**Cell Signaling by the Type IV Pili of Pathogenic Neisseria***

Helena Källström§, Md. Shahidul Islam¶, Per-Olof Berggren§, and Ann-Beth Jonsson‡

*From the §Microbiology and Tumorbiology Center, Karolinska Institute, S-171 77, Stockholm, Sweden and ¶The Rolf Luft Center for Diabetes Research, Department of Molecular Medicine, Karolinska Institute, Karolinska Hospital, S-171 76 Stockholm, Sweden

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**Neisseria gonorrhoeae and Neisseria meningitidis** are Gram-negative bacterial pathogens that infect human mucosal epithelia. Type IV pilus-mediated adherence of these bacteria is a crucial early event for establishment of infection. In this work, we show that the type IV pili transduce a signal into the eucaryotic host cell. Purified adherent pili, but not pili from a low binding mutant, trigger an increase in the cytosolic free calcium ([Ca$^{2+}$])$_i$ in target epithelial cells, a signal known to control many cellular responses. The [Ca$^{2+}$]$_i$ increase was blocked by antibodies against CD46, a putative pilus receptor, suggesting a role for this protein in signal transduction. Pilus-mediated attachment was inhibited by depletion of host cell intracellular Ca$^{2+}$ stores but not by removal of extracellular Ca$^{2+}$. Further, kinase inhibition studies showed that pilus-mediated adherence is dependent on casein kinase II. In summary, these data reveal a novel function of the type IV pili, namely induction of signal transduction pathways in host cells.

**Neisseria gonorrhoeae**, the etiological agent of gonorrhea, and Neisseria meningitidis, which causes sepsis and/or meningitis, are two human-specific organisms. Bacterial adherence to epithelial cell surfaces plays an important role in the establishment of infection. Pili, or fimbriae, are assembled and expressed on the surface of many Gram-negative bacteria and have been shown to establish an important link in communication between the bacteria and the target cells.

Type IV pili of pathogenic Neisseria are essential during the initial stage of infection (1). Studies with human volunteers showed that non-piliated variants of *N. gonorrhoeae* are avirulent (2, 3). The pilus consists of a major pilus subunit protein, PilE, and a minor pilus-associated protein, PilC. The adherence to epithelial cells is dependent on expression of PilC, and on sequence variation in PilE (4–10). Most strains carry two pilC alleles, pilC1 and pilC2 (7, 11). In *N. gonorrhoeae* MS11 mutants expressing either PilC1 or PilC2 adhere equally well to epithelial cells, whereas in *N. meningitidis* strain FAM20 or strain 8013, PilC1+ pilC2– mutants, but not pilC1–, pilC2– mutants, adhere well to cells (8, 9, 11, 12). PilC has been suggested to be located at the tip of the pilus, and purified PilC inhibits adherence of both gonococci and meningococci (13).

However, PilC is also found in the bacterial membranes and is associated with the bacterial cell surface (11).

CD46 (membrane cofactor protein) acts as a eucaryotic receptor for gonococcal and meningococcal pili (14). CD46 is an abundant transmembrane glycoprotein involved in complement regulation on host cells and is expressed on virtually every human cell type except erythrocytes (15). Antibodies directed against CD46 as well as purified recombinant CD46 block binding of pathogenic *Neisseria* to target cells. Further, pilated, but not non-piliated, bacteria adhere to Chinese hamster ovary cells expressing human CD46 (14). It is likely that CD46, which is a human-specific protein, determines the host specificity of the pathogenic *Neisseria* species.

Colonization of epithelial cells by *N. gonorrhoeae* and *N. meningitidis* is followed by cellular invasion. The opacity proteins (Opa) are a family of invasion-associated outer membrane proteins that bind to CD66 (16–19) and heparan sulfate proteoglycan receptors on human cells (20, 21). The invasion of gonococci into HEC-1-B cells is enhanced by preincubation with fixed target cells, suggesting an induction of invasion-related functions upon contact with epithelial cells (22). It has also been shown that interaction between pilated and/or Opa-expressing *N. gonorrhoeae* and epithelial cells leads to activation of nuclear factor-κB, the activator protein 1, and production of inflammatory cytokines (23).

The mechanism behind bacterial signaling during adhesion and invasion has also been studied in enteropathogenic *Escherichia coli* (EPEC) and species of *Salmonella*, *Shigella*, and *Yersinia*. In these systems, entry into nonphagocytic cells involves induction of host signal transduction mechanisms (24). The pathogenic *Neisseria* colonize the mucosal epithelia, invade the target cells, and disseminate into the blood stream. The [Ca$^{2+}$]$_i$ in the extracellular space and in the blood are in the millimolar range. Within the eucaryotic cell, the [Ca$^{2+}$]$_i$ plays a central role in signal transduction. In a resting epithelial cell, the [Ca$^{2+}$]$_i$ is around 100 nM. High storages of Ca$^{2+}$ are kept in the endoplasmic reticulum (ER) and are released upon signals and/or receptor activation.

To better understand the mechanism(s) involved in the induction of the host cell response to neisserial attachment, we examined the role of Ca$^{2+}$ signaling in the interaction of these bacteria with epithelial cells. We provide the first evidence showing that neisserial pili stimulate a Ca$^{2+}$ signal in host cells. Type IV pili from an adhesive strain, but not pili from a low binding pilC mutant, trigger mobilization of cytosolic free Ca$^{2+}$ in target epithelial cells. The [Ca$^{2+}$]$_i$ transient is associated with the pilus, which is then a novel Ca$^{2+}$ signaling factor.

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¶ To whom correspondence should be addressed. Tel.: 46-8-728-71-74; Fax: 46-8-34-26-51; E-mail: Ann-Beth.Jonsson@mct.ki.se.

1 The abbreviations used are: EPEC, enteropathogenic *E. coli*; ER, endoplasmic reticulum; MBP, maltose binding protein; PKC, protein kinase C; PKG, protein kinase G; CaMK, calmodulin kinase; PKA, protein kinase A; MLCK, myosin light chain kinase; CK, casein kinase; IP$_3$, inositol 1,4,5-triphosphate; AM, acetoxymethyl ester; HBS, Hepes-buffered saline; cff, colony-forming units.
**EXPERIMENTAL PROCEDURES**

**Bacterial Strains—** *N. gonorrhoeae* MS11 mk(P1) and MS11 mk(P2), deleted in the 5’ end of *pilE*, have been described (3). The MS11mk strain used in our studies is designated MS11 mk(P1–5’ end) and is referred to in the text as MS11. Piliated (P1) and non-piliated (P2) variants were distinguished by colony morphology under a binocular microscope. FAM20 mutant strains FAM20.1 and FAM20.2 with mutations in *pilC1* and *pilC2*, respectively, have previously been described (11). The bacteria used did not express detectable levels of Opa, as detected by SDS-polyacrylamide gel electrophoresis of outer membrane preparations. Bacteria were grown on GCB-agar supplemented with Kellogg’s complement (2) at 37 °C in 5% CO2 atmosphere and passed every 18–20 h.

**Flux and Outer Membrane Preparations—** Preparation of pili and outer membranes were performed as described previously (7). The pil preparations used (1 mg/ml) were crystallized and solubilized three times and contain less than 1% of minor proteins detected in Coomassie Blue-stained gels (shown in Ref. 7). For detection of Opa, outer membranes were heated to 100 or 37 °C, subjected to 12% SDS-polyacrylamide gels and blotted to nitrocellulose membranes. Outer membranes were performed as described previously (7). The pili from MS11 were partially purified and used for imaging of ME180 cells in an ATP-generating system containing 10 mM phosphocreatine and 10 units/ml creatine phosphatase.

**Preparation of Defined Buffers—** Defined Ca2+ buffer, an excess of CaCl2 was added to one of the wells during chemical treatment or infection. The buffer used for permeabilization was prepared as described previously (11). The bacteria used did not express detectable levels of Opa, as detected by SDS-polyacrylamide gel electrophoresis of outer membrane preparations. Bacteria were grown on GCB-agar supplemented with Kellogg’s complement (2) at 37 °C in 5% CO2 atmosphere and passed every 18–20 h.

**Measurement of Intracellular Free Ca2+—** The cellular content of Ca2+ was measured using the fluorescent Ca2+ indicator fura-2. ME180 cells, grown on polystyrene plates for 2–3 days until each well contained about 10^5 cells, were cultured in plastic bottles. To make a defined Ca2+ buffer, an excess of CaCl2 was added to one of the wells during chemical treatment or infection. The buffer used for permeabilization was prepared as described previously (11). The bacteria used did not express detectable levels of Opa, as detected by SDS-polyacrylamide gel electrophoresis of outer membrane preparations. Bacteria were grown on GCB-agar supplemented with Kellogg’s complement (2) at 37 °C in 5% CO2 atmosphere and passed every 18–20 h.

**RESULTS**

**Adherent Type IV Pili Trigger a Cytosolic Free Ca2⁺ Transient in Target Cells—** The effects of highly purified pili of *N. gonorrhoeae* MS11 on Ca2⁺ signaling in ME180 epithelial cells were examined using a spectrofluorometric instrument. The pili were introduced with a flow rate of 0.02 ml/min. After 6 min of perfusion, the pili (20 μg/ml) induced a cytosolic free Ca2⁺ ([(Ca2⁺])i) increase from about 90 nM to 450 nM (Fig. 1A). The [(Ca2⁺)]i responses were not elicited by control buffer (HBS) or by outer membrane preparations of MS11(P2) (Fig. 1B).

**Antibodies Directed Against CD46 Block the [Ca2⁺]i Response—** We have previously shown that antibodies directed against the extracellular domain of CD46 block the Ca2⁺ transient triggered by pili (27). The CD46 antibody used in these studies was harvested from transgenic mice expressing human CD46 under the control of the RH promoter (27). For this purpose, murine B cells were infected with these transgenic mice and the antibody was purified from the cell supernatant. The antibody was used at a concentration of 1 μg/ml.

**Confluent Imaging—** ME180 cells were grown on glass chambers (LAB-TEK). Upon assay, 80 μM BAPTAVM (Calbiochem) was added to the cells and incubated at 37 °C for 30 min. The solution was removed and fresh medium was added followed by an additional incubation of 15 min. Bacteria were allowed to bind for 60 min. Bacteria were detected with antiserum against MS11 diluted 1:100 and goat anti-rabbit IgG-fluorescein isothiocyanate diluted 1:500 (Sigma). We used the MultiProbe 2001 CLSM confocal laser scanning system (Molecular Dynamics) equipped with a diapht 200 inverted microscope (Nikon). An extinction filter of 488 nm and the emission filter 510 EFLP was used. The images were visualized by a 60 X objective. The data were collected in a stack of 30 layers with a Z-stepsize of 1 μm. Each image was then further processed by Photoshop 4.0 (Adobe Systems).

**Permeabilization of Cells—** For the permeabilization of cells, nonconfluent layers of ME180 cells were washed 3 × 5 min in P-HBS. The cells were permeabilized with 0.5 μg/ml digitonin in P-HBS for 15 min at 37 °C (28). Trypan blue (0.01%) was added in a control well as an indicator of successful permeabilization. Binding assays were carried out in an ATP-generating system containing 10 mM phosphocreatine and 10 units/ml creatine phosphatase.

**Binding Assays—** The cells were grown in 24-well tissue culture plates for 2–3 days until each well contained about 10^5 cells. The monolayers were carefully washed three times in 500 μl of HBS with 1.5 mM MgCl2, 10 mM Hepes, and 5 mM D-glucose. The buffer used for permeabilization was P-HBS containing 1.5 mM free Ca2⁺.

**Kinase Inhibition Assays—** The used kinase inhibitors were staurosporine (Sigma), genistein (Sigma), H-89 (Calbiochem), and DRB (5,6-dichloro-1-beta-D-ribofurano-sylbenzimidazole, Calbiochem). IC50 values and used concentrations are shown in Table II. For the assay, nonconfluent layers of ME180 cells were preincubated with the inhibitor for 30 min at 37 °C prior to addition of bacteria. For adherence of bacteria in Ca2⁺ free extracellular medium, the cells were first preincubated for 15 min in 5 mM EGTA. The binding assay was carried out in EGTA containing HBS. As a control, 0.01% trypan blue was added to one of the wells during chemical treatment or infection. Only 1% of the ME180 cells were permeable for trypan blue, which is not more than what is seen for uninfected or untreated cells.
against CD46 block pilus-mediated adherence of pathogenic Neisseria to ME180 cells (14). Consequently, we tested whether antibodies directed against CD46 could block the Ca\(^{2+}\) signaling. As shown in Fig. 3, ME180 cells pretreated with polyclonal or monoclonal antibodies against CD46 did not respond with a [Ca\(^{2+}\)] transient when exposed to pili. Pili still triggered a [Ca\(^{2+}\)] transient in ME180 cells preincubated with monoclonal antibodies directed against CD55 (decay accelerating factor) (Fig. 3C) or normal rabbit serum (data not shown). Adherence of piliated Neisseria to ME180 cells is not blocked by CD55 antibodies (data not shown). These data suggest that the Ca\(^{2+}\) signal is transduced by the transmembrane cellular pilus receptor, CD46.

**Ca\(^{2+}\) Is Released from Intracellular Stores**—The induction of [Ca\(^{2+}\)] transients exclusively by pili from adherent Neisseria suggested that Ca\(^{2+}\) signaling was necessary for adherence. Accordingly, the cells were treated in several ways to inhibit the [Ca\(^{2+}\)] signals and then were exposed to bacteria in adherence assays. The [Ca\(^{2+}\)] transients in mammalian cells can be mediated by release of Ca\(^{2+}\) from intracellular stores and/or by open channels in the plasma membrane. Thapsigargin, an irreversible inhibitor of the ER Ca\(^{2+}\)-ATPase (29), induces release of intracellular Ca\(^{2+}\), resulting in depletion of the ER stores. Preincubation of ME180 cells with thapsigargin for 30 min blocked attachment of MS11 P\(^+\) (Fig. 4). Further, ME180 monolayers were pretreated with dantrolene, a drug that prevents release of Ca\(^{2+}\) from IP\(_3\)-sensitive stores (30, 31). As demonstrated in Fig. 4, the binding of MS11 P\(^+\) was reduced in dantrolene-treated cells. Spectrofluorometric [Ca\(^{2+}\)] measurements showed that both thapsigargin and dantrolene block the pilus-mediated rises in [Ca\(^{2+}\)] (data not shown). However, removal of external free Ca\(^{2+}\) with 5 mM EGTA did not affect adherence of MS11 P\(^-\) to ME180 cells, indicating that Ca\(^{2+}\) ions did not enter through channels in the plasma membrane (Fig. 4).

To further address the requirement for intracellular calcium elevation in bacterial attachment, the ME180 cells were preincubated with the membrane-permeable calcium chelator BAPTA/AM. This agent is trapped inside cells after complexing with membrane cholesterol and other unconjugated \(\beta\)-hydroxysterols (28). As shown in Fig. 5, A and B, chelation of the cytosolic free Ca\(^{2+}\) with BAPTA reduced the adhesion of MS11 P\(^+\) to the host cells. Taken together, our data argue that Ca\(^{2+}\) from intracellular stores is mobilized in response to binding of P\(^+\) bacteria.

**The Cytosolic Free [Ca\(^{2+}\)] of the Host Cell Affects MS11 P\(^+\) Adherence**—To examine the direct role of Ca\(^{2+}\) in pilus-mediated adherence, we used permeabilized ME180 cells and buffers with defined free [Ca\(^{2+}\)]. Chemical permeabilization with digitonin creates pores of 8–10 nm in the plasma membrane by complexing with membrane cholesterol and other unconjugated \(\beta\)-hydroxysterols (28). As shown in Fig. 5, C and D, show the adherence of MS11 P\(^+\) to permeabilized ME180 cells in P-HBS conditions.
taining 10 and 0 μM Ca\(^{2+}\), respectively. The adherence of MS11 P\(^+\) is directly dependent on the [Ca\(^{2+}\)] in the buffer. Further, Fig. 6 shows that the free intracellular [Ca\(^{2+}\)] clearly influences the adherence of MS11 P\(^+\) to cells in a dose-dependent manner. At cytosolic [Ca\(^{2+}\)] of 200 nM, the binding was low; however, at 800 nM the bacterial adherence was close to that observed with intact cells. These data argue that efficient binding of piliated Neisseria to target epithelial cells requires an elevated cytosolic free [Ca\(^{2+}\)].

Neisserial Adhesion Is Dependent on Casein Kinase II—We employed kinase inhibitors of varying substrate specificity to gain further insight into the host cell signaling pathway. The effects of the various kinase inhibitors upon bacterial attachment is summarized in Table II. Staurosporine, genistein, or BIMM I did not block adherence of MS11 P\(^+\) to ME180 cells. Staurosporine, a broad range inhibitor, affects protein kinase A (PKA), protein kinase C (PKC), protein kinase G (PKG), Ca\(^{2+}\)/calmodulin kinase (CaMK), and myosin light chain kinase (MLCK) (32). Genistein inhibits PKA, PKC, PKG, and tyrosine kinases (33). BIMM I inhibits PKC, PKG, and tyrosine kinases (34). In contrast, both H-89 (35) and DRB (36) reduced MS11 P\(^+\) adherence (Table II). H-89 blocked bacterial binding at concentrations known to inhibit casein kinase I (CK-I), casein kinase II (CK-II), CaMK, and MLCK. The possible involvement of CaMK and MLCK could be excluded, as staurosporine had no inhibitory effect. The highly specific CK-II inhibitor, DRB, clearly inhibited adherence of the bacteria, suggesting that CK-II takes part in the signal transduction event during pilus-dependent adherence. Finally, spectrofluorometric analysis showed that the pilus-induced Ca\(^{2+}\) peak occurred in ME180 cells pretreated with DRB (data not shown). Thus, the release of intracellular Ca\(^{2+}\) may be followed by the action of CK-II.
We suggest that the \([\text{Ca}^{2+}]\) transient induced by pili is needed as an initial step to establish a stable contact between the bacteria and the host cells. If the calcium-dependent signal is blocked, the bacteria will not form a secondary tight interaction with the host, and the interaction with the cells will be lost during the washing procedure. The \([\text{Ca}^{2+}]\) signal was detected in the epithelial cells after 6 min of perfusion. As the flow of pili into the chamber was 0.02 ml/min, there was a delay of at least 5 min until the concentration of pili in the chamber reached 20 \(\mu\text{g/ml}\). It is possible that the development of a successful signal transduction is concentration dependent and that the process may involve interactions with several domains of the pilus, or several pilus rods.

When using permeabilized cells, at least 800 nm \([\text{Ca}^{2+}]\) was needed to induce a strong pilus-mediated adherence. Therefore, it is likely that the pilus-induced \([\text{Ca}^{2+}]\) mobilization results in a local \([\text{Ca}^{2+}]\) significantly higher than the 450 nm peak detected, which was representing the total concentration within the whole cell. The \([\text{Ca}^{2+}]\), transient caused by pili was blocked by antibodies directed against the putative neisserial pilus receptor, CD46. Because the pilus receptor CD46 is a transmembrane protein, it may transfer a signal across the host cell membrane. Such signals may prime the host cells for bacterial uptake. CD55 (also called decay accelerating factor) shares homologies with the putative repetitive domains of the CD46 protein (15). CD55 has no transmembrane domain or cytoplasmic tail. Preincubation of the host cells with antibodies against CD55 did not interfere with the pilus-induced \([\text{Ca}^{2+}]\) signal. The exact pilus component responsible for the signal remains to be determined. However, because a PilC1 mutant failed to trigger \([\text{Ca}^{2+}]\) release, it is likely that the bacteria are, directly or indirectly, dependent on PilC1 for signaling. Whether the signal is mediated by PilC, PilE, or another pilus protein remains to be determined.

Pathogenic bacteria have developed various mechanisms to interact with host tissue. Many bacteria that cause disease have the capacity to enter into and survive within eucaryotic cells. Most mechanisms for this involve subversion and exploitation of host cell functions. Entry into nonphagocytic cells involves in many cases triggering of host signal transduction mechanisms to accomplish a bacterial uptake, \(i.e.,\) to induce rearrangements of the host cell cytoskeleton to stimulate protein synthesis or phosphorylation of host cell proteins. \(Salmonella\ typhimurium\) and EPEC are known to elevate \([\text{Ca}^{2+}]\), in target cells. Upon attachment to epithelial cells, EPEC induces a signal transduction cascade involving host cell IP3 formation followed by \([\text{Ca}^{2+}]\), release from IP3-sensitive stores (37). However, the \([\text{Ca}^{2+}]\), of the cells was measured an hour or more after bacterial infection. Though extracellular \([\text{Ca}^{2+}]\) was removed, EPEC could still adhere to the cells, suggesting that the \([\text{Ca}^{2+}]\) was released from internal stores (38). Also \(S.\ typhi-

\text{murium}\) infection of cultured cells is accompanied by a marked increase in \([\text{Ca}^{2+}]\), (39, 40). The \([\text{Ca}^{2+}]\) rise did no longer occur in strains carrying mutations in genes responsible for invasion, and chelators of intracellular \([\text{Ca}^{2+}]\), but not extracellular \([\text{Ca}^{2+}]\), block the entry of \(S.\ typhimurium\) into cultured epithelial cells (41). Also, \(Trypanosoma\ cruzi\), an intracellular parasite that causes Chagas’ disease in humans, produces a soluble factor that induces rapid and repetitive \([\text{Ca}^{2+}]\), transients in host cells (42).

Kinase inhibitors are widely used in all kinds of combinations to evaluate the pathway in which a signal is transduced. By using kinase inhibitors with overlapping specificities, we show that PKC, PKA, PKG, CaMK, and MLCK are most likely not involved in pilus-mediated adhesion of \(Neisseria\) to epithelial ME180 cells, as staurosporine, genistein, or BIMM I af-

**Fig. 6. The free \([\text{Ca}^{2+}]\) affects adherence of \(N.\ gonorrhoeae\) to ME180 cells.** Bacteria, suspended in P-HBS, were added to permeabilized cells. After 60 min of infection, the cells were washed, treated with saponin, and plated onto GCB plates. Percentage of bacterial adherence was calculated as follows: 100 \(\times\) cfu per well/cfu per well for MS11 P+ in McCoy’s 5A medium using intact cells. The cfu per well for MS11 P+ was 5.5 \(\times\) 106. Shown are the average of three independent experiments and standard deviations.

**Table II**

| Adherence of \(N.\ gonorrhoeae\) MS11 P+ to ME180 cells preincubated with different kinase inhibitors |
|---|
| Inhibitor | Kinase | \( \text{IC}_{50} \) | Used concentration | Adherence |
|---|---|---|---|---|
| Control | — | — | — | 100 ± 11 |
| Staurosporine | CaMK | 0.02 | 100 | 92 ± 13 |
| | MLCK | 0.013 | 100 | 92 ± 13 |
| | PKA | 0.007 | 100 | 92 ± 13 |
| | PKC | 0.007 | 100 | 92 ± 13 |
| | PKG | 0.085 | 100 | 92 ± 13 |
| Genistein | PKA | 100 | 250 | 87 ± 106 |
| | PKC | 100 | 250 | 87 ± 106 |
| | PKR | 100 | 250 | 87 ± 106 |
| | PKG | 100 | 250 | 87 ± 106 |
| BIMM I* | PKC | 0.01 | 1 | 75 ± 416 |
| | PKR | 2.0 | 10 | 101 ± 44 |
| H-89 | PKA | 0.048 | 100 | 92 ± 13 |
| | PKG | 0.48 | 100 | 92 ± 13 |
| MLCK | 28.3 | 3 | 81 ± 186 |
| CaMK | 29.7 | 300 | 1.1 ± 0.5 |
| PKC | 31.7 | 300 | 1.1 ± 0.5 |
| CR-I | 38.3 | 300 | 1.1 ± 0.5 |
| CR-II | 137 | 300 | 1.1 ± 0.5 |

* BIMM I, bisindolylmaleimide I; DRB, 5,6-dichloro-1-b-β-ribofuranosyl-benzimidazole; tyr-P, broad range tyrosine kinases.
* The difference between this value and the control is not statistically significant.

**DISCUSSION**

In this study, we characterize signal transduction events that occur in human epithelial cells during pilus-dependent adherence of pathogenic \(Neisseria\). We show that cytosolic \([\text{Ca}^{2+}]\) elevations occur in the host cells as they are exposed to pili and that the signal is most likely transduced by the pilus receptor, CD46. The increased \([\text{Ca}^{2+}]\) is due to release from intracellular stores since the depletion of intracellular \([\text{Ca}^{2+}]\) with thapsigargin or treatment with dantrolene inhibited adherence of the \(\text{P}^+\) bacteria to host cells. In addition, removal of extracellular \([\text{Ca}^{2+}]\) by EGTA did not affect the binding. Thus, the ability of the cells to release \([\text{Ca}^{2+}]\) from intracellular stores must play an essential role in pilus-mediated attachment.

Pilus-mediated adherence is a rapid event, resulting in bacteria that are firmly attached to the host cell within 5–10 min.
fected the adherence. However, II-89 and DRB with distinct substrate profiles significantly reduced binding at concentrations known to block CK-II. Sequence analysis shows a possible threonine phosphorylation site for CK-II at the cytoplasmic tail of CD46 (43). Thus, it is tempting to speculate that these sites might be phosphorylated by CK-II upon neisserial attachment. If so, the pilus-mediated mobilization of intracellular Ca\(^{2+}\) is followed by the phosphorylation(s) event, as DRB did not inhibit the \([Ca^{2+}]\) transient.

Among other bacteria able to phosphorylate host cell proteins, *S. typhimurium* stimulates the epithelial growth factor receptor, initiating a signal transduction cascade resulting in the tyrosine phosphorylation and activation of the mitogen-activated protein kinase. In contrast to pathogenic *Neisseria*, the adherence of EPEC to host cells could be inhibited by staurosporine and genistein (44).

In summary, our data show that pili play a novel role as an inducer of signaling pathways in the target cells. The pilus-mediated adherence is an initial event that involves interaction with CD46. Additionally, the pili transduce a signal into the host cell, involving \([Ca^{2+}]\), mobilization and CK-II activity. The detailed pathways in host cell signaling and the possible threonine phosphorylation of the CD46 cytoplasmic tail upon neisserial attachment is currently under investigation.

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