INFECTION AND IMMUNITY, July 1996, p. 2857–2860 Vol. 64, No. 7
0019-9567/96/$04.00+0

The GafD Protein of the G (F17) Fimbrial Complex Confers Adhesiveness of *Escherichia coli* to Laminin

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Received 22 January 1996/Returned for modification 29 February 1996/Accepted 25 April 1996

***Escherichia coli* IHE11088(pRR-5) expressing the G (F17) fimbria adhered to immobilized laminin as well as to reconstituted basement membranes. No adhesion was seen with the plasmidless strain IHE11088 or with the deletion derivative IHE11088(pHUB110), which expresses the G-fimbrial filament with a defective GafD lectin and lacks *N*-acetyl-D-glucosamine-specific binding. Adhesion of IHE11088(pRR-5) to laminin and to reconstituted basement membranes was specifically inhibited by *N*-acetyl-D-glucosamine, and adhesion was abolished after *N*-glycosidase F treatment of laminin. The results show that the GafD lectin binds to laminin carbohydrate and suggest a novel function for the F17 fimbria in binding to mammalian basement membranes.**

The G fimbriae were originally described as occurring in a pyelonephritogenic *Escherichia coli* strain of the O2 serogroup and named on the basis of their binding specificity to terminal *N*-acetyl-D-glucosamine residues of glycoproteins (16, 23). Subsequent work indicated that the G fimbriae share serological cross-reactivity as well as binding specificity with the F17 fimbriae on *E. coli* strains associated with bovine infections (7, 8). Three fimbrial antigens in the F17 family have been described, the G, F17, and F17b fimbriae (2, 7, 16). These fimbrial types exhibit serological variability but share *N*-acetyl-D-glucosamine-specific binding, which is mediated by a minor fimbrial protein, named GafD, F17-G, or F17b-G in the different fimbrial complexes (2, 8, 17). The primary structures of GafD, F17-G, and F17b-G are highly homologous, which is in agreement with their closely related binding specificities. The F17 fimbrial gene clusters encode four proteins, of which GafA (F17-A) is the major structural subunit responsible for the antigenic properties of the filament.

The G fimbriae are rare among *E. coli* strains from human urinary tract infections (23), whereas the F17 fimbriae occur frequently on *E. coli* strains associated with diarrhea and septicemia in newborn calves (7, 8). In particular, there is a close association of the expression of F17-like fimbriae with that of the cytotoxic necrotizing factor type 2 in bovine *E. coli* isolates (2, 13). F17 fimbriae bind to calf intestinal epithelium (7, 8, 12, 13, 19), which suggests a pathogenic function for these fimbriae in promoting the colonization of *E. coli* in the calf intestine.

A number of fimbrial and nonfimbrial adhesins of enterobacterial pathogens have recently been shown to bind to the mammalian extracellular matrix (ECM) and basement membranes (for a review, see reference 25). Such adherence may have a role in bacterial penetration through tissue barriers (6) and appears to be a major virulence factor of *Yersinia enterocolitica* infections in mice (20). As F17 fimbriae are closely associated with septicemic diarrheal diseases in which one would expect bacterial invasion beyond the epithelial barrier, we undertook the present study to assess their capacity to mediate adhesiveness of *E. coli* to the mammalian basement membrane.

The *gaf* gene cluster encoding the G variant of F17 fimbriae has been characterized (16, 17). In the K-12 background, expression of *gafD* is associated with strong autoaggregation most likely resulting from the binding of GafD to the lipopolysaccharide of the host strain. We therefore electroporated (18) into the *E. coli* strain IHE11088 plasmid pRR-5, which contains the *gaf* gene cluster on a 7-kb DNA fragment from the uropathogenic *E. coli* O2 strain IHE11165 (16). Strain IHE11088 is a cystitis isolate and was chosen as the expression host because it is nonfimbriate and of serogroup O2, which does not give autoaggregation by the GafD lectin. Strains IHE11165 and IHE11088 belong to the set of uropathogenic *E. coli* isolates characterized in detail in our laboratory (14, 22, 23). The adhesion tests were also performed with the plasmidless strain IHE11088 as well as *E. coli* IHE11088(pHUB110). pHUB110 contains a 6-bp deletion within the coding region of *gafD* resulting in G-fimbrial filaments lacking the GlcNAc-binding property (17). The strains were grown on Luria agar plates supplemented with tetracycline (10 μg/ml) for the recombinant strains. Hemagglutination tests with endo-β-galactosidase-treated human O erythrocytes (23) showed that *E. coli* IHE11088(pRR-5) caused a GlcNAc-inhibitable agglutination, whereas strain IHE11088(pHUB110) and the plasmidless strain IHE11088 failed to agglutinate. In contrast to the plasmidless *E. coli* IHE11088, strains IHE11088(pRR-5) and IHE11088(pHUB110) were agglutinated in an antiserum against the G fimbriae (17) and were fimbriate as determined by examination by transmission electron microscopy of negatively stained cells (17).

Adherence of the bacteria to ECM proteins. The adhesiveness of the *E. coli* strains to ECM proteins used to coat glass was assessed as described previously (5). The bacteria were used at a concentration of 1×10^8 to 5×10^9 cells per ml in phosphate-buffered saline, pH 7.1 (PBS). The surface concentration, 2.5 pmol per well, of type I, IV, and V collagens (Sigma Co., St. Louis, Mo.), type III collagen (Collaborative Research, Bedford, Mass.), laminin (Upstate Biotechnology Inc., Lake Placid, N.Y.), and human plasma fibronectin (Collaborative Research) was achieved as described earlier (26). The control proteins fetuin and bovine serum albumin (BSA; Sigma) were used to coat glass from a solution of 25 μg/ml. The adherent

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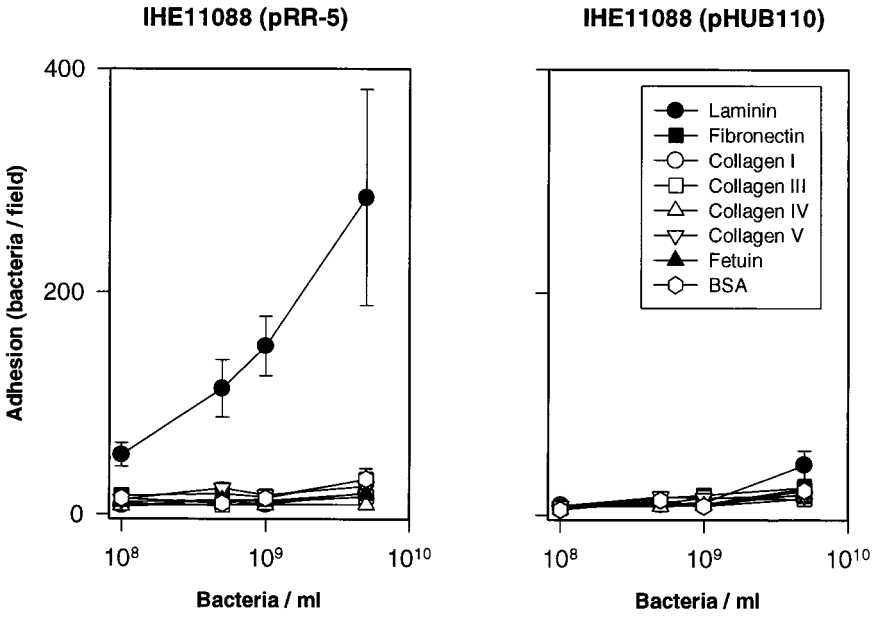

FIG. 1. Adherence of the G-fimbriate *E. coli* strains to ECM and control proteins immobilized on glass. IHE11088(pRR-5) expresses the complete *gaf* gene cluster, whereas IHE11088(pHUB110) is a *gafD* deletion derivative lacking *N*-acetyl-D-glucosamine binding. Means and standard deviations of bacterial numbers in 20 randomly chosen microscopic fields of 4.8×10^3 μm^2 are shown.

bacteria were stained with methylene blue and visualized in a BX50 microscope (Olympus, Tokyo, Japan) equipped with a charged-coupled device camera (4912-5000; Cohu, San Diego, Calif.), and the images were digitized by using an LG-3 (Scion, Frederick, Md.) Scientific Frame Grabber and a Macintosh 7100/80 MHz computer using the public domain NIH Image 1.55 program. The number of bacteria in 20 microscopic fields of 4.8×10^3 μm^2 were determined by density slicing.

The *E. coli* strain IHE11088(pRR-5) adhered efficiently to immobilized laminin but showed only negligible adhesiveness to the other ECM proteins as well as to fetuin and BSA (Fig. 1). No significant adhesion to any of the test proteins was seen with strain IHE11088(pHUB110) (Fig. 1) or the plasmidless strain IHE11088 (data not shown). The failure of *E. coli* IHE11088(pHUB110) to adhere suggested that the observed adhesion to laminin was mediated by the functional GafD protein expressed on strain IHE11088(pRR-5).

Inhibition of adhesion. We next assessed the effect of the receptor analog GlcNAc (16) on the adhesion of IHE11088 (pRR-5). Prior to the adhesion assays, the bacteria (2.5×10^9 cells per ml) were incubated with 4 mM GlcNAc or GalNAc (Sigma) solution in PBS for 30 min over crushed ice. GlcNAc caused a 67% inhibition of adhesion, whereas the same concentration of the closely related carbohydrate GalNAc had no effect on adhesion by IHE11088(pRR-5) (Fig. 2).

Laminin contains 15% carbohydrate exclusively as N-linked oligosaccharides (1, 3). We therefore tested the effect of *N*-glycosidase F treatment of laminin on adhesion; these tests were performed with 10^9 cells per ml in PBS. The enzyme was from Boehringer Mannheim (Mannheim, Germany), and 0.6 U in 40 μl of buffer was used according to the manufacturer's instructions to deglycosylate 2.5 pmol of laminin immobilized on glass. As a control, we used immobilized laminin that was incubated for the same time period in buffer alone. The enzyme treatment caused an 81% inhibition of the adherence of *E. coli* IHE11088(pRR-5) (Fig. 2, bars e and f).

Adherence of bacteria to reconstituted basement membranes. Laminin is a major glycoprotein of basement membranes (9), and we assessed whether GafD can mediate adherence of *E. coli* to basement membranes as well. As a target, we used Matrigel (Collaborative Research), a reconstituted mouse sarcoma basement membrane preparation containing mainly laminin, type IV collagen, and heparan sulfate proteoglycan (4). Under physiological conditions, Matrigel forms a gel-like structure resembling in ultrastructure the lamina densa zone of basement membranes. Matrigel has biological activity

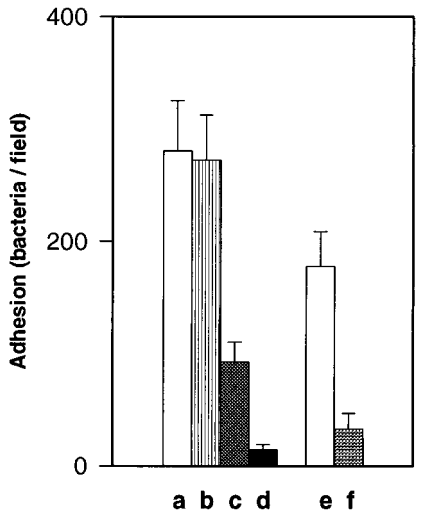

FIG. 2. Inhibition of the adherence of *E. coli* IHE11088(pRR-5) to laminin immobilized on glass. Bar a shows the adherence of IHE11088(pRR-5) (2.5×10^9 cells per ml) in the absence of carbohydrate, bar b shows adhesion in the presence of 4 mM GalNAc, and bar c shows adhesion in the presence of 4 mM GlcNAc. Bar d shows the background adhesion to BSA-coated glass. Bar f shows the adhesiveness of *E. coli* IHE11088(pRR-5) (10^9 cells per ml) to laminin treated with *N*-glycosidase F, and the control adhesion to laminin incubated in the buffer alone is shown by bar e. Means and standard deviations of bacterial numbers in 20 randomly chosen microscopic fields of 4.8×10^3 μm^2 are shown.

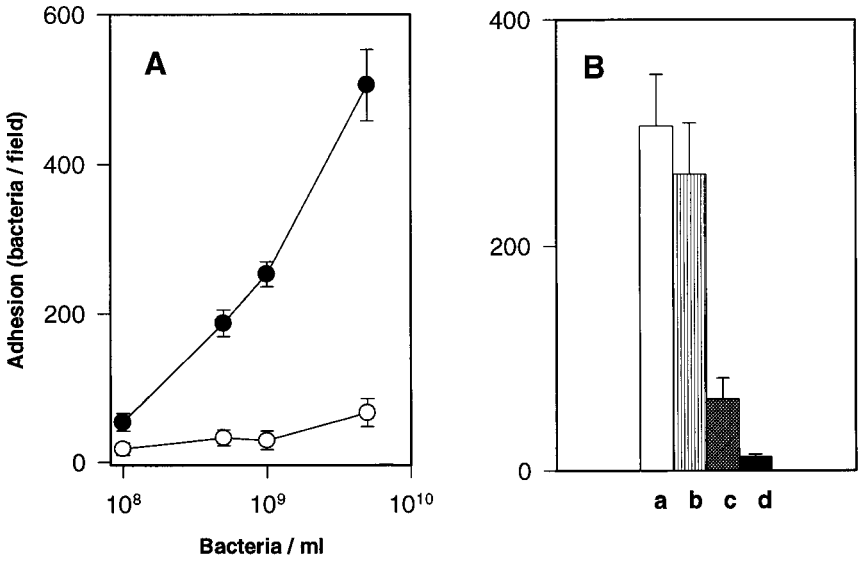

FIG. 3. Adherence of the *E. coli* strains to basement membranes reconstituted on glass. (A) Adherence of strain IHE11088(pRR-5) (solid circles) and strain IHE11088(pHUB110) (open circles). (B) Inhibition of adherence of strain IHE11088(pRR-5) (2.5×10^9 cells per ml) by 4 mM GalNAc (bar b) and GlcNAc (bar c). Adherence in the absence of carbohydrate is shown by bar a, and bar d shows the background adhesiveness to BSA-coated glass. Means and standard deviations of bacterial numbers in 20 randomly chosen microscopic fields of 4.8×10^3 μm^2 are shown.

in stimulating the growth and differentiation of eukaryotic cells, and it has also been used in adhesion and invasion studies of cancer cells (for a review, see reference 11) as well as pathogenic bacteria (5, 20). The adhesion tests using Matrigel were performed as detailed earlier (5, 20). We used 1×10^8 to 5×10^9 bacteria per ml, and the adherence was quantified as described above except that we did not stain adherent cells with methylene blue. *E. coli* IHE11088(pRR-5) adhered efficiently to the reconstituted basement membrane preparation, whereas the adhesiveness of strain IHE11088(pHUB110) was only poor (Fig. 3A) and similar to that shown by the plasmidless strain IHE11088 (data not shown). Tested at the 4 mM concentration, GlcNAc inhibited by 80% the adherence of IHE11088(pRR-5) to Matrigel, whereas 4 mM GalNAc had no significant effect on the adhesion (Fig. 3B).

Our results demonstrate that the GafD lectin protein of the F17 family of fimbrial adhesins confers adhesiveness of *E. coli* to laminin and basement membranes. IHE11088(pRR-5) showed a slightly higher degree of adhesiveness to Matrigel than to laminin; this most likely is due to a larger amount of laminin in Matrigel wells. Laminin is a multidomain glycoprotein produced by a variety of cell types, including epithelial, endothelial, and muscle cells (for a review, see reference 9). It is a major protein of basement membranes and has several molecular interactions with eukaryotic cells and within the ECM (10). Laminin is highly glycosylated and contains N-linked carbohydrates in a high-mannose-type oligosaccharide and in nine different complex oligosaccharide chains which further vary in regard to substitutions by terminal galactose and sialic acid residues (1, 3, 21). We demonstrate here that the targets for GafD on laminin and Matrigel are the laminin carbohydrates. This conclusion is supported by the efficient and specific inhibition of adhesion by GlcNAc and the *N*-glycosidase F treatment of laminin, as well as by the critical dependency of the adhesion on a functional GafD protein encoded by pRR-5. The core structures of laminin oligosaccharides contain internal GlcNAc residues and are thought to also contain terminal GlcNAc residues in short side chains of the core structures (21). The latter structures are the probable binding sites for GafD in laminin, as GafD and the related F17 adhesins have a high affinity to oligosaccharides with terminal GlcNAc residues and bind only poorly to internal GlcNAc residues of N-linked oligosaccharides (12, 23) present in, e.g., human plasma fibronectin (15). Laminin and basement membrane carbohydrate are recognized by other fimbrial types as well. It has previously been shown that the high-mannose chains (5) and the sialyloligosaccharides (24) of laminin function as binding sites for the *E. coli* type 1 and S fimbriae, respectively.

It is well established that F17 fimbriae bind to GlcNAc-containing glycoconjugates at intestinal brush borders (2, 7, 8, 12, 13, 19). This adhesion is inhibited by GlcNAc and promotes colonization of *E. coli* in the calf intestine. It thus appears that, similar to other enterobacterial fimbrial types (25), F17 fimbriae recognize both epithelial and basement membrane receptors. GafD-mediated adhesion to basement membranes may have important pathogenetic functions for F17-positive *E. coli*. F17 fimbriae are mainly associated with septicemia and intestinal infections, and adherence to basement membranes may increase the colonization potential of the bacteria at damaged intestinal tissue sites. Adhesiveness to ECM has been proposed to promote bacterial penetration through tissue barriers (6), and it may be that adherence to laminin potentiates the translocation of F17-positive *E. coli* through epithelial barriers from the intestine into the circulation.

This study was supported by the Academy of Finland, the Sigrid Jusélius Foundation, and the University of Helsinki.

We thank Kaarina Lähteenmäki for help in drawing the figures and Raili Lameranta for skilled technical assistance.

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Editor: B. I. Eisenstein