH (25), C4bp (41), and vitronectin (17) has been demonstrated in H. influenzae. Protein E has been shown to be the receptor for vitronectin, whereas the receptor (s) for fH and C4bp are currently unknown. In Y. enterocolitica, the outer membrane proteins YadA and Ail are capable of binding both fH (28) and C4bp (42), although in the case of Ail, binding was observed only in the absence of the distal regions of fH. Whether Rck has the ability to bind to multiple complement regulatory proteins is currently not known and is under further investigation.

Heffner and colleagues (8) observed that Rck expression in Salmonella or E. coli conferred complete resistance to NHS in both smooth and rough strains. Furthermore, expression of Rck in smooth E. coli or rough Salmonella resulted in increased release of C9 following trypsin treatment compared with the background strain, suggesting that in both cases, the MAC complex is not properly inserted into the outer membrane. These results suggest that Rck may function in a similar manner in both Salmonella and E. coli. Nevertheless, it is important to emphasize that the results


