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S Fimbriae of Uropathogenic *Escherichia coli* Bind to Primary Human Renal Proximal Tubular Epithelial Cells but Do Not Induce Expression of Intercellular Adhesion Molecule 1

BURKHARD KREFT,[1]* MARTIN PLACZEK,[1] CHRISTIAN DOEHN,[2] JÖRG HACKER,[3] GÜNTHER SCHMIDT,[4] GEORG WASENAUER,[5] MOHAMMED R. DAHA,[6] FOKKO J. VAN DER WOUDE,[6] AND KLAUS SACK[1]

Klinik für Innere Medizin[1] und Klinik für Urologie[2] der Medizinischen Universität zu Lübeck, 23538 Lübeck, Institut für Molekulare Infektionsbiologie der Universität Würzburg, 97070 Würzburg,[3] Institut für Experimentelle Biologie und Medizin, Forschungsinstitut Borstel, 23845 Borstel,[4] and Institut für Medizinische Mikrobiologie und Hygiene, Universität Ulm, 89081 Ulm,[5] Germany, and Department of Nephrology, Academisch Ziekenhuis Leiden, 2300 Leiden, The Netherlands[6]

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We have recently reported an increase of expression of the intercellular adhesion molecule 1 by renal carcinoma cells in response to S fimbriae of *Escherichia coli*. Now we demonstrate that *E. coli* expressing S and P fimbriae strongly binds to human proximal tubular epithelial cells. However, in primary and simian virus 40-transfected renal tubular epithelial cells S fimbriae do not enhance the expression of intercellular adhesion molecule 1.

In different infectious diseases, the expression of cell adhesion molecules (e.g., intercellular adhesion molecule 1 [ICAM-1]) is enhanced (5, 21, 26). Under several conditions, ICAM-1 hyperexpression is thought to increase the adhesion of lymphocytes to endothelial and epithelial cells (22). Thus, ICAM-1 expression may play a key role in the initiation of inflammation in infectious diseases. Different inflammatory renal diseases are characterized by an enhanced tubular epithelial expression of ICAM-1 (4). However, factors which may induce the expression of cell adhesion molecules by tubular epithelial cells (TEC) in the early phase of renal bacterial infections have not been described. We recently reported enhanced expression of ICAM-1 in cultured renal carcinoma cells challenged with S fimbriae of uropathogenic *Escherichia coli* (14). Since it cannot be excluded that renal carcinoma cells demonstrate a different biological response to pathogens and purified bacterial virulence factors in vitro, we tested the hypothesis that in human primary renal proximal tubular cells ICAM-1 expression is also upregulated in the presence of S fimbriae.

Human recombinant gamma interferon (IFN-γ), peroxidase-conjugated goat anti-mouse immunoglobulin G, peroxidase-conjugated goat anti-rabbit immunoglobulin G, and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ammonium salt (ABTS) were from Sigma (Munich, Germany). The murine monoclonal anti-human ICAM-1 antibody was purchased from Dianova (Hamburg, Germany). Tissue culture media and reagents were obtained from Biochrom, Berlin, Germany. Cell culture flasks and microtiter plates were from Becton Dickinson, Heidelberg, Germany. The *E. coli* wild-type strain 536/21wt was isolated from a patient suffering from a urinary tract infection (2). It was identified by standard methods and characterized as described previously (16). Strain 536/21wt (serotype O6:K15:H31) exhibits a mannose-resistant, neuraminidase-sensitive, S-specific hemagglutination (MrH^+) and production of protein fimbriae. The spontaneous mutant 536/21del has lost the ability to produce fimbriae (Fim^-) and to agglutinate (MrH^-). Strain 536/21del was transformed with S- and P-fimbrial adhesin recombinant plasmids (pANN 801-4 encoding the S-specific MrH^+ Fim^+ phenotype [8] and pRHU 845 encoding the P-fimbrial adhesins). Human proximal TEC were generated as described previously (30) and cultured according to the protocol of Detrisac et al. (6). Transformation of secondary TEC culture was done by standard calcium phosphate transfection procedure (24) with the pSV3 gpt plasmid containing the simian virus 40 (SV40) early region with a functional origin of replication and the bacterial gene for xanthine-guanine phosphoribosyltransferase (used as a selection

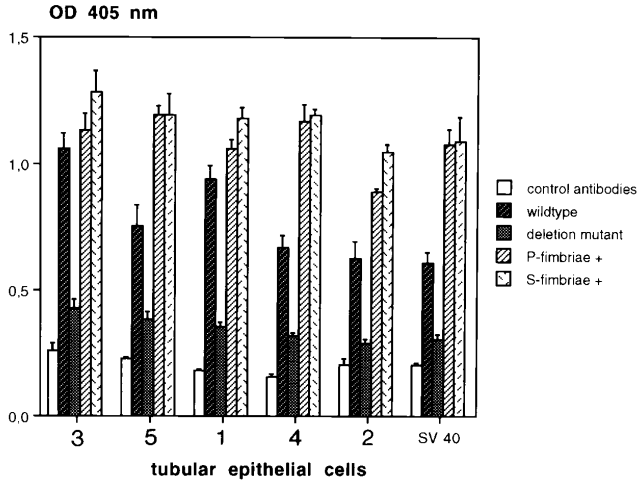

FIG. 1. Adhesion of different clones of *E. coli* 536/21 to primary proximal TEC from five patients (no. 3, 5, 1, 4, and 2) and SV40 TEC, respectively. Means ± standard deviations are from a representative experiment; n = six detections per column. Wild type, *E. coli* 536/21wt; deletion mutant, *E. coli* 536/21del; P fimbriae +, *E. coli* 536/21 pRHU 845; S fimbriae +, *E. coli* 536/21 pANN 801-4. OD, optical density.

* Corresponding author. Mailing address: Klinik für Innere Medizin, Medizinische Universität zu Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany. Phone: 0049 451 500 6400. Fax: 0049 451 500 6334.

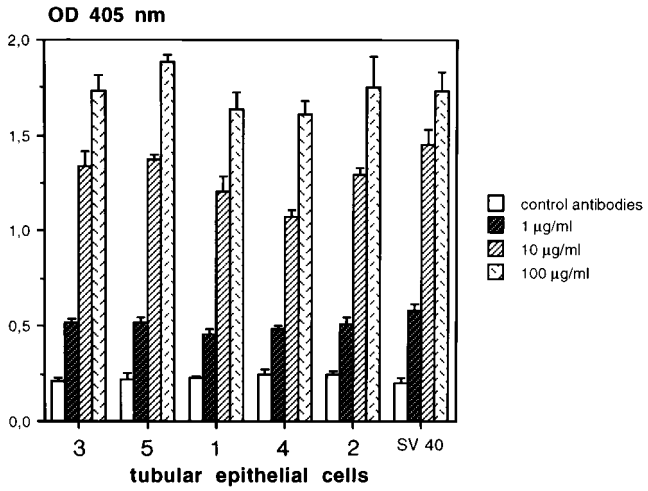

FIG. 2. S-fimbrial binding to primary (patients no. 3, 5, 1, 4, and 2, respectively) and SV40-transfected human renal proximal TEC. Means ± standard deviations are from a representative experiment; n = six detections per concentration. OD, optical density.

marker for transfection). For all experiments, passage numbers 3 to 6 were used. TEC and SV40 TEC were characterized by binding of a monoclonal antibody directed against the epithelial membrane antigen (Dakes, Glostrup, Denmark) and two other monoclonal antibodies (1071 and 1072) directed against adenosine deaminase-binding proteins (kindly provided by W. N. Dinjens, University Hospital, Maastricht, The Netherlands) (30). Both immunohistology and light microscopy of SV40 TEC were identical compared with those of TEC. Adhesion of *E. coli* to TEC and SV40 TEC was detected as described previously (11, 17). Killing of bacteria with formaldehyde was performed according to the method of Agace et al. (1). S fimbriae were purified from the recombinant *E. coli* clones HB101 pANN 801-4, carrying the S-fimbrial adhesin specific for NeuAca2-3Gal from *E. coli* 536 (7, 14). The fimbriae were isolated and purified by gradient ultracentrifugation (27). Specific antiserum against S fimbriae was produced in rabbits by intravenous injection of 100 μg of S-fimbrial protein on days 0, 4, and 10 and after 6 weeks. Gel electrophoresis analysis of S-fimbrial solutions was done by the method of Laemmli (15). Adherence of S fimbriae was determined by an enzyme-linked immunosorbent assay (ELISA) according to the method of Parkkinen et al. (18) with minor modifications (14). For the detection of ICAM-1 expression by ELISA, TEC and SV40 TEC (20,000 cells per well) were grown in 96-well flat-bottom microtiter plates. After 6 h of incubation at 37°C, the cells were stimulated with IFN-γ (0 to 500 U/ml), S fimbriae, or bacteria killed by formaldehyde (1) for 24 h at 37°C. ICAM-1 cell surface expression was detected by a standard protocol (10, 14). For the detection of soluble ICAM-1 (sICAM-1), supernatants of SV40 TEC were generated in 24-well plates. Cells were plated at a density of 100,000 per well and cultured to confluency for 24 h. The supernatant was removed, and conditioned medium (IFN-γ, S fimbriae, medium controls) was added. After 24 h of incubation at 37°C, supernatants were collected and stored at −80°C. Detection of sICAM-1 in the supernatants by a test kit was performed according to the manufacturer's protocol (Boehringer, Mannheim, Germany). Each experiment consisted of four to eight determinations and was repeated up to three times. Statistical analysis was performed by the U test of Wilcoxon, Mann, and Whitney (23). Differences were considered significant for $P < 0.05$; results of representative experiments expressed as means and standard deviations are shown.

The binding of *E. coli* 536/21wt (expressing S and P fimbriae) to SV40 TEC was dependent on both the number of SV40 TEC representing the substrate of adhesion and the number of bacteria inoculated (data not shown). We found a distinctive pattern of adhesion of *E. coli* to TEC generated from the kidneys of five patients and to SV40 TEC (Fig. 1). A weak adherence of the deletion mutant (536/21del, fimbria negative) of the wild-type strain was detected in all patients. The wild-type strain and mutants expressing S fimbriae (536/21 pANN 801-4) and P fimbriae (563/21 pRHU 845) were more adhesive to TEC than the deletion mutant. *E. coli* expressing S and P fimbriae demonstrated a similar intensity of adherence. A significant difference of adherence of these strains was found in only one patient (no. 2). Therefore, S as well as P fimbriae mediate a significant binding of *E. coli* to TEC generated from different individuals. Studying the adhesion of *E. coli* to TEC of different passage numbers, no differences among passage numbers 3 through 6 were detected (data not shown). When SV40 TEC were analyzed, there was no difference concerning the adhesion of different *E. coli* strains compared with TEC (Fig. 1). We next investigated the binding of purified S fimbriae to TEC. S fimbriae were isolated and purified from *E. coli* and analyzed by polyacrylamide gel electrophoresis. The S-fimbrial preparation used in our experiments represented a single protein band of 16 kDa. In the ELISA, we found a significant binding of S fimbriae (1, 10, 100 μg/ml) to all TEC and SV40

TABLE 1. ICAM-1 expression in primary (patients no. 1, 3, 4, and 5) and SV40-transfected human TEC in response to IFN-γ and S fimbriae of *E. coli*[a]

TEC	Control		IFN-γ (250 U/ml)		S fimbriae (μg/ml)			
					10		100	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Patient no. 1	1.256	0.055	1.487[b]	0.095	1.213	0.113	1.153	0.198
Patient no. 3	1.097	0.072	1.619[b]	0.069	ND[c]	ND	1.136	0.075
Patient no. 4	0.908	0.031	1.215[b]	0.061	0.938	0.060	0.887	0.025
Patient no. 5	0.617	0.037	0.820[b]	0.064	0.652	0.029	0.711	0.044
SV40	1.194	0.039	1.499[b]	0.084	ND	ND	1.121	0.039
SV40	0.931	0.072	1.456[b]	0.041	0.926	0.031	0.895	0.064

[a] Data from representative experiments (optical density at 405 nm) are shown as means ± standard deviations. Control, ICAM-1 expression in the absence of stimulation.
[b] $P < 0.05$ versus control.
[c] ND, not done.

TABLE 2. ICAM-1 expression in primary (patients no. 3, 2, and 1) and SV40-transfected human TEC in response to IFN-γ and to *E. coli* killed by formaldehyde[a]

Stimulation	TEC (patient no. 3)		TEC (patient no. 2)		TEC (patient no. 1)		SV40		SV40	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
E. coli 536/21wt	0.470	0.068	0.942	0.060	1.179	0.064	1.060	0.059	1.148	0.027
E. coli 536/21del	0.476	0.069	0.880	0.034	1.205	0.042	0.989	0.034	1.113	0.033
E. coli 536/21 (pRHU 845)	0.456	0.033	0.830	0.142	1.154	0.055	1.032	0.044	1.190	0.067
E. coli 536/21 (pANN 801-4)	0.408	0.064	0.839	0.034	1.361	0.014	1.093	0.014	1.175	0.060
Control	0.405	0.062	0.845	0.050	1.097	0.072	1.051	0.054	1.125	0.089
IFN-γ (250 U/ml)	0.830[b]	0.102	1.792[b]	0.044	1.619[b]	0.069	1.244[b]	0.054	1.325[b]	0.100

[a] Data from representative experiments (optical density at 405 nm) are shown as means ± standard deviations. Control, ICAM-1 expression in the absence of stimulation.
[b] $P < 0.05$ versus control.

TEC (Fig. 2). Since we have recently reported an increase of ICAM-1 expression in renal carcinoma cells in response to the binding of S fimbriae of *E. coli*, we tested the hypothesis that adhesion of S fimbriae to TEC may enhance ICAM-1 expression. As presented in Table 1, in none of the primary tubular cell cultures did incubation with S fimbriae significantly enhance ICAM-1 expression. In addition, no increase of ICAM-1 immunoreactivity of SV40 TEC in the presence of S fimbriae was detected in repeated experiments (Table 1). However, we found a significant constitutive expression of ICAM-1 immunoreactivity in all cells tested (data not shown). Furthermore, formaldehyde-killed bacteria expressing P fimbriae (*E. coli* 536/21 pRHU 845) or S fimbriae (*E. coli* 536/21 pANN 801-4) did not enhance cell surface ICAM-1 expression (Table 2).

sICAM-1 was increased in the supernatants of SV40 TEC stimulated with IFN-γ (250 U/ml). When SV40 TEC were incubated with S fimbriae (100 μg/ml), we did not detect any enhanced expression of sICAM-1 in the supernatants of these cells (Fig. 3) compared with medium controls. Because of limited material, we did not analyze the supernatants of primary TEC.

In different renal diseases, the expression of ICAM-1 by tubular cells is increased (4) and may enhance the infiltration of the kidney by T lymphocytes (3, 29). We recently reported that in renal carcinoma cells purified S fimbriae of *E. coli* enhance the production of ICAM-1 (14). Thus, one might speculate that in human renal bacterial infections S fimbriae may stimulate the expression of this adhesion molecule. This might be of relevance since ICAM-1 is a costimulatory signal in antigen presentation (9). Thus, antigen presentation by TEC (28) might be modified by S fimbriae. In this report, we demonstrate that a distinct binding of *E. coli* expressing S and P fimbriae to TEC and SV40 TEC is detectable and that purified S fimbriae bind to these cells equally. However, ICAM-1 and sICAM-1 expression by TEC or SV40 TEC was not enhanced in the presence of S fimbriae.

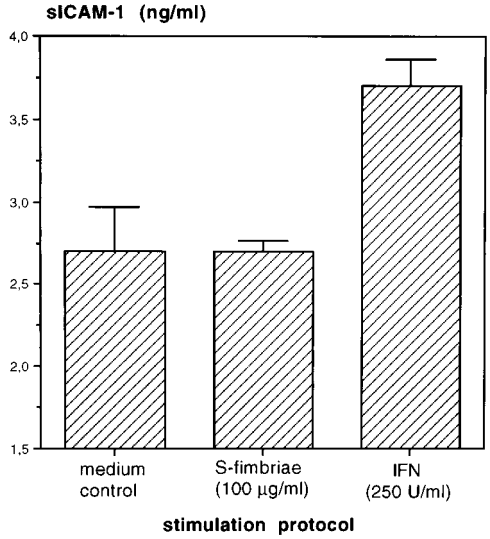

FIG. 3. Detection of soluble ICAM-1 in supernatants from SV40-transfected human renal proximal TEC incubated with medium (negative control), S fimbriae (100 μg/ml), or IFN-γ (250 U/ml, positive controls) for 24 h. Means ± standard deviations of four detections per stimulation are shown.

Our data lead to the following conclusions. First, S fimbriae mediate a significant adhesion of *E. coli* to cultured primary and SV40-transfected human renal proximal tubular cells. Korhonen et al. have reported a distinct binding of *E. coli* expressing S fimbriae to renal tubular cells in frozen kidney sections (12). However, *E. coli* expressing S fimbriae are infrequently found in urinary tract infections (13, 19). Therefore, mechanisms reducing the incidence of acute pyelonephritis caused by *E. coli* producing S fimbriae seem to be relevant (20). Tamm-Horsfall glycoprotein had been identified as a urinary protein reducing the binding of *E. coli* S adhesions to sialyloligosaccharide chains of erythrocytes (20). The absence of Tamm-Horsfall glycoprotein, which is produced by distal TEC, might explain the prominent binding of S fimbriae to proximal TEC used in this study. Second, our data confirm recent studies demonstrating a strong binding of *E. coli* expressing P fimbriae to cultured TEC (25). Third, since ICAM-1 expression by renal carcinoma cells but not by primary TEC and SV40 TEC is upregulated in the presence of S fimbriae the relevance of carcinoma cell lines for in vitro studies on the expression of cell adhesion molecules in response to pathogens or purified virulence factors is limited. Further experiments are currently performed to evaluate the use of SV40 TEC in studies on the immune response of TEC to bacterial challenge. The use of SV40 TEC may overcome the malady of limited cellular material in studies using primary TEC and may be closer to clinical relevance than carcinoma cells of renal origin.

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