CEACAM engagement by human pathogens enhances cell adhesion and counteracts bacteria-induced detachment of epithelial cells

Petra Muenzner,[1] Manfred Rohde,[2] Susanne Kneitz,[3] and Christof R. Hauck[1]

[1]Zentrum für Infektionsforschung, Universität Würzburg, 97070 Würzburg, Germany
[2]Gesellschaft für Biotechnologische Forschung, 38124 Braunschweig, Germany
[3]Institut für Klinische Biochemie und Pathobiochemie, Universität Würzburg, 97078 Würzburg, Germany

Exfoliation, which is the detachment of infected epithelial cells, is an innate defense mechanism to prevent bacterial colonization. Indeed, infection with *Neisseria gonorrhoeae* induced epithelial detachment from an extracellular matrix (ECM) substrate in vitro. Surprisingly, variants of *N. gonorrhoeae* that bind to human carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) failed to induce detachment and, instead, promoted enhanced host cell adhesion to the ECM. Microarray analysis revealed that CEACAM engagement by several human pathogens triggers expression of CD105. Blockage of CD105 expression by antisense oligonucleotides abolished infection-induced cell adhesion. The expression of full-length CD105 promoted cell adhesion to the ECM and was sufficient to prevent infection-induced detachment. The CD105-mediated increase in cell adhesion was dependent on the presence and function of integrin β1. CD105 expression did not elevate cellular integrin levels but caused a dramatic increase in the ECM-binding capacity of the cells, suggesting that CD105 affects integrin activity. The exploitation of CEACAMs to trigger CD105 expression and to counteract infection-induced cell detachment represents an intriguing adaptation of pathogens that are specialized to colonize the human mucosa.

Introduction

Colonization of mucosal surfaces, which is the initial key step in the pathogenesis of diverse bacterial pathogens, requires the attachment of microorganisms to host surface structures (Finlay and Falkow, 1997; Abraham et al., 1998). As a countermeasure, epithelial cells can undergo exfoliation, which is an innate defense mechanism that is characterized by detachment of the infected cell (Mulvey et al., 2000). Exfoliation occurs as a rapid cellular response to bacteria and effectively lowers the infectious load (Apicella et al., 1996; Mulvey et al., 1998, 2000). Also, in the case of *Neisseria gonorrhoeae*, which is the causative agent of gonorrhea and a paradigmatic colonizer of human mucosa, the exfoliation of infected epithelial cells has been documented in vitro and in vivo (Ward et al., 1974; Evans, 1977; Melly et al., 1981; Apicella et al., 1996; Mosleh et al., 1997). Although the nature of the signal that induces exfoliation in vivo is unknown, the detachment of epithelial cells can be induced by soluble Neisserial products such as lipo-oligosaccharide in organ cultures (Melly et al., 1981).

Despite host cell exfoliation, *N. gonorrhoeae* is highly successful in establishing itself on epithelial surfaces of the human body. Gonococci express an array of adhesive factors that together allow for multiple, independent host cell interactions (Dehio et al., 2000). Although the exquisite host adaptation of *N. gonorrhoeae* has restricted in vivo experimentation, a limited number of volunteer studies and extensive in vitro experiments have suggested an orchestrated sequence of events upon infection. In particular, these investigations have pointed to type IV pili, which are filamentous surface appendages of microorganisms, to establish initial bacterial contact with the mucosa (Dehio et al., 2000). Furthermore, the expression of another group of outer membrane proteins, the so-called colony opacity-associated (Opa) proteins, appears to mediate intimate binding of bacteria to host cells. Although Opa protein expression is subject to phase variation, the opaque phenotype dominates in vivo because mainly Opa-expressing variants are reisolated from volunteers even when nonopaque gonococci are used for infection (Swanson et al., 1988; Jerse et al., 1994).

Correspondence to Christof R. Hauck: christof.hauck@mail.uni-wuerzburg.de

Abbreviations used in this paper: CEACAM, carcinoembryonic antigen-related cell adhesion molecule; CS, calf serum; CV, crystal violet; MOI, multiplicity of infection; Opa, opacity associated; ZRP-1, zyxin-related protein 1.

The online version of this article contains supplemental material.

The Journal of Cell Biology, Vol. 170, No. 5, August 29, 2005 825–836
http://www.jcb.org/cgi/doi/10.1083/jcb.200412151

Work over the last decade has identified cellular receptors that are targeted by Opa proteins from *N. gonorrhoeae* and its close relative *Neisseria meningitidis*, which is a major cause of bacterial meningitis and is a common colonizer of nasopharyngeal mucosa (for review see Hauck and Meyer, 2003). Besides a few Opa proteins that recognize heparansulfate proteoglycans (Opa_{HSPG}), most gonococcal and meningococcal Opa proteins that have been characterized so far recognize members of the human carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family (Opa_{CEA}). Opa_{CEA}–CEACAM association involves two of the four extracellular loops of outer membrane–embedded Opa proteins and the unglycosylated face of the NH_2-terminal Ig variable-like domain of CEACAM1, CEACAM3, CEA (the product of the CEACAM5 gene), or CEACAM6 (Bos et al., 1999, 2002; Virji et al., 1999).

Epithelial cells express the transmembrane receptor CEACAM1 as well as glycosylphosphatidylinositol-linked CEA and CEACAM6, which usually localize to the apical membrane domain (Hammarstrom, 1999). The in vivo function of CEACAMs on epithelia is not clear, though some family members can mediate cell–cell adhesion in vitro (Benchimol et al., 1989). It is important to note that only a few CEACAM homologues are present in rodents, whereas the CEACAM family has expanded considerably during primate evolution and is still diversifying (Hammarstrom and Baranov, 2001; Zhou et al., 2001). As these glycoproteins are recognized by a variety of gram-negative bacteria that are found in association with human mucosa (Leusch et al., 1991; Sauter et al., 1993; Virji et al., 1996, 2000; Toleman et al., 2001; Hill and Virji, 2003), it has been speculated that CEACAMs regulate bacterial colonization; however, direct evidence for this hypothesis is lacking (Hammarstrom and Baranov, 2001).

In this study, we demonstrate that CEACAM engagement by bacterial pathogens triggers enhanced host cell adhesion to the ECM, thereby abrogating cell detachment, which is an essential component of the exfoliation response. Enhanced cell adhesion depends on de novo expression of CD105, a TGF-β1 receptor. Moreover, bacteria-triggered CD105 surface expression is necessary and sufficient to prevent detachment of infected cells by modulating integrin affinity. These results not only provide, for the first time, molecular insight into microbial strategies that counteract epithelial exfoliation but, in addition, help to explain the prevalence of CEACAM recognition amongst human pathogens.

Results

Opa_{CEA}-expressing gonococci induce enhanced epithelial cell adhesion

To analyze the interaction of *N. gonorrhoeae* with target tissues, we used the human cervix-derived epithelial cell line ME-180, which is an established model system that allows pilus- and Opa protein–mediated gonococcal adhesion (Rudel et al., 1992; Kupsch et al., 1993; Scheuerpflug et al., 1999). When confluent monolayers of ME-180 cells were infected for >8 h with piliated gonococci, the infected cells detached from the culture dish, whereas ME-180 cells that were infected with

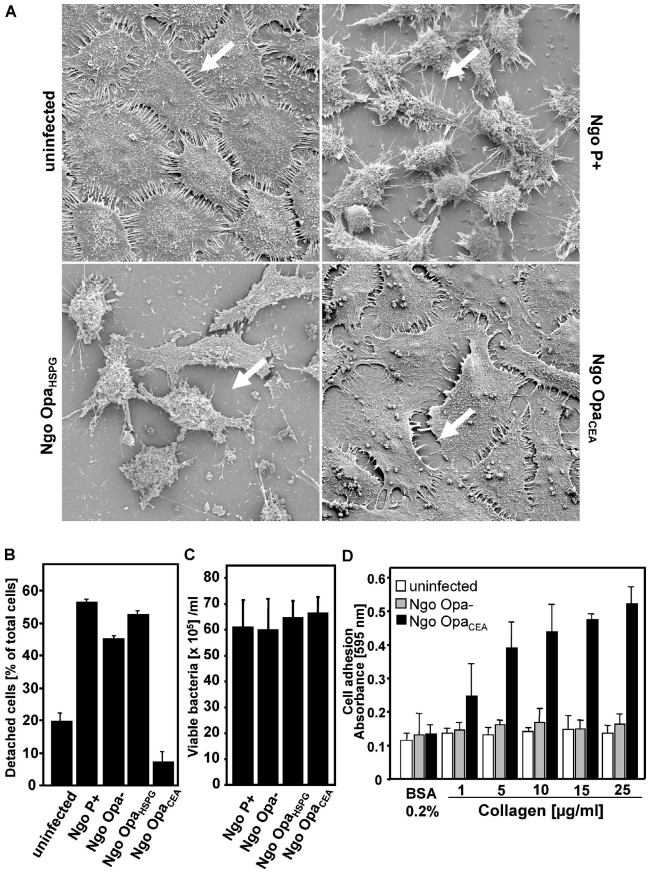

Figure 1. **Opa_{CEA}-expressing *N. gonorrhoeae* triggers enhanced epithelial cell adhesion.** (A) Confluent layers of ME-180 cells were left uninfected or were infected for 14 h with piliated, nonopaque (Ngo P+), nonpiliated Opa_{HSPG}-expressing (Ngo Opa_{HSPG}), or nonpiliated Opa_{CEA}-expressing *N. gonorrhoeae* MS11 (Ngo Opa_{CEA}). Cultures were fixed in situ and were analyzed by scanning EM. Arrows highlight cell–cell contacts. (B) Confluent layers of ME-180 cells were infected for 14 h with the indicated bacterial strains. Cells were used in detachment assays, were fixed and stained, and the percentage of detached cells was determined. (C) Aliquots of detachment assays performed as in B were plated after 14 h of infection onto GC agar plates, and the resulting bacterial colonies were counted. (D) ME-180 cells were infected with the indicated gonococcal variants or were left uninfected. After 8 h, cells were used in adhesion assays. Adherent cells were fixed and stained with CV. Error bars represent means ± SD of five wells (B) and four determinations (C) or represent mean staining intensity ± SD of five wells (D).

Opa_{CEA}-expressing gonococci did not detach (unpublished data). To investigate this cellular response in more detail, scanning EM was performed on monolayers of ME-180 cells that were infected for 14 h. In contrast to uninfected cells, cells that were infected with piliated gonococci or nonpiliated Opa_{HSPG}-expressing pathogens released cell–cell contacts and acquired a rounded morphology that was consistent with bacteria-induced cell detachment (Fig. 1 A). Strikingly, cells that were infected with CEACAM-binding gonococci remained attached throughout the 14 h of infection, although they showed reduced numbers of cell–cell contacts (Fig. 1 A, arrows). When the samples were inverted and centrifuged before fixation to remove loosely attached cells, ~45–60% of ME-180 cells that were infected with Opa_{HSPG}-expressing nonopaque, or piliated gonococci had detached (Fig. 1 B). Centrifugation also removed

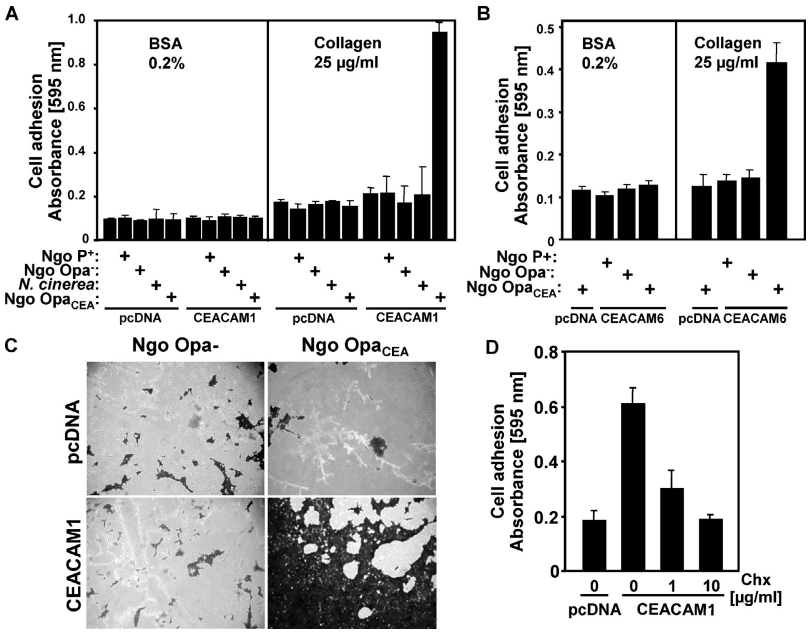

Figure 2. **Enhanced cell adhesion upon infection depends on host cell CEACAMs and de novo protein synthesis.** (A) 293T cells were transfected with control vector (pcDNA) or CEACAM1; were infected or left uninfected for 8 h with piliated *N. gonorrhoeae* (Ngo P+), nonopaque *N. gonorrhoeae* (Ngo Opa−), Opa$_{CEA}$ *N. gonorrhoeae* (Ngo Opa$_{CEA}$), or *N. cinerea;* and were used in an adhesion assay. (B) 293T cells were transfected with empty vector (pcDNA) or CEACAM6, were infected for 8 h with indicated bacteria, and were used in an adhesion assay. (C) 293T cells were transfected with control vector (pcDNA) or CEACAM1 and were infected for 14 h with indicated bacteria. Infected cultures were gently washed to remove loosely attached cells, and adherent cells were fixed and stained with CV. Pictures show representative fields of view. (D) 293T cells transfected as in C were infected for 8 h with Ngo Opa$_{CEA}$ in the presence of increasing amounts of cycloheximide (Chx) and were used in adhesion assays. (A, B, and D) Error bars represent means ± SD of five wells.

∼15–20% of ME-180 cells from uninfected cultures; however, cells infected with Opa$_{CEA}$-expressing gonococci almost completely stayed attached to collagen-coated culture dishes (Fig. 1 B). Importantly, all gonococcal variants exhibited the same growth properties in the cell culture medium (Fig. 1 C), suggesting that the lack of cell detachment after infection with Opa$_{CEA}$-expressing bacteria was not a result of differences in total bacterial load. As infection with Opa$_{CEA}$-expressing gonococci seemed to reduce the amount of cell–cell contacts (Fig. 1 A), we speculated that these bacteria stimulate increased cell matrix adhesion. Indeed, when ME-180 cells were used in an adhesion assay 8 h after infection, cells that were infected with Opa$_{CEA}$ gonococci showed dramatically enhanced cell adhesion to collagen compared with uninfected cells or with cells infected with nonopaque gonococci (Fig. 1 D).

Gonococcus-induced epithelial cell adhesion requires CEACAM-initiated signaling and host gene expression

To investigate whether Opa$_{CEA}$-mediated stimulation of epithelial CEACAMs was responsible for increased cell adhesion, we infected the CEACAM-negative human 293T cell line with Opa$_{CEA}$ gonococci (Ergun et al., 2000; Schmitter et al., 2004). Adhesion of these cells was unaltered by bacterial infection (Fig. 2 A). However, when 293T cells were transfected with cDNA encoding either CEACAM1 or 6, infection with Opa$_{CEA}$ gonococci led to increased cell adhesion on a collagen matrix (Fig. 2, A and B). In contrast, neither isogenic nonopaque or piliated gonococci nor nonpathogenic *Neisseria cinerea* induced enhanced adhesion of CEACAM-expressing cells. Clearly, CEACAM1 or 6 expression in the absence of bacterial infection did not lead to alterations in cell adhesion (Fig. 2, A and B). Also, cell adhesion to an uncoated cell culture dish was not affected by any of the aforementioned conditions (Fig. 2, A and B). Increased cell adhesion in response to Opa$_{CEA}$ *N. gonorrhoeae* correlated with the ability of CEACAM1-expressing 293T cells to withstand detachment after prolonged infection, whereas infection of these cells with nonopaque gonococci resulted in cell detachment (Fig. 2 C). Interestingly, the increase in 293T cell adhesion in response to Opa$_{CEA}$ bacteria was abolished when host cell kinases were inhibited by staurosporine (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200412151/DC1) or when protein translation in the eukaryotic cell was blocked by cycloheximide (Fig. 2 D). These results suggested that CEACAM engagement by Opa$_{CEA}$ proteins of pathogenic *N. gonorrhoeae* stimulates signaling and gene expression events in the host cell that lead to altered cell adhesion and prevent detachment of infected cells.

Bacterial engagement of CEACAMs stimulates expression of specific host cell genes

To identify factors that are responsible for enhanced host cell adhesion, we analyzed bacteria-triggered gene expression in CEACAM1- or 6-transfected cells by cDNA microarrays. RNA was isolated from CEACAM1- or 6-expressing 293T cells that were infected or left uninfected with Opa$_{CEA}$ *N. gonorrhoeae*. mRNA was transcribed in cDNA and labeled with Cy3 or Cy5, respectively, and the labeled samples were compared with each other as outlined in Fig. S2 (available at http://www.jcb.org/cgi/content/full/jcb.200412151/DC1). In total, the expression of 20 genes was consistently up-regulated >1.8-fold in response to CEACAM1 stimulation by Opa$_{CEA}$ gonococci, whereas the expression of 57 genes was enhanced 90 min after infection of CEACAM6-expressing cells. When the two datasets were combined, a common set of nine genes was identified to be up-regulated >1.8-fold upon bacterial CEACAM stimulation (Table I). Assuming that the factor responsible for enhanced cell adhesion is identical in both CEACAM1- and 6-expressing cells and that it is connected to

Table I. Gene expression events that are common to Opa_{CEA}-mediated engagement of CEACAM1 or 6

Gene	Description	-fold induction[a] CEACAM1	CEACAM6
CRIM	Cysteine-rich motor neuron	2.2 ± 0.1	4.6 ± 1.7
ENG	CD105 (endoglin)	1.9 ± 0.3	2.9 ± 0.5
EPHB3	EphB3	2.1 ± 0.3	3.0 ± 0.9
GIT1	G protein–coupled receptor kinase interactor 1	1.9 ± 0.3	2.6 ± 0.9
KIAA1321	KIAA1321 protein	2.2 ± 0.5	3.0 ± 1.2
KIAA0720	KIAA0720 protein	2.7 ± 0.6	2.2 ± 0.6
MMP17	Matrix metalloprotease 17	1.9 ± 0.3	2.5 ± 0.4
SQV7L	Nucleotide sugar transporter similar to *C. elegans* sqv7	2.3 ± 0.5	2.6 ± 0.4
TCEB1	Transcription elongation factor B (SIII) polypeptide 1	2.5 ± 0.1	3.3 ± 0.4

Gene expression analysis by cDNA microarrays was performed as described in Microarray analysis. Only genes that were found to be induced >1.8-fold upon bacterial stimulation of both CEACAM1 and 6 are listed (Fig. S2).
[a]Values represent means of -fold induction (compared with uninfected cells) ± SD of three comparisons performed in different dye configurations.

cell adhesion, we concentrated on CD105 (endoglin), which is a member of the TGF-β1 receptor family that has been linked to cell migration and organization of cell adhesion sites (Liu et al., 2002; Conley et al., 2004; Sanz-Rodriguez et al., 2004). CD105 mRNA was increased about two- to threefold in CEACAM1- or 6-expressing cells, respectively, 90 min after infection with Opa_{CEA} gonococci (Table I and Fig. S2).

CEACAM1 and 6 engagement stimulate CD105 expression

To verify the results of the microarray screen, semiquantitative RT-PCR was performed on RNA samples that were isolated from control- (pcDNA) or CEACAM1-transfected 293T cells before or after infection. CD105 mRNA levels were found to specifically increase about four- to sixfold 8 h after infection of CEACAM1-expressing cells with CEACAM-binding gonococci (Fig. 3 A). As an internal control, an ∼350-bp fragment of β-actin was coamplified. In addition, the same set of primers was used on a plasmid template containing full-length CD105 cDNA as a positive control (Fig. 3 A). Western blotting of lysates from transfected 293T cells confirmed the expression of CEACAM1 (Fig. 3 B). When ME-180 cervix carcinoma cells that endogenously express CEACAM1 (Fig. 3 B) were infected with Opa_{CEA}-expressing gonococci, a similar up-regulation of CD105 mRNA was observed (Fig. 3 A). The up-regulation of CD105 was also observed after the infection of CEACAM6-expressing cells with Opa_{CEA} gonococci, which is consistent with the microarray analysis (Fig. 3 D). CEACAM6 is anchored to the plasma membrane via a glycosylphosphatidylinositol moiety, suggesting that a cytoplasmic domain is not required to initiate CD105 up-regulation. Indeed, deletion of the cytoplasmic domain of CEACAM1 did not alter its ability to induce an increase in CD105 mRNA in response to infection with Opa_{CEA} *N. gonorrhoeae* (Fig. 3 E). Together, these data corroborated the findings of the microarray analysis. Furthermore, the results suggested that, as reported for CEACAM1- and 6-mediated bacterial internalization (Billker et al., 2002), CEACAM-initiated CD105 expression does not require the presence of a cytoplasmic domain.

Diverse CEACAM-binding human pathogens trigger CD105 expression

To verify that CD105 mRNA up-regulation in CEACAM-expressing cells was a specific result of CEACAM engagement by pathogens, cells were infected with different bacterial strains. Importantly, the increase of CD105 mRNA in CEACAM1-positive cells depended on the correct phenotype of the bacteria, as piliated but nonopaque gonococci did not induce CD105 expression (Fig. 4 A). In addition to *N. gonorrhoeae* and the closely related *N. meningitidis* that use Opa proteins to engage CEACAMs, several other human-specific bacterial pathogens

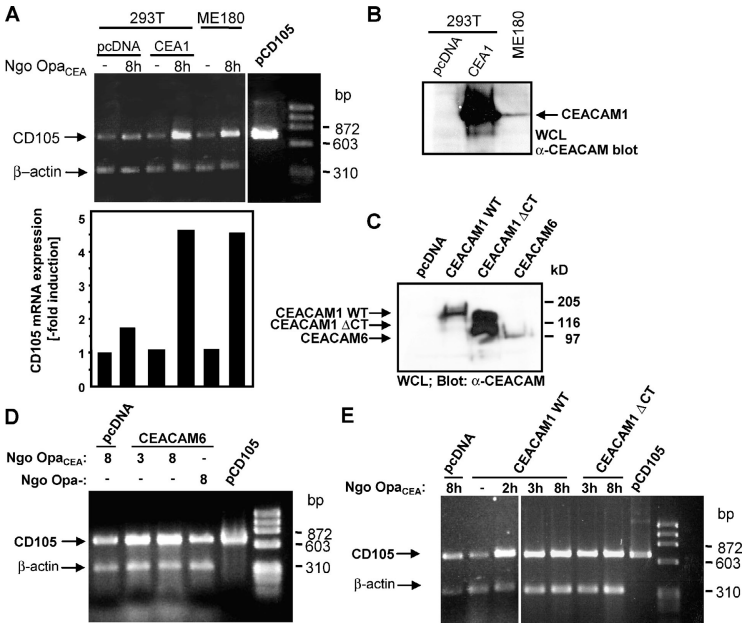

Figure 3. **CEACAM1 and 6 engagement stimulate CD105 expression.** (A) Control-transfected (pcDNA) or CEACAM1 (CEA1)-expressing 293T or ME-180 cells were left uninfected or were infected for 8 h with Opa_{CEA} *N. gonorrhoeae* (Ngo $Opa-_{CEA}$). After RNA isolation, fragments of CD105 and β-actin mRNA were coamplified by RT-PCR (top), and the ratio of both signals from each sample was determined by densitometry (bottom). Bars represent the fold induction of infected compared with uninfected cells. A plasmid encoding full-length CD105 served as a positive control (pCD105). (B) Expression of CEACAM1 by the cells used in A, as analyzed by Western blotting of whole cell lysates (WCL) with mAb α-CEACAM. (C) Expression of CEACAM1 WT, CEACAM1 ΔCT, and CEACAM6 by the cells used in D and E (analyzed as in B). (D) Transfected 293T cells were infected for the indicated times (in hours) with Ngo Opa_{CEA} or nonopaque gonococci (Ngo Opa−). CD105 mRNA levels were detected as in A. (E) Transfected 293T cells were infected for the indicated times with Ngo Opa_{CEA}. CD105 mRNA levels were detected as in A.

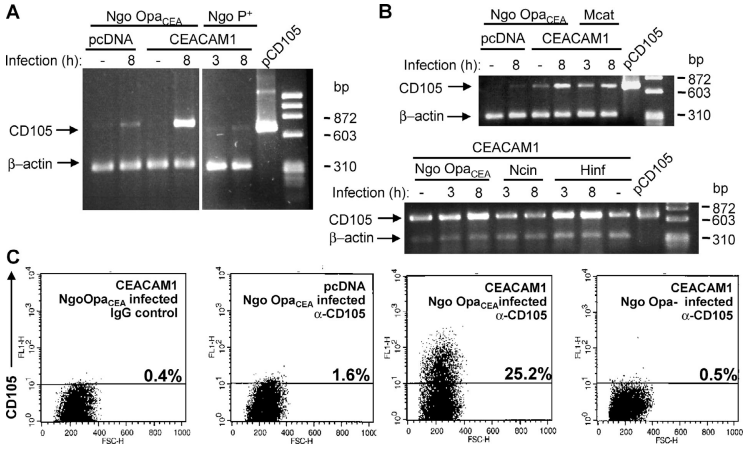

Figure 4. **CEACAM engagement by human pathogens triggers up-regulation of CD105 mRNA and protein levels.** (A) Control-transfected (pcDNA) or CEACAM1-expressing 293T cells were left uninfected or were infected with Opa$_{CEA}$ *N. gonorrhoeae* (Ngo Opa$_{CEA}$) or piliated *N. gonorrhoeae* (Ngo P+) for the indicated times. After RNA isolation, fragments of CD105 and β-actin mRNA were coamplified by RT-PCR. A plasmid encoding full-length CD105 served as a positive control (pCD105). (B) 293T cells transfected as in A were left uninfected or were infected with Ngo Opa$_{CEA}$, *M. catarrhalis* (Mcat), *N. cinerea* (Ncin), or *H. influenzae* (Hinf) for the indicated times. CD105 mRNA levels were analyzed as in A. (C) 293T cells transfected as in A were left uninfected or were infected as indicated for 14 h. Cells were detached, stained with monoclonal α-CD105 or control mouse IgG (control), and analyzed by flow cytometry. FSC-H, forward scatter; FL1-H, intensity of the signal detected in fluorescence channel 1.

have been described to firmly bind to CEACAMs (Virji et al., 2000). Examples include *Haemophilus influenzae* and *Moraxella catarrhalis*, which target human CEACAMs by distinct adhesins (Hill et al., 2001; Hill and Virji, 2003). Therefore, CEACAM1-expressing 293T cells were infected with *H. influenzae*, *M. catarrhalis*, or nonpathogenic *N. cinerea* for 3 or 8 h, and CD105 mRNA levels were detected by RT-PCR. Importantly, both CEACAM-binding pathogens led to a marked increase in CD105 mRNA within 3–8 h after infection, whereas non-CEACAM–binding *N. cinerea* did not influence CD105 mRNA levels (Fig. 4 B). Enhanced levels of CD105 mRNA were directly correlated to the presence of detectable amounts of CD105 on the surface of CEACAM-expressing cells after infection with Opa$_{CEA}$ gonococci (Fig. 4 C). Infection of CEACAM-deficient cells with Opa$_{CEA}$ gonococci or infection of CEACAM1-expressing cells with nonopaque bacteria or Opa$_{HSPG}$-expressing gonococci did not result in elevated CD105 levels (Fig. 4 C and Fig. S3, available at http://www.jcb.org/cgi/content/full/jcb.200412151/DC1). Together, these data corroborated the findings of the microarray analysis and demonstrated that engagement of CEACAMs by several bacterial pathogens triggers characteristic gene expression events that lead to elevated levels of CD105 on human epithelial cells.

Suppression of CD105 up-regulation abrogates enhanced cell adhesion upon bacterial CEACAM engagement

To investigate whether the observed up-regulation of CD105 could be required for bacteria-induced cell adhesion, 293T cells were cotransfected with CEACAM1 cDNA, and an antisense oligonucleotide was directed against CD105. The antisense oligonucleotide was complementary to sequences spanning the AUG start codon on the CD105 mRNA and has been shown to specifically suppress CD105 expression in human vascular endothelial cells (Li et al., 2000). In contrast to untreated cells or to cells receiving a scrambled control oligonucleotide, CEACAM1-expressing 293T cells that were treated with the CD105 antisense oligonucleotide did not exhibit CD105 up-regulation upon infection with CEACAM-binding gonococci (Fig. 5 A and Fig. S4, available at http://www.jcb.org/cgi/content/full/jcb.200412151/DC1). Importantly, the blockage of CD105 up-regulation by antisense oligonucleotide treatment also abolished increased cell adhesion in response to Opa$_{CEA}$ gonococci in a dose-dependent manner (Fig. 5 B). The cotransfection of 293T cells with the scrambled control oligonucleotide had no effect on bacteria-induced cell

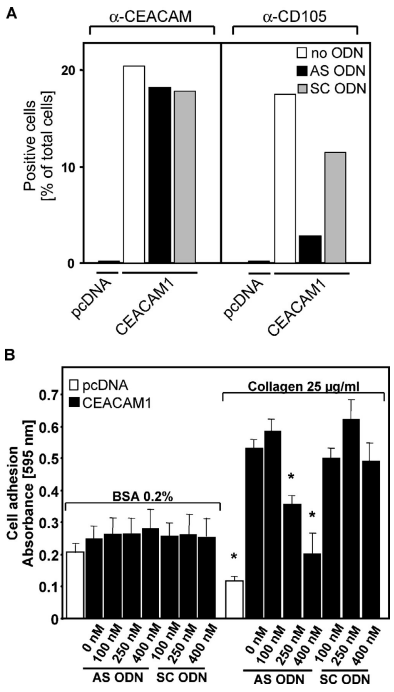

Figure 5. **CD105 up-regulation is necessary to mediate increased cell adhesion.** (A) 293T cells were cotransfected with the empty vector (pcDNA) or CEACAM1 and 400 nM of either CD105 antisense oligonucleotide (AS ODN) or a scrambled control oligonucleotide (SC ODN), respectively. After 8 h of infection with Ngo Opa$_{CEA}$, CEACAM1 and CD105 expression were determined by flow cytometry. Bars represent the percentage of CEACAM1- or CD105-positive cells of a representative experiment repeated three times with similar results. (B) 293T cells were treated as in A using the indicated amounts of antisense or scrambled control oligonucleotides, respectively. After 8 h of infection with Ngo Opa$_{CEA}$, cells were used in adhesion assays. Error bars represent means ± SD of eight samples. Statistical significance was determined by a two-tailed *t* test. Collagen-plated samples that differed significantly ($P < 0.001$) from the infected CEACAM1-expressing cells in the absence of oligonucleotides are marked by an asterisk.

adhesion (Fig. 5 B). These results suggested that CD105 upregulation is necessary to promote cell adhesion upon bacterial engagement of CEACAMs.

CD105 expression is sufficient to promote host cell adhesion and to prevent bacteria-induced cell detachment

To investigate whether enhanced expression of CD105 is sufficient to promote epithelial cell adhesion, 293T cells were transfected with cDNA encoding full-length human CD105, and their adhesive properties were compared with CEACAM1-transfected cells before and after infection with Opa_{CEA} gonococci. Strikingly, CD105 overexpression in 293T cells in the absence of bacterial infection led to increases in cell adhesion similar to those observed after infection of CEACAM-positive cells with Opa_{CEA} gonococci (Fig. 6 A). CD105 overexpression enhanced cell adhesion to collagen and also increased cell adhesion to gelatine, matrigel, and, to a lesser extent, fibronectin (Fig. S5 A, available at http://www.jcb.org/cgi/content/full/jcb.200412151/DC1). 293T cells overexpressing CD105 were resistant to bacteria-induced detachment even after prolonged infection with both CEACAM-binding and nonbinding microorganisms, which is consistent with a role for CD105 in the prevention of epithelial cell detachment (Fig. 6 B). Altogether, these results demonstrated that enhanced surface expression of CD105 is sufficient to mediate increased cell adhesion to ECM substrates and to prevent epithelial cell detachment after bacterial engagement of CEACAMs.

The COOH-terminal domain of CD105 is required to promote host cell adhesion and to prevent bacteria-induced cell detachment

Recently, several molecular connections between the cytoplasmic domain of CD105 and focal adhesion–associated molecules have been reported that allow CD105 to modulate cytoskeletal organization and focal adhesion composition (Conley et al., 2004; Sanz-Rodriguez et al., 2004). Therefore, we generated variants of CD105 with either a deletion of the complete 47–amino acid COOH-terminal cytoplasmic tail (CD105 ΔCT) or a deletion of the last 35 amino acids (CD105 Δ35). In contrast to 293T cells that were transfected with wild-type CD105 (CD105 WT), the expression of these CD105 variants did not result in elevated cell adhesion on collagen (Fig. 6 C), although they were expressed at similar levels compared with CD105 WT (Fig. S5 B). In addition, compromising the cytoplasmic domain of CD105 also abolished the ability of CD105-expressing cells to withstand bacteria-induced cell detachment (Fig. 6 D). This suggests that molecular connections of the CD105 COOH-terminal domain that influence focal adhesion composition are important to modify cell adhesive properties and to counteract cell detachment in response to bacterial infection.

CD105 requires the presence of β1 integrins to enhance cell adhesion

Because the enhanced cell adhesion that was triggered by CD105 was most pronounced on surfaces coated with ligands

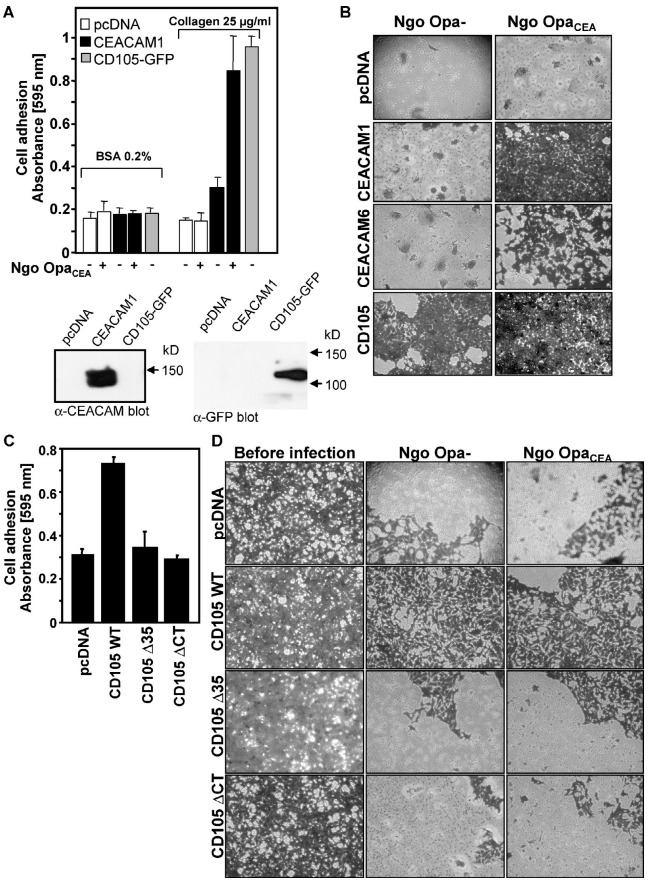

Figure 6. **CD105 is sufficient to promote cell adhesion and to prevent bacteria-induced cell detachment.** (A) 293T cells were transfected as indicated and were infected or left uninfected for 8 h with Opa_{CEA}-expressing *N. gonorrhoeae*. Cells were used in adhesion assays. Bottom panel depicts Western blots of whole cell lysates (WCL) from transfected cells. (B) 293T cells transfected with empty vector (pcDNA), CEACAM1, CEACAM6, or CD105-GFP were infected for 14 h with the indicated bacterial strains. Cells were used in a detachment assay before microphotographs were taken. (C) 293T cells were transfected with empty vector (pcDNA), CD105 WT, CD105 ΔCT, or CD105 Δ35 and were used in adhesion assays. Error bars represent means ± SD of five samples (A) or wells (C). (D) 293T cells transfected as in C were seeded in 24-well plates, and photographs were taken before infection. Cells were then infected for 14 h with the indicated bacterial strains and were used in a detachment assay before remaining cells were photographed.

for β1 integrins, we wondered whether this process involved integrins. Cell adhesion assays that were performed in the presence of monoclonal antibodies directed against either CD105, integrin β1, or the hyaluronate receptor CD44 demonstrated that interference with both CD105 or integrin β1 abolished enhanced cell adhesion after bacterial infection or CD105 expression (Fig. 7 A). In contrast, anti-CD44 antibodies or control mouse IgG had no influence on cell adhesion to collagen (Fig. 7 A). Importantly, CD105 expression did not lead to enhanced cell adhesion in cells that were genetically deficient for integrin β1, whereas reexpression of human integrin β1 restored the ability of these cells to display enhanced cell adhesion upon CD105 expression, demonstrating that integrin β1 is essential for this process (Fig. 7 B). Flow cytometry revealed that the levels of integrin β1 are not modulated by the presence of

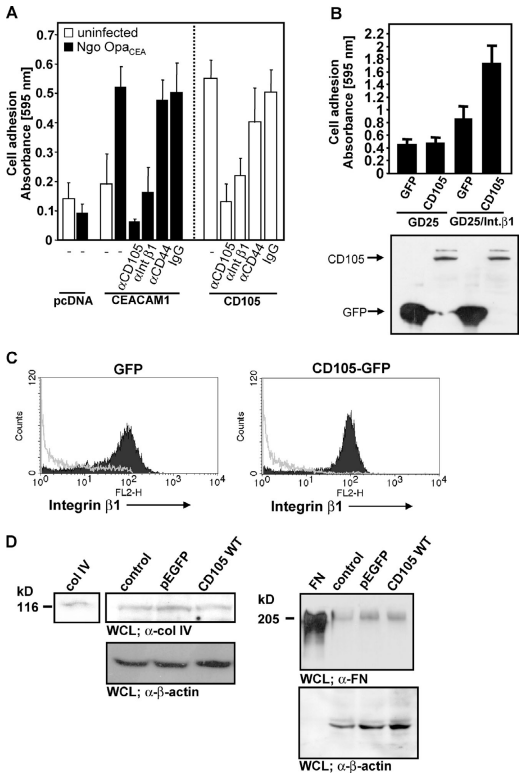

Figure 7. **CD105 cooperates with integrin β1 to mediate enhanced cell adhesion.** (A) 293T cells were transfected with control vector (pcDNA), CEACAM1, or CD105 WT; infected (black bars) or left uninfected (white bars) for 8 h with Ngo Opa$_{CEA}$; and used in adhesion assays in the absence or presence of mAbs against CD105, integrin β1 (αIntβ1), CD44, or with control mouse IgG. (B) Integrin-deficient fibroblasts (GD25 cells) or GD25 cells expressing human integrin β1 were transfected with CD105-GFP or the empty vector control (pEGFP) and were used in adhesion assays. Bars represent means ± SD of five (A) or eight wells (B). Bottom panel shows Western blotting of whole cell lysates with anti-GFP antibody. (C) 293T cells transfected as in B were stained with anti–integrin β1 antibodies (clone P5D2) and phycoerythrin (PE)-conjugated secondary antibodies (black). Controls (gray lines) were stained with isotype-matched control IgG. Upon gating of GFP-positive cells, phycoerythrin-derived fluorescence was measured in 10,000 transfected cells. A representative experiment that was repeated three times is shown. FL2-H, intensity of the fluorescence signal detected in channel 2. (D) 293T cells were transfected as in C or were left untransfected (control). 2 d later, whole cell lysates as well as purified collagen type IV (Col) and fibronectin (FN) as positive controls were analyzed by Western blotting with antibodies against either collagen type IV (top left) or fibronectin (top right). After stripping, the membrane was developed with anti–β-actin antibodies demonstrating equal loading (bottom).

CD105 (Fig. 7 C). Furthermore, CD105 expression did not alter the production of ECM proteins, such as collagen or fibronectin, by 293T cells (Fig. 7 D), suggesting that CD105 exerts its effect by altering the ligand-binding properties of integrin β1.

CD105 modulates integrin activity

A characteristic property of integrins is their ability to switch from a low to a high affinity state with respect to ligand binding in a process termed integrin activation (Hynes, 2002). Integrin activation can be promoted by signals from within the cell (inside-out signaling) by long-range conformational changes that reposition the ligand-binding site of the heterodimeric receptor and allow substrate binding (Xiao et al., 2004). To investigate whether CD105 expression has an influence on integrin activation, we incubated cells expressing GFP, CD105 WT, or CD105 ΔCT with microsphere beads coated with either BSA as a control or integrin β1 ligand collagen. After gentle washing, GFP-positive cells as well as cell-bound beads were visualized to allow an estimate of integrin activation on the single cell level (Fig. 8 A). Strikingly, there was a strong and selective binding of collagen-coated beads to CD105 WT–transfected cells. Untransfected, GFP-transfected, or CD105 ΔCT–expressing cells exhibited very little binding of collagen-coated beads that was indistinguishable from the low background binding of BSA control beads (Fig. 8 A). In addition, beads that were coated with an antibody directed against integrin β1 bound equally to all transfected as well as untransfected cells, demonstrating that integrin levels were not altered by CD105 expression and further indicating that CD105 influences integrin activity (Fig. 8 A). Quantification of the mean number of beads that were bound per cell corroborated the view that CD105 WT–expressing cells have a higher collagen-binding capacity as a result of increased integrin activity and not as a

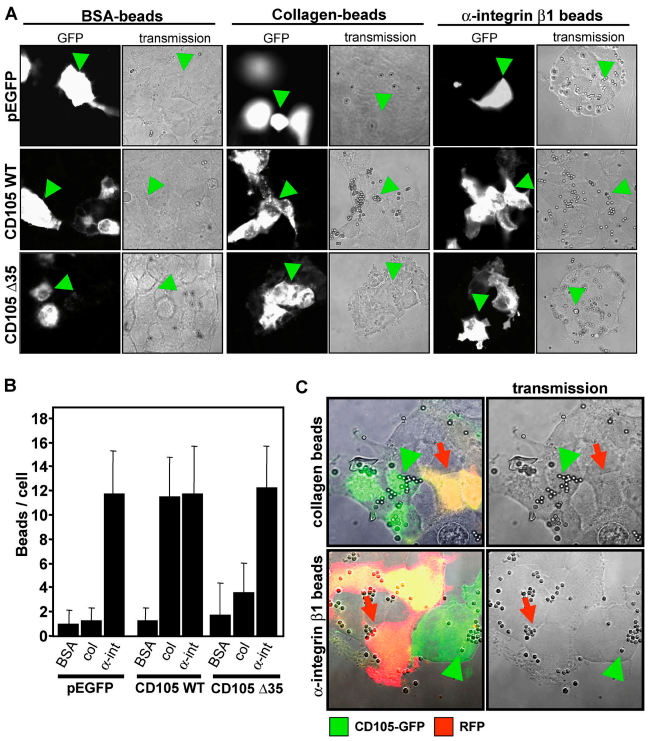

Figure 8. **CD105 expression results in enhanced integrin activity.** (A) 293T cells were transfected as indicated and seeded on glass coverslips. The cells were either incubated with collagen- (col), α–integrin β1- (α-int), or BSA-coated microspheres for 2 h at 37°C. Nonadherent beads were removed by washing with PBS, and the cells were fixed and analyzed by microscopy. Arrowheads indicate transfected cells. (B) Quantification of cell-associated beads from samples shown in A. Bars represent the mean number of beads per transfected cell ± SD observed in three independent experiments in which 60 transfected cells for each sample were counted. (C) 293T cells expressing either RFP (red arrows) or CD105-GFP (green arrowheads) were mixed and plated together on glass coverslips. Cells were incubated with collagen- or α–integrin β1 antibody–coated microspheres for 2 h, washed, fixed, and analyzed by microscopy. Shown are representative fields of view.

result of changes in integrin expression (Fig. 8 B). To finally demonstrate the cis-acting properties of CD105 on cell adhesion, cells expressing either RFP or CD105 WT–GFP were mixed, plated, and incubated with collagen-coated or anti–integrin β1-coated microspheres (Fig. 8 C). Whereas beads coated with the integrin β1 antibody bound equally well to both cells, the collagen-coated beads bound with high affinity almost exclusively to the CD105 WT–GFP-expressing cells (Fig. 8 C). Together, these results support the idea that CD105 positively influences integrin activation via its cytoplasmic domain, thereby increasing integrin affinity for ECM proteins and leading to enhanced cell adhesion.

Various CEACAM-binding bacteria enhance host cell adhesion

Up-regulation of CD105 via CEACAM engagement could be a general mechanism that is used by multiple human-specific pathogens to prevent epithelial cell detachment. To analyze whether other CEACAM-binding bacteria are able to trigger enhanced cell adhesion to collagen, 293T cells were transfected with CEACAM1 and were infected with a variety of gram-negative CEACAM-binding bacteria, including *N. meningitidis*, *H. influenzae*, and *M. catarrhalis*. As a positive control, cells were transfected with human CD105. As observed previously, the expression of human CD105 was sufficient to enhance 293T cell adhesion to collagen (Fig. 9). Plating of the infected cells onto collagen-coated surfaces again revealed a strong increase in cell adhesion in response to Opa_{CEA}-expressing, but not to nonopaque, gonococci (Fig. 9). Importantly, all CEACAM-binding microorganisms were able to significantly induce cell adhesion to collagen, whereas an attachment to uncoated cell culture surface was unaltered (Fig. 9). Together, these results demonstrate that a variety of CEACAM-binding bacteria is able to trigger enhanced epithelial cell adhesion to ECM proteins and suggest that this might be a common strategy to facilitate the colonization of epithelial cell layers of their human host.

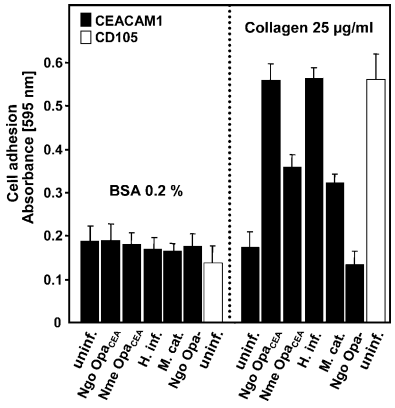

Figure 9. **Diverse CEACAM-binding human-specific microorganisms trigger enhanced cell adhesion.** 293T cells were transfected with CEACAM1 (black bars) or CD105-GFP (white bars). Cells were left uninfected or were infected for 8 h with Opa_{CEA} *N. gonorrhoeae* (Ngo Opa_{CEA}), Opa-expressing *N. meningitidis* (Nme Opa_{CEA}), *H. influenzae* (H. inf.), *M. catarrhalis* (M. cat.), or nonopaque *N. gonorrhoeae* (Ngo Opa−) and were used in adhesion assays. Error bars represent means ± SD of five wells.

Discussion

Interestingly, several gram-negative microbes such as *H. influenzae*, *N. gonorrhoeae*, *N. meningitidis*, or *M. catarrhalis*, which colonize human mucosal surfaces as their sole natural habitat, exploit members of the human CEACAM family (Hauck, 2002). Therefore, it has been suggested that CEACAM recognition might represent a specific adaptation of these bacteria to successfully occupy their narrow environmental niche (Virji et al., 2000; Hauck, 2002). Indeed, according to the results presented in this study, CEACAM engagement not only allows intimate attachment to host tissues but may also provide these microbes with means to blunt an innate defense mechanism of the mucosa (i.e., the exfoliation response of epithelial cells). In particular, we demonstrate that bacterial stimulation of CEACAMs triggers enhanced host cell adhesion to the ECM, thereby counteracting the infection-induced detachment of epithelial cells.

Microorganisms have elaborated an array of adhesive factors, allowing them to firmly attach to host surfaces (Finlay and Falkow, 1997; Abraham et al., 1998). However, stratified epithelial tissues can rapidly respond to bacterial attachment with accelerated renewal, including detachment of superficial cell layers as well as differentiation of basal and intermediate cells (Mulvey et al., 2000; Mysorekar et al., 2002). Infection of the mouse bladder with uropathogenic *Escherichia coli* has demonstrated that within 6 h after bacterial inoculation, detachment of bacteria-laden superficial epithelial cells can be detected in vivo (Mulvey et al., 1998). In the same time frame, urothelial gene expression is altered to reflect the microbe-induced renewal of the epithelial strata (Mysorekar et al., 2002). It is interesting to note that CD105 up-regulation and increased adhesion of epithelial cells after CEACAM stimulation can be observed within 3–8 h upon infection. This is consistent with the idea that the impaired detachment of epithelial cells colonized via CEACAMs could operate to effectively counteract the exfoliation response.

CD105 is a nonsignaling member of the TGF-β1 receptor family (type III receptor) that is known to modulate cellular responses to this growth factor (Lebrin et al., 2004). Although the role of CD105 in TGF-β1 signaling is far from understood (Fonsatti et al., 2001), CD105 has an essential function in establishing a well-differentiated vasculature (Li et al., 1999; Sorensen et al., 2003). Accordingly, mutations in CD105 are found in hereditary hemorrhagic telangiectasia type 1, which is a human disease characterized by vascular malformations (McAllister et al., 1994). In addition, an antiapoptotic function has been reported for CD105 in endothelial cells, where CD105 is induced upon hypoxia (Li et al., 2003; Zhu et al., 2003). However, we did not observe differences in apoptosis upon infection of CEACAM1-expressing cells with Opa_{CEA}-expressing gonococci compared with cells that were infected with nonopaque bacteria (unpublished data). Clearly, de novo CD105 expression in human epithelial cells has a positive influence on cell adhesion to the ECM in the absence of bacterial infection, further suggesting that the ability of CD105 to counteract the detachment of infected cells is not caused by an antiapoptotic effect.

Interestingly, the adhesion-promoting activity of CD105 mapped to the last 35 residues of the 47–amino acid-long cyto-

plasmic domain. These results are in accordance with recent investigations that have revealed a novel function for CD105 in modulating the actin cytoskeleton and cell migration (Liu et al., 2002; Conley et al., 2004; Sanz-Rodriguez et al., 2004). In particular, two recent studies have demonstrated that the cytoplasmic tail of CD105 interacts with the LIM (Lin-11, Isl-1, and Mec-3) domains of zyxin and ZRP-1 (zyxin-related protein 1), respectively, and the presence of CD105 redistributes zyxin and ZRP-1 from their regular localization at focal adhesion sites (Conley et al., 2004; Sanz-Rodriguez et al., 2004). Zyxin and ZRP-1 bind to members of the $p130^{CAS}$ family of adaptor molecules that, together with their binding partners FAK, c-Src, and c-Crk, play important roles in cell motility and are involved in the turnover of focal adhesion structures (Honda et al., 1998; Klemke et al., 1998; O'Neill et al., 2000; Yi et al., 2002). Importantly, CD105 expression not only disturbs the focal adhesion association of zyxin and ZRP-1 but also disturbs the subcellular distribution of $p130^{CAS}$ and c-Crk (Conley et al., 2004). These molecular interactions might allow CD105 to influence focal adhesion composition and, thereby, impact cell adhesion.

It is important to note that CD105-triggered adhesion was most pronounced on substrates encompassing integrin β1 ligands such as collagen type I or IV and laminin (the major constituents of matrigel; Hynes, 2002). Whereas CD105 expression had no influence on the expression of matrix proteins or on the surface expression levels of integrin β1, enhanced adhesion could be blocked by antibodies directed against this integrin. In addition, cells that were genetically deficient for integrin β1 showed no increase in cell adhesion upon CD105 expression. The affinity of integrins for their substrate can be altered by signals from within the cell (integrin activation) that modulate the association of focal adhesion proteins such as talin with integrin β1 cytoplasmic domains (Tadokoro et al., 2003). As CD105 expression did not affect the total amount of integrin β1 but affected the ligand-binding capabilities of integrins, we suggest that CD105 positively influences integrin activity by modulating the protein composition of integrin-dependent cell attachment sites, thereby influencing integrin activity by inside-out signaling.

The intriguing molecular cross talk that is revealed in this study sheds light onto an unexplored process in the establishment of infectious disease; namely, how bacterial pathogens overcome the exfoliation response of epithelial cells. A surprising, yet simple, solution to this challenge might be the pathogen-triggered modulation of host cell adhesion, as examplified in this study in the case of diverse CEACAM-binding human-specific microorganisms. In vivo, bacteria-triggered up-regulation of CD105 via CEACAM engagement and the resulting suppression of epithelial cell detachment could provide CEACAM-binding microorganisms with a selective advantage during initial colonization of the human mucosa. It is interesting to speculate that such an advantage could be the major driving force behind the convergent evolution of distinct CEACAM-binding adhesins in several gram-negative species of the genera *Neisseria*, *Haemophilus,* and *Moraxella* (Hill et al., 2001; Hauck and Meyer, 2003; Hill and Virji, 2003). Aside from bacterial colonization, the functional link between CEACAMs, CD105, and integrin activation could also play important roles in physiological settings such as angiogenesis (Ergun et al., 2000; Lebrin et al., 2004) and warrants further investigation.

Materials and methods

Cell culture and transfection

The human cervix carcinoma cell line ME-180 (American Type Culture Collection) and the embryonic kidney cell line 293T were cultured in DME containing 10% calf serum (CS). 14 h before adhesion assays or before infection, cells were serum starved with DME containing 0.5% CS. 293T cells were transfected by calcium phosphate coprecipitation using 5 μg of plasmid DNA for each 10-cm culture dish. In some experiments, cells were cotransfected with plasmid DNA and 0.1, 0.25, or 0.4 μM of either CD105 antisense oligonucleotide or a scrambled control oligonucleotide (Li et al., 2000). Integrin β1–deficient mouse embryo fibroblasts (GD25 cells) and GD25 cells reexpressing human β1 integrins were provided by R. Fässler (Max-Planck Institute for Biochemistry, Munich, Germany) and were cultured as previously described (Wennerberg et al., 1996). Fibroblasts were transfected using LipofectAMINE Plus (Invitrogen) according to manufacturer's instructions.

Bacteria and infection

The following strains were provided by T. Meyer (Max-Planck Institut für Infektionsbiologie, Berlin, Germany): Opa_{52}-expressing (Opa_{CEA}) nonpiliated *N. gonorrhoeae* MS11-B2.1 (strain N309); Opa_{50}-expressing (Opa_{HSPG}) nonpiliated *N. gonorrhoeae* MS11-B2.1 (strain N303); nonopaque, piliated gonococci (strain N280); nonpiliated, nonopaque gonococci (strain N302); and commensal *N. cinerea*. Opa-expressing nonencapsulated *N. meningitidis* (SiaD mutant of strain MC58) was obtained from M. Frosch (Institut für Hygiene und Mikrobiologie, Universität Würzburg, Germany). *M. catarrhalis* strain 11994 was obtained from DSMZ. *M. catarrhalis* and all *Neisseriae* species were grown on GC agar plates (Difco BRL) supplemented with vitamins at 5% CO_2 and 37°C and were subcultured daily. The unencapsulated variant of *H. influenzae* strain RD was obtained from A. Reidl (Zentrum für Infektionsforschung, Universität Würzburg, Germany). *H. influenzae* was grown on brain–heart infusion agar at 5% CO_2 37°C. For infection, bacteria were suspended in DME, the OD of the suspension was used to estimate the number of microorganisms, and bacteria were added to cells at the indicated multiplicity of infection (MOI).

Recombinant DNA constructs

Mammalian expression plasmids encoding cDNAs of human CEACAM1-4L (CEACAM1) and CEACAM6 were provided by W. Zimmermann (Universitätsklinikum Grosshadern, München, Germany). CEACAM1 ΔCT was constructed by PCR amplification with primers CEACAM1 sense (5′-GGGAAGCTTGCCATGGGGCACCTCTCAGCCCCACTTCAC-3′) and CEA1-HA-ΔCT antisense (5′-GGGGACGTCATAGGGATATTTCCCGAAATGCAGAAAACATGCCAGGGC-3′) and were cloned into the HindIII-AatII–digested plasmid pBluescript CEACAM3-HA (Schmitter et al., 2004) before further subcloning via HindIII-NotI into pcDNA3.1 Hygro (Invitrogen). Plasmid pDS red encoding RFP was from BD Biosciences. A cDNA clone encoding human CD105 (endoglin) was obtained from Deutsches Ressourcenzentrum für Genomforschung GmbH (RZPD; clone ID IMAGp958J07537QZ). Amplification of full-length CD105 was performed by using the primer pair EndoIF sense (5′-GAAGTTATCAGTCGATACCATGGACCGCGGCACGCTCCCTCTGGC-3′) and EndoIF antisense (5′-ATGGTCTAGAAAGCTTCCTGCCATGCTGCTGGTGGAGCAGGGGGTGC-3′). CD105 variants with deletions in the cytoplasmic domain were generated by amplification from the same template using the primer EndoIF sense combined with either EndoIF ΔCT antisense (5′-ATGGTCTAGAAAGCTTCCCCAGAGTGCAGCAGTGAGCAGGGC-3′) or EndoIF Δ35 antisense (5′-ATGGTCTAGAAAGCTTTCCCGCTTGCTGGGGGAACGCGTGTGCG-3′) to result in CD105 ΔCT or CD105 Δ35, respectively. The resulting PCR fragments were cloned into pDNR-Dual using the In-Fusion PCR Cloning Kit (BD Biosciences) and were transferred by Cre-mediated recombination into pLPS-3′ EGFP (BD Biosciences), resulting in GFP fused to the COOH terminus of the expressed proteins.

EM

ME-180 cells were seeded at 2.5×10^5 cells/well in 24-well plates on acid-washed glass coverslips that were coated with 25 μg/ml collagen. The next day, medium was replaced with DME and 0.5% CS for 8 h.

Then, cells were infected for 14 h at an MOI of 20 or were left uninfected. Samples were fixed in situ with 2% glutaraldehyde/3% formaldehyde in 0.1 M cacodylate, 0.09 M sucrose, 0.01 M $CaCl_2$, and 0.01 M $MgCl_2$, pH 6.9, for at least 1 h at 4°C. The samples were washed with 20 mM Tris and 1 mM EDTA, pH 7.0, and were dehydrated in a graded series of aceton on ice. After critical point drying from liquid CO_2, samples were sputter coated with 10-nm gold and examined at 5 kV of accelerating voltage in a field emission scanning electron microscope (model DSM982 Gemini; Carl Zeiss MicroImaging, Inc.) using Everhardt Thornley and inlense secondary electron detectors (Carl Zeiss MicroImaging, Inc.) in a 1:1 ratio. Images were digitally recorded and processed in Adobe Photoshop 6.

Detachment assay

ME-180 cells were seeded at 2×10^4 cells/well onto collagen-coated (16 h at 4°C) wells of 96-well plates. The next day, growth medium was replaced with DME containing 0.5% CS, and after 8 h, the confluent monolayers were infected or left uninfected for 14 h with the indicated bacteria at an MOI of 20. In some cases, aliquots of sample that were infected for 14 h were used for the determination of viable bacteria by dilution plating onto GC agar. After infection, plates were either fixed in situ or inverted and centrifuged for 5 min at 2,500 rpm to remove detached cells and medium before fixation with 4% PFA (20 min at RT). Samples were stained with 0.1% crystal violet in 0.1 M borate, pH 9.0 (CV stain). The staining intensity was measured in a microplate reader (Bio-Rad Laboratories) at 570 nm. The percentage of detached cells was determined by comparing samples fixed before and after centrifugation. 293T cells were seeded at 10^5 cells/well in a 24-well plate coated with 25 μg/ml collagen type I (in PBS for 16 h at 4°C) and infected with an MOI of 20 for 14 h. Cells were washed with PBS, fixed, stained with CV stain, and photographed.

Cell adhesion assay

The wells of 96-well plates were coated with 100 μl PBS containing the indicated concentrations of collagen (collagen type 1 from calf skin [ICN Biomedicals], gelatine [Merck], matrigel [BD Biosciences], and bovine fibronectin [ICN Biomedicals]) or 0.2% BSA, respectively, for 24 h at 4°C. Serum-starved cells were infected or left uninfected with the indicated bacteria at an MOI of 30 for 8 h in the absence or presence of cycloheximide or staurosporine (Calbiochem). Then, the cells were detached by limited trypsin/EDTA digestion that was stopped by the addition of 0.5 mg/ml of soybean trypsin inhibitor in DME. Detached cells were kept in suspension medium (DME and 0.2% BSA) for 1 h at 37°C and were replated at 2×10^4 cells/well onto protein-coated wells in replicates of five. Where indicated, 1 μg/ml mAbs against CD105 (clone P4A4; Developmental Studies Hybridoma Bank [DSHB], under the auspices of the National Institutes of Child Health and Human Development), integrin β1 (clone P5D2; DSHB), CD44 (clone H4C4; DSHB), or mouse IgG were present during replating. After 90 min at 37°C, nonadherent cells were removed by washing with PBS, adherent cells were fixed and stained with CV stain, and the staining intensity was measured.

Microarray analysis

Total RNA was isolated from pcDNA, CEACAM1, or CEACAM6-transfected 293T cells 90 min after infection with Opa_{CEA}-expressing *N. gonorrhoeae* or from the corresponding uninfected cells using the RNeasy kit (QIAGEN). 15 μg RNA was reverse transcribed into cDNA in the presence of fluorescently labeled nucleotides (Cy3 or Cy5) using the CyScribe First Strand cDNA kit (GE Healthcare). For each sample, reverse transcription and labeling was performed once in the presence of Cy3- and Cy5-labeled nucleotides. Labeled cDNA populations from an infected sample and the corresponding uninfected sample were hybridized in both dye configurations to custom-made cDNA microarrays using a Slide Pro station (Lucidea; GE Healthcare). The arrays contained PCR products with a mean size of 1,000 bp, which was derived from 4,500 mostly cancer-associated genes (generated from cDNA templates that were obtained from RZPD). A scheme of the different hybridizations is outlined in Fig. S2. After extensive washing, fluorescence of the Cy3- as well as the Cy5-labeled cDNA population that was hybridized to the slide was recorded with a ScanArray (model 4000; PerkinElmer), and 16-bit images for each fluorescence channel were generated. The resulting raw data were transformed into spot intensities by ScanAlyze 2 software (obtained from M. Eisen, University of California, Berkeley, Berkeley, CA). The data obtained for each hybridization experiment were normalized by using the whole set of measurements, and genes that were up-regulated >1.8-fold independently of the dye configuration in two repetitions of the experiment were regarded as induced by bacterial infection.

RT-PCR analysis

Total RNA was isolated from the indicated cells at different time points after infection by using the RNeasy kit and were further treated with RNase-free DNase-I (QIAGEN). 2 μg RNA were reverse transcribed into cDNA using SuperscriptIIRT and oligonucleotide (desoxythymidine) primers (Invitrogen). RNA that was used directly for PCR amplification did not yield any PCR product, indicating that signals were not caused by chromosomal DNA contamination. The amplification of a 640-bp fragment of human CD105 was performed using primers 5′-CGGTGGTAGGCTGCAGACCTCACC-3′ and 5′-CCTATGGACTTCCTGGTCTTGAGACC-3′ with 30 cycles of amplification and an annealing temperature of 58°C. Simultaneously, a 350-bp fragment of β-actin (primers β-actin sense, 5′-AGCGGGAAATCGTGCGTG-3′; and β-actin antisense, 5′-GGGTACATGGTGGTGCCG-3′) was coamplified and served as an internal control.

Flow cytometry and Western blotting

Analyses were performed as described previously (Schmitter et al., 2004) by using mAbs against CEACAM (clone D14HD11; Genovac), CD105 (clone P4A4; DSHB), fibronectin or β-actin (clone F 3648 or AC-74; Sigma-Aldrich), and GFP (clone JL-8; BD Biosciences), pAbs against collagen IV (H-243; Santa Cruz Biotechnology, Inc.), and rabbit pAbs against *N. gonorrhoeae* (gift of T.F. Meyer, Max Planck Institut für Infektionsbiologie). Secondary antibodies were obtained from Jackson ImmunoResearch Laboratories.

Covalent coupling of proteins to microspheres and microsphere-binding assay

1.2×10^8 polybead carboxylate microspheres (1 μm in diameter; Polysciences) were sonicated and covalently coated with either 1 mg/ml collagen type 1, 100 μg/ml mAb α–integrin β1 (P5D2; DSHB), or 4 mg/ml BSA for 2 h in coupling buffer (0.2 M $NaHCO_3$ and 0.5 M NaCl, pH 8.6). Unreacted binding sites were saturated with 20 mg/ml BSA in coupling buffer for 1 h before extensive washing with PBS. To determine bead attachment to cells, transfected 293T cells were seeded at 2.5×10^5 cells/well in 24-well plates on acid-washed glass coverslips and were incubated with 2.5×10^7 coated microspheres. After 2 h at 37°C, nonadherent beads were removed by washing with PBS, and the cells were fixed and analyzed by microscopy to determine the number of beads that bound to transfected cells.

Online supplemental material

Fig. S1 shows that bacteria-triggered cell adhesion is blocked by the kinase inhibitor staurosporine. Fig. S2 presents the general layout of microarray analysis and provides original data of hybridized microarray slides. Fig. S3 provides evidence that Opa_{HS}-expressing gonococci are unable to induce CD105 up-regulation, as analyzed by flow cytometry. Fig. S4 demonstrates the blockage of bacteria-triggered CD105 up-regulation by CD105-directed antisense oligonucleotides, as analyzed by flow cytometry. Fig. S5 shows that CD105 promotes cell adhesion to different ECM proteins and presents the expression of CD105 variants with COOH-terminal deletions in transfected 293T cells. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200412151/DC1.

We thank T.F. Meyer and M. Frosch for the *Neisseria* strains that were used in this study, W. Zimmermann for CEACAM cDNAs, R. Fässler for integrin β1–deficient cells, and D. Deininger for expert technical assistance.

This study was supported by funds from the Bundesministerium für Bildung und Forschung and Deutschen Forschungsgemeinschaft (grant Ha2568/3-1) to C.R. Hauck.

Submitted: 23 December 2004
Accepted: 20 July 2005

References

Abraham, S.N., A.B. Jonsson, and S. Normark. 1998. Fimbriae-mediated host-pathogen cross-talk. *Curr. Opin. Microbiol.* 1:75–81.

Apicella, M.A., M. Ketterer, F.K.N. Lee, D. Zhou, P.A. Rice, and M.S. Blake. 1996. The pathogenesis of gonococcal urethritis in men: confocal and immunoelectron microscopic analysis of urethral exsudates from men infected with *Neisseria gonorrhoeae*. *J. Infect. Dis.* 173:636–646.

Benchimol, S., A. Fuks, S. Jothy, N. Beauchemin, K. Shirota, and C.P. Stanners. 1989. Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. *Cell.* 57:327–334.

Billker, O., A. Popp, V. Brinkmann, G. Wenig, J. Schneider, E. Caron, and T.F. Meyer. 2002. Distinct mechanisms of internalization of *Neisseria gonorrhoeae* by members of the CEACAM receptor family involving Rac1- and Cdc42- dependent and -independent pathways. *EMBO J.* 21:560–571.

Bos, M.P., D. Hogan, and R.J. Belland. 1999. Homologue scanning mutagenesis reveals CD66 receptor residues required for neisserial Opa protein binding. *J. Exp. Med.* 190:331–340.

Bos, M.P., D. Kao, D.M. Hogan, C.C. Grant, and R.J. Belland. 2002. Carcinoembryonic antigen family receptor recognition by gonococcal Opa proteins requires distinct combinations of hypervariable Opa protein domains. *Infect. Immun.* 70:1715–1723.

Conley, B.A., R. Koleva, J.D. Smith, D. Kacer, D. Zhang, C. Bernabeu, and C.P. Vary. 2004. Endoglin controls cell migration and composition of focal adhesions: function of the cytosolic domain. *J. Biol. Chem.* 279: 27440–27449.

Dehio, C., S.D. Gray-Owen, and T.F. Meyer. 2000. Host cell invasion by pathogenic *Neisseriae*. *Subcell. Biochem.* 33:61–96.

Ergun, S., N. Kilik, G. Ziegeler, A. Hansen, P. Nollau, J. Gotze, J.H. Wurmbach, A. Horst, J. Weil, M. Fernando, and C. Wagener. 2000. CEA-related cell adhesion molecule 1: a potent angiogenic factor and a major effector of vascular endothelial growth factor. *Mol. Cell.* 5:311–320.

Evans, B.A. 1977. Ultrastructural study of cervical gonorrhea. *J. Infect. Dis.* 136:248–255.

Finlay, B.B., and S. Falkow. 1997. Common themes in microbial pathogenicity revisited. *Microbiol. Mol. Biol. Rev.* 61:136–169.

Fonsatti, E., L. Del Vecchio, M. Altomonte, L. Sigalotti, M.R. Nicotra, S. Coral, P.G. Natali, and M. Maio. 2001. Endoglin: an accessory component of the TGF-beta-binding receptor-complex with diagnostic, prognostic, and bioimmunotherapeutic potential in human malignancies. *J. Cell. Physiol.* 188:1–7.

Hammarstrom, S. 1999. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. *Semin. Cancer Biol.* 9:67–81.

Hammarstrom, S., and V. Baranov. 2001. Is there a role for CEA in innate immunity in the colon? *Trends Microbiol.* 9:119–125.

Hauck, C.R. 2002. Cell adhesion receptors - signaling capacity and exploitation by bacterial pathogens. *Med. Microbiol. Immunol. (Berl.).* 191:55–62.

Hauck, C.R., and T.F. Meyer. 2003. 'Small' talk: Opa proteins as mediators of *Neisseria*-host-cell communication. *Curr. Opin. Microbiol.* 6:43–49.

Hill, D.J., and M. Virji. 2003. A novel cell-binding mechanism of *Moraxella catarrhalis* ubiquitous surface protein UspA: specific targeting of the N-domain of carcinoembryonic antigen-related cell adhesion molecules by UspA1. *Mol. Microbiol.* 48:117–129.

Hill, D.J., M.A. Toleman, D.J. Evans, S. Villullas, L. Van Alphen, and M. Virji. 2001. The variable P5 proteins of typeable and non-typeable *Haemophilus influenzae* target human CEACAM1. *Mol. Microbiol.* 39:850–862.

Honda, H., H. Oda, T. Nakamoto, Z. Honda, R. Sakai, T. Suzuki, T. Saito, K. Nakamura, K. Nakao, T. Ishikawa, et al. 1998. Cardiovascular anomaly, impaired actin bundling and resistance to Src-induced transformation in mice lacking p130Cas. *Nat. Genet.* 19:361–365.

Hynes, R.O. 2002. Integrins: bidirectional, allosteric signaling machines. *Cell.* 110:673–687.

Jerse, A.E., M.S. Cohen, P.M. Drown, L.G. Whicker, S.F. Isbey, H.S. Seifert, and J.G. Cannon. 1994. Multiple gonococcal opacity proteins are expressed during experimental urethral infection in the male. *J. Exp. Med.* 179:911–920.

Klemke, R.L., J. Leng, R. Molander, P.C. Brooks, K. Vuori, and D.A. Cheresh. 1998. CAS/Crk coupling serves as a "molecular switch" for induction of cell migration. *J. Cell Biol.* 140:961–972.

Kupsch, E.-M., B. Knepper, T. Kuroki, I. Heuer, and T.F. Meyer. 1993. Variable opacity (Opa) outer membrane proteins account for the cell tropisms displayed by *Neisseria gonorrhoeae* for human leukocytes and epithelial cells. *EMBO J.* 12:641–650.

Lebrin, F., M.J. Goumans, L. Jonker, R.L. Carvalho, G. Valdimarsdottir, M. Thorikay, C. Mummery, H.M. Arthur, and P. ten Dijke. 2004. Endoglin promotes endothelial cell proliferation and TGF-beta/ALK1 signal transduction. *EMBO J.* 23:4018–4028.

Leusch, H.G., Z. Drzeniek, Z. Markos-Puztai, and C. Wagener. 1991. Binding of *Escherichia coli* and *Salmonella* strains to members of the carcinoembryonic antigen family: differential binding inhibition by aromatic glycosides of mannose. *Infect. Immun.* 59:2051–2057.

Li, C., I.N. Hampson, L. Hampson, P. Kumar, C. Bernabeu, and S. Kumar. 2000. CD105 antagonizes the inhibitory signaling of transforming growth factor beta1 on human vascular endothelial cells. *FASEB J.* 14:55–64.

Li, C., R. Issa, P. Kumar, I.N. Hampson, J.M. Lopez-Novoa, C. Bernabeu, and S. Kumar. 2003. CD105 prevents apoptosis in hypoxic endothelial cells. *J. Cell Sci.* 116:2677–2685.

Li, D.Y., L.K. Sorensen, B.S. Brooke, L.D. Urness, E.C. Davis, D.G. Taylor, B.B. Boak, and D.P. Wendel. 1999. Defective angiogenesis in mice lacking endoglin. *Science.* 284:1534–1537.

Liu, Y., B. Jovanovic, M. Pins, C. Lee, and R.C. Bergan. 2002. Over expression of endoglin in human prostate cancer suppresses cell detachment, migration and invasion. *Oncogene.* 21:8272–8281.

McAllister, K.A., K.M. Grogg, D.W. Johnson, C.J. Gallione, M.A. Baldwin, C.E. Jackson, E.A. Helmbold, D.S. Markel, W.C. McKinnon, J. Murrell, et al. 1994. Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. *Nat. Genet.* 8:345–351.

Melly, M.A., C.R. Gregg, and Z.A. McGee. 1981. Studies of toxicity of *Neisseria gonorrhoeae* for human fallopian tube mucosa. *J. Infect. Dis.* 143:423–431.

Mosleh, I.M., H.J. Boxberger, M.J. Sessler, and T.F. Meyer. 1997. Experimental infection of native human ureteral tissue with *Neisseria gonorrhoeae*: adhesion, invasion, intracellular fate, exocytosis, and passage through a stratified epithelium. *Infect. Immun.* 65:3391–3398.

Mulvey, M.A., Y.S. Lopez-Boado, C.L. Wilson, R. Roth, W.C. Parks, J. Heuser, and S.J. Hultgren. 1998. Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*. *Science.* 282:1494–1497.

Mulvey, M.A., J.D. Schilling, J.J. Martinez, and S.J. Hultgren. 2000. Bad bugs and beleaguered bladders: interplay between uropathogenic *Escherichia coli* and innate host defenses. *Proc. Natl. Acad. Sci. USA.* 97:8829–8835.

Mysorekar, I.U., M.A. Mulvey, S.J. Hultgren, and J.I. Gordon. 2002. Molecular regulation of urothelial renewal and host defenses during infection with uropathogenic *Escherichia coli*. *J. Biol. Chem.* 277:7412–7419.

O'Neill, G.M., S.J. Fashena, and E.A. Golemis. 2000. Integrin signalling: a new Cas(t) of characters enters the stage. *Trends Cell Biol.* 10:111–119.

Rudel, T., J.P.M. van Putten, C.P. Gibbs, R. Haas, and T.F. Meyer. 1992. Interaction of two variable proteins (PilE and PilC) required for pilus-mediated adherence of *Neisseria gonorrhoeae* to human epithelial cells. *Mol. Microbiol.* 6:3439–3450.

Sanz-Rodriguez, F., M. Guerrero-Esteo, L.M. Botella, D. Banville, C.P. Vary, and C. Bernabeu. 2004. Endoglin regulates cytoskeletal organization through binding to ZRP-1, a member of the LIM family of proteins. *J. Biol. Chem.* 279:32858–32868.

Sauter, S.L., S.M. Rutherfurd, C. Wagener, J.E. Shively, and S.A. Hefta. 1993. Identification of the specific oligosaccharide sites recognized by type 1 fimbriae from *Escherichia coli* on nonspecific cross-reacting antigen, a CD66 cluster granulocyte glycoprotein. *J. Biol. Chem.* 268:15510–15516.

Scheuerpflug, I., T. Rudel, R. Ryll, J. Pandit, and T.F. Meyer. 1999. Roles of PilC and PilE proteins in pilus-mediated adherence of *Neisseria gonorrhoeae* and *Neisseria meningitidis* to human erythrocytes and endothelial and epithelial cells. *Infect. Immun.* 67:834–843.

Schmitter, T., F. Agerer, L. Peterson, P. Muenzner, and C.R. Hauck. 2004. Granulocyte CEACAM3 is a phagocytic receptor of the innate immune system that mediates recognition and elimination of human-specific pathogens. *J. Exp. Med.* 199:35–46.

Sorensen, L.K., B.S. Brooke, D.Y. Li, and L.D. Urness. 2003. Loss of distinct arterial and venous boundaries in mice lacking endoglin, a vascular-specific TGFbeta coreceptor. *Dev. Biol.* 261:235–250.

Swanson, J., O. Barrera, J. Sola, and J. Boslego. 1988. Expression of outer membrane protein II by gonococci in experimental gonorrhea. *J. Exp. Med.* 168:2121–2129.

Tadokoro, S., S.J. Shattil, K. Eto, V. Tai, R.C. Liddington, J.M. de Pereda, M.H. Ginsberg, and D.A. Calderwood. 2003. Talin binding to integrin beta tails: a final common step in integrin activation. *Science.* 302:103–106.

Toleman, M., E. Aho, and M. Virji. 2001. Expression of pathogen-like Opa adhesins in commensal *Neisseria*: genetic and functional analysis. *Cell. Microbiol.* 3:33–44.

Virji, M., S.M. Watt, S. Barker, K. Makepeace, and R. Doyonnas. 1996. The N-domain of the human CD66a adhesion molecule is a target for Opa proteins of *Neisseria meningitidis* and *Neisseria gonorrhoeae*. *Mol. Microbiol.* 22:929–939.

Virji, M., D. Evans, A. Hadfield, F. Grunert, A.M. Teixeira, and S.M. Watt. 1999. Critical determinants of host receptor targeting by *Neisseria meningitidis* and *Neisseria gonorrhoeae*: identification of Opa adhesiotopes on the N-domain of CD66 molecules. *Mol. Microbiol.* 34:538–551.

Virji, M., D. Evans, J. Griffith, D. Hill, L. Serino, A. Hadfield, and S.M. Watt. 2000. Carcinoembryonic antigens are targeted by diverse strains of typable and non-typable *Haemophilus influenzae*. *Mol. Microbiol.* 36:784–795.

Ward, M.E., P.J. Watt, and J.N. Robertson. 1974. The human fallopian tube: a laboratory model for gonococcal infection. *J. Infect. Dis.* 129:650–659.

Wennerberg, K., L. Lohikangas, D. Gullberg, M. Pfaff, S. Johansson, and R. Fassler. 1996. β1 integrin-dependent and -independent polymerization of fibronectin. *J. Cell Biol.* 132:227–238.

Xiao, T., J. Takagi, B.S. Coller, J.H. Wang, and T.A. Springer. 2004. Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. *Nature.* 432:59–67.

Yi, J., S. Kloeker, C.C. Jensen, S. Bockholt, H. Honda, H. Hirai, and M.C. Beckerle. 2002. Members of the Zyxin family of LIM proteins interact with members of the p130Cas family of signal transducers. *J. Biol. Chem.* 277:9580–9589.

Zhou, G.Q., Y. Zhang, and S. Hammarstrom. 2001. The carcinoembryonic antigen (CEA) gene family in non-human primates. *Gene.* 264:105–112.

Zhu, Y., Y. Sun, L. Xie, K. Jin, N. Sheibani, and D.A. Greenberg. 2003. Hypoxic induction of endoglin via mitogen-activated protein kinases in mouse brain microvascular endothelial cells. *Stroke.* 34:2483–2488.