

## Binding of *Escherichia coli* S Fimbriae to Human Kidney Epithelium

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**Purified S fimbriae and an *Escherichia coli* strain carrying the recombinant plasmid pANN801-4 that encodes S fimbriae were tested for adhesion to frozen sections of human kidney. The fimbriae and the bacteria bound to the same tissue domains, and in both cases the binding was specifically inhibited by the receptor analog of S fimbria, sialyl( $\alpha$ 2-3)lactose. S fimbriae bound specifically to the epithelial elements in the kidneys; to the epithelial cells of proximal and distal tubules as well as of the collecting ducts and to the visceral and parietal glomerular epithelium. In addition, they bound to the vascular endothelium of glomeruli and of the renal interstitium. No binding to connective tissue elements was observed. The results suggest that the biological function of S fimbriae is to mediate the adhesion of *E. coli* to human epithelial and vascular endothelial cells.**

*Escherichia coli* is known to express many types of fimbriae, which are characterized by binding specificity, serological properties, serotype of the strains carrying the fimbriae, and the associated clinical situation. Some fimbriae, like those of the P type associated with human pyelonephritogenic *E. coli* strains (25) and the K88 and K99 antigens of enterotoxigenic strains (4) are known to enhance the virulence of the bacteria, while the functions and the biological significance of many of the recognized fimbrial antigens remain uncertain.

In addition to P fimbriae, a number of mannose-resistant adhesins have been identified in *E. coli* strains associated with human urinary tract or septic infections (27). One of them, a fimbrial adhesin termed S, was recently characterized (14). It binds to sialyl( $\alpha$ 2-3)galactosides on human erythrocytes and various glycoproteins (20) and occurs only on some *E. coli* strains, notably those of the serogroup O18ac:K1:H7 (13). The genetic determinants coding for the S fimbriae adhesin (*sfa*; previously termed the X determinant) have been cloned from the chromosome of a uropathogenic O6:K15 strain (5), and the contribution of the S fimbria to nephropathogenicity in an experimental rat pyelonephritis model was recently demonstrated (R. Marre, J. Hacker, W. Henke, and W. Goebel, submitted for publication). Although the S fimbria has been characterized in detail for receptor specificity (14, 20), no information is available about its adhesion properties or tissue specificity in human tissues. The kidneys represent a readily available and well-characterized human tissue source (2, 6, 7, 9, 26) composed of a variety of specific cell types and have previously been successfully used for bacterial binding studies (17). In this communication we describe the binding characteristics of S fimbriae, showing particular tissue tropism, to human urinary tract epithelium by using human kidney as the target tissue.

### MATERIALS AND METHODS

**Bacteria.** *E. coli* HB101 (1) was cultivated overnight at 37°C on Luria agar plates, and the recombinant strain HB101(pANN801-4) was grown on Luria agar plates supplemented with tetracycline (25  $\mu$ g/ml). The recombinant plasmid pANN801-4 consists of the vector pBR322 and a 9-kilobase (kb) *Pst*I fragment originally derived from the chromosome of uropathogenic *E. coli* 536 (O6:K15:H31) and carrying the structural genes of the *sfa* determinant (5). The bacteria were labeled with fluorescein isothiocyanate (FITC; Sigma Chemical Co., St. Louis, Mo.) as described previously (28). Briefly,  $8 \times 10^{10}$  bacteria were incubated for 30 min at room temperature with occasional mixing in 2 ml of 0.1 M Na<sub>2</sub>CO<sub>3</sub>, pH 9.0, containing 0.9% (wt/vol) NaCl and 75  $\mu$ g of FITC per ml. The suspension was diluted to 20 ml with phosphate-buffered saline (PBS), pH 7.1, containing 0.05% (vol/vol) Tween-20 (BDH Chemicals Ltd., Poole, U.K.), and the bacteria were collected by centrifugation (20 min, 2,000  $\times$  g, 4°C) and washed once with 20 ml of PBS-Tween. The cells were then suspended in 1 ml of PBS and stored at -20°C in 100- $\mu$ l portions.

**Kidney samples.** Four kidney samples were obtained from the macroscopically normal pole of kidneys carrying renal adenocarcinoma at the opposite pole, as described earlier (7). Two of the kidneys were from males and two from females of blood groups BRh<sup>-</sup>, B, ORh<sup>+</sup>, and O. The kidney samples have been characterized earlier for their lectin-binding domains (7). Frozen sections were cut in an LKB (Bromma, Sweden) cryostat and sections (4  $\mu$ m thick) were mounted on glass slides. The sections were fixed for 10 min at room temperature with cold 3.5% (wt/vol) paraformaldehyde (E. Merck AG, Darmstadt, F.R.G.) in PBS and washed thrice with 50 ml of PBS.

**Bacterial adhesion.** Adhesion of HB101(pANN801-4) and of the plasmidless strain HB101 to the tissue sections was tested essentially as described previously (17, 28). Briefly, FITC-labeled bacteria were thawed and diluted in PBS

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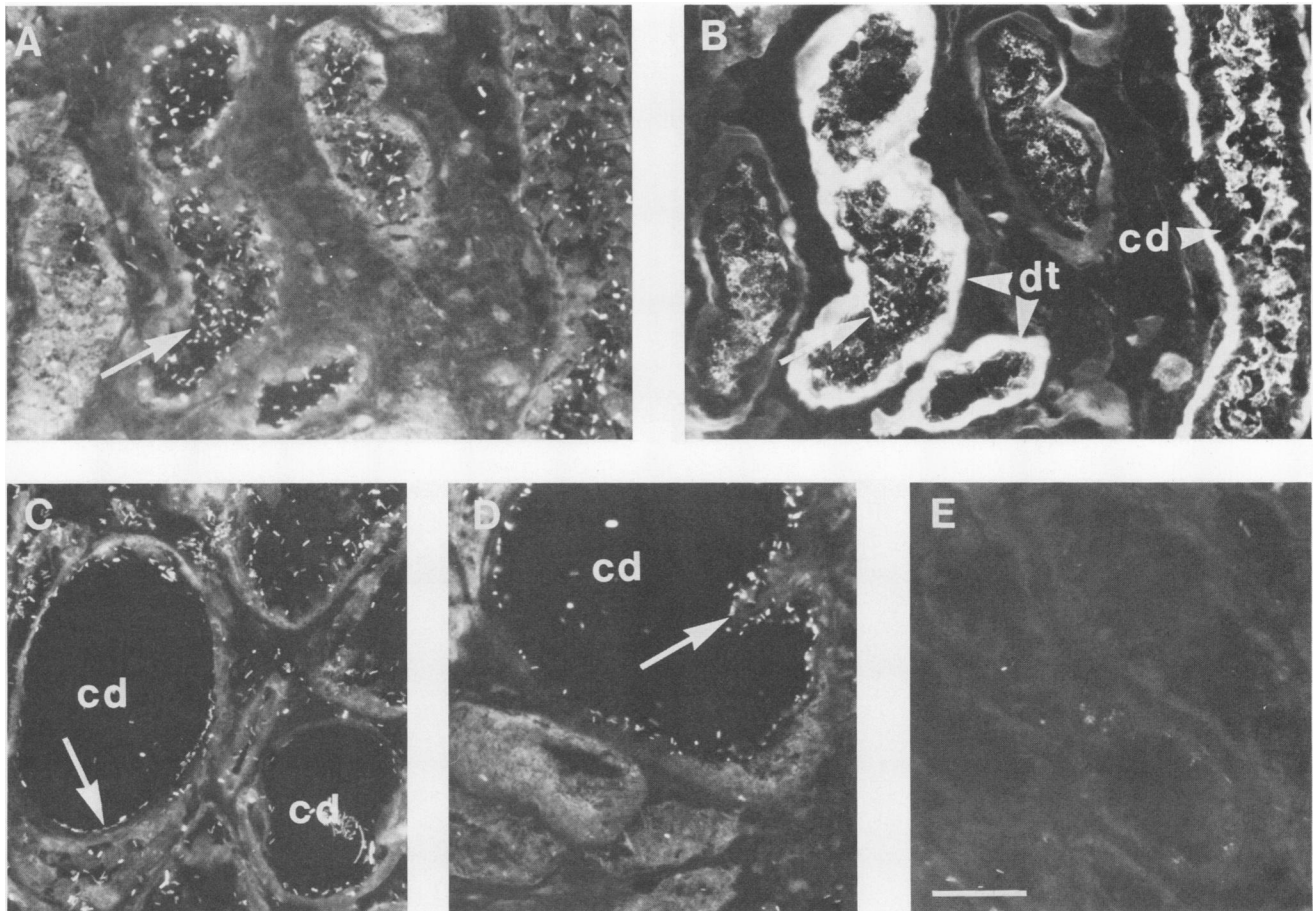


FIG. 1. Adhesion of HB101(pANN801-4) to tubular structures in human kidney. (A) Adhesion of FITC-labeled bacteria to tubular epithelium (arrow). (B) The same field double-stained with TRITC-PNA for the identification of distal tubules (dt) (positive for PNA) and cortical collecting ducts (cd) (binding of PNA to the lumen and basement membrane). The bacteria adhered to the luminal aspect of the tubules. (C and D) Adhesion of HB101(pANN801-4) to the lumen of inner medullary collecting ducts (cd). Done in the presence of free sialic acid and lactose (A-D) or in the presence of sialyl(2-3)lactose (E). Note the complete inhibition of adhesion in panel E. Bar, 10  $\mu$ m.

containing 0.01% (vol/vol) Tween-20 and 50 mM sialic acid and lactose (for controls) (Sigma) or 50 mM sialyl( $\alpha$ 2-3)lactose (for inhibition) to give  $10^{10}$  bacteria per ml. Sialyl( $\alpha$ 2-3)lactose, purified from human urine, was available from previous work (19). The carbohydrate solutions were adjusted to pH 7.1 before use. The bacteria and carbohydrate were kept for 15 min in an ice bath, and then 50  $\mu$ l of the bacterial suspension was pipetted onto tissue sections on glass slides and incubated in a moist chamber at 4°C for 40 min before being washed in 50 ml of PBS thrice for 5 min each under gentle agitation.

For the identification of various segments of the distal nephron (7), the tissue sections with adherent bacteria were subsequently stained with tetramethylrhodamine B-isothiocyanate (TRITC)-conjugated *Arachis hypogaea* lectin (PNA lectin; E-Y Laboratories, San Mateo, Calif.) as recently described (17). The tissue sections were incubated with TRITC-PNA (50  $\mu$ g/ml in Dulbecco solution) at room temperature for 30 min and then washed three times with PBS as above.

Nicethamid was used as the mounting medium as described previously (7). A Zeiss standard microscope equipped with an epi-illuminator and filter systems for FITC and TRITC fluorescence was used for microscopy. When the effect of sialyl( $\alpha$ 2-3)lactose on bacterial adhesion was eval-

uated, the number of adherent bacteria in control and inhibited tissue sections was counted from photographs of the same magnification and showing morphologically similar cortical fields. Five fields from each experiment were counted.

**Binding of purified S fimbriae to tissue sections.** S fimbriae of the recombinant strain HB101(pANN801-4) were purified by using deoxycholate and concentrated urea (12). The fimbrial preparation gave only one peptide band, with an apparent molecular weight of 17,000 (14), in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (not shown). Antiserum against S fimbriae purified from strain IH3084 was available from previous work (14). Binding of S fimbriae to the tissue sections was assayed by indirect immunofluorescence. Purified fimbriae were diluted to 1 mg/ml in PBS containing either 50 mM lactose and free neuraminic acid (for control) or 50 mM sialyl( $\alpha$ 2-3)lactose, and the suspension was kept for 15 min in an ice bath. Fifty microliters of the suspension was pipetted onto tissue sections on glass slides and incubated at 4°C overnight. The slides were washed in 50 ml of ice-cold PBS thrice for 5 min each and fixed for 10 min with ice-cold paraformaldehyde. After being washed with PBS, the slides were incubated with an anti-S-fimbria serum (diluted 1:20 in PBS) at room temperature for 1 h, washed with PBS, and incubated with FITC-conjugated

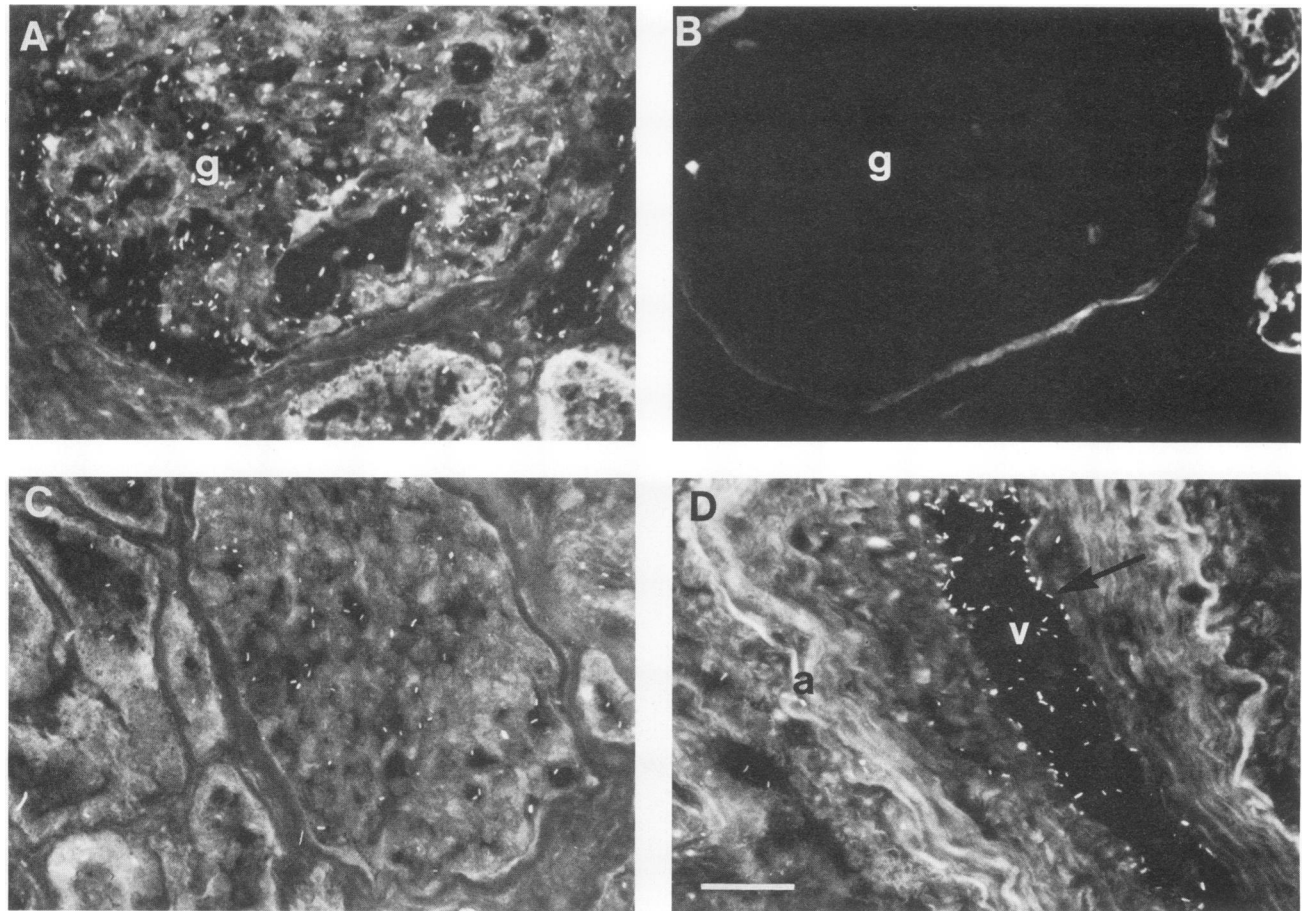


FIG. 2. (A) Adhesion of HB101(pANN801-4) to glomerulus (g). (B) In double-staining with TRITC-PNA, only Bowman's capsule was positive. (C) Adhesion in the presence of sialyl( $\alpha$ 2-3)lactose. (D) Adhesion of HB101(pANN801-4) to the endothelium (arrow) of a large vessel (v). Note the typical autofluorescence (a) pattern of the various layers of the vessel wall. Bar, 10  $\mu$ m.

swine anti-rabbit immunoglobulin G (IgG) (Dakopatts a/s, Glostrup, Denmark; diluted 1:40 in PBS) for 30 min. The slides were then washed with PBS, mounted, and examined in the microscope.

**Hemagglutination assays.** Hemagglutination of human OP<sub>1</sub> and OP<sub>2</sub> erythrocytes by the bacteria was tested as described previously (10).

## RESULTS

**Adhesion of *E. coli* HB101(pANN801-4).** The recombinant strain *E. coli* HB101(pANN801-4) carried plasmid pANN 801-4, which contains the *sfa* gene cluster encoding fimbriae responsible for neuraminidase-sensitive hemagglutination (5). When testing various sialyl oligosaccharides for hemagglutination inhibition (details not shown), we observed that the inhibition pattern with HB101(pANN801-4) was identical to that with IH3084, the S-fimbriated model strain (14). Moreover, HB101(pANN801-4) was strongly agglutinated by the hyperimmune serum raised against purified S fimbriae of *E. coli* IH3084. The plasmidless strain HB101 did not cause hemagglutination, nor did it react with the antiserum. It was concluded that the S fimbriae of *E. coli* strains HB101(pANN801-4) and IH3084 are functionally and antigenically similar. Hemagglutination assays with intact and FITC-labeled HB101(pANN801-4) cells showed no signifi-

cant quantitative differences, indicating that the labeling procedure did not damage the S fimbriae.

Strain HB101(pANN801-4) adhered strongly and selectively to the epithelial elements of frozen sections of human kidney (Fig. 1A), whereas, similarly to our previous findings (17), HB101 at the same bacterial concentration did not show significant binding (data not shown). Results are shown for one kidney sample only, since no differences in the binding pattern of the S fimbriae could be observed between different tissue samples.

HB101(pANN801-4) adhered strongly to the apical aspect of tubular cells but not to the interstitial elements. The strain appeared to adhere to both distal and proximal tubules, identified both morphologically (26) and by binding of TRITC-PNA (7), as shown in Fig. 1A and B. The strain adhered also to the luminal aspects of the cells of collecting ducts (Fig. 1A through D). The experiments shown were done in the presence of free neuraminic acid and lactose; similar assays in the presence of sialyl( $\alpha$ 2-3)lactose showed hardly any adherent bacteria (Fig. 1E), indicating that the adhesion observed was due to S fimbriae (14, 20).

Strain HB101(pANN801-4) adhered specifically to some glomerular elements as well (Fig. 2A and B), and sialyl ( $\alpha$ 2-3)lactose inhibited the adhesion by 60% (Fig. 2C), as calculated by the number of bound bacteria. In the glomeruli, the bacteria adhered preferentially to capillary

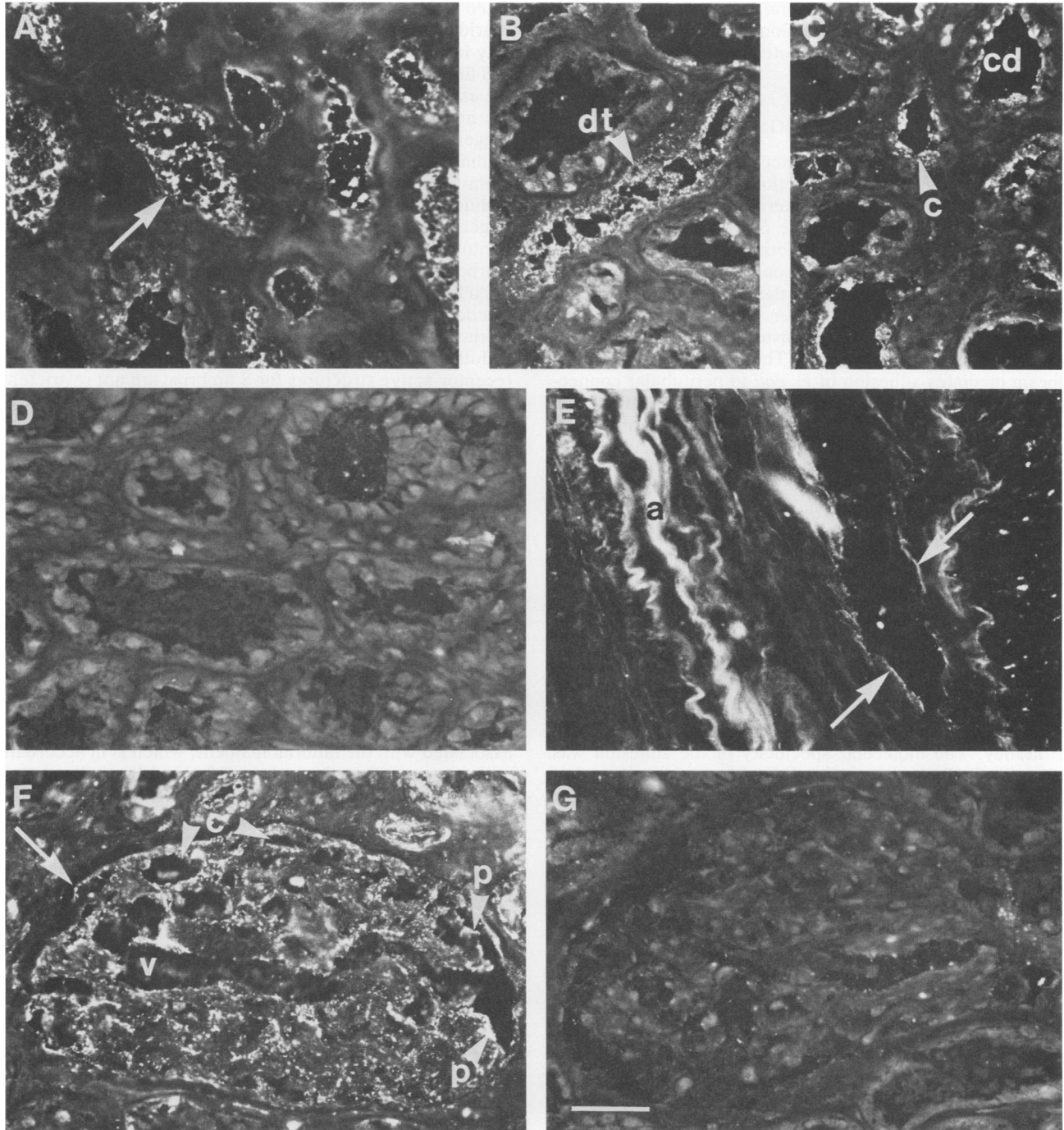


FIG. 3. Binding of purified S fimbriae to human kidney. The fimbriae bound strongly (arrow) to the luminal and cytoplasmic aspects of tubules and capillaries (A–C), and this was inhibited completely by sialyl( $\alpha$ 2-3)lactose (D). Abbreviations: dt, distal tubulus; cd, collecting duct; c, capillary. (E) Binding (arrows) of S fimbriae to the endothelium of a large vessel; a, autofluorescence of the muscular layer of the vessel (compare with Fig. 2D). (F) Binding of S fimbriae to a glomerulus; a strong binding to capillary endothelium, to podocyte (p) surfaces, and to the parietal glomerular epithelium (arrow) can be seen. V, Vessel. (G) Sialyl( $\alpha$ 2-3)lactose completely inhibited fimbrial binding to the glomerulus. Bar, 10  $\mu$ m.

walls but not to Bowman's capsule (Fig. 2A and B), and also in interstitial vessels a strong adhesion to vessel walls could be seen (Fig. 2D).

**Binding of purified S fimbriae.** We then tested whether purified S fimbriae showed similar characteristics of binding to the tissue sections. Results with the purified fimbriae were similar to those obtained with HB101(pANN801-4) bacteria.

The fimbriae bound efficiently to the luminal aspect of tubular cells and to the capillary endothelia of renal interstitium (Fig. 3A through C), and the binding was completely inhibited by sialyl( $\alpha$ 2-3)lactose (Fig. 3D). Similarly to the HB101(pANN801-4) bacteria, the purified S fimbriae bound to the vascular endothelium of vessels (Fig. 3E).

S fimbriae bound strongly to the walls of glomerular



capillaries as well as to the visceral and parietal glomerular epithelium (Fig. 3F). Also at the glomeruli, the binding of the S fimbriae was completely inhibited by sialyl( $\alpha$ 2-3)lactose (Fig. 3G).

## DISCUSSION

Bacterial adhesion to host surfaces is an important step in the pathogenesis of many infectious diseases. The best-known examples of specific bacterial adhesion include the attachment of pyelonephritogenic *E. coli* strains to human uroepithelium, mediated by P fimbriae (11, 17, 25), and K88, K99, and colonization factor antigen-mediated attachment of enterotoxigenic *E. coli* strains to the intestinal epithelium of animals and humans (4). However, not all *E. coli* adhesins bind to epithelial cells, but can instead show tissue tropism to some other tissue elements. Thus, the so-called O75X adhesin mediates adhesion of *E. coli* to peritubular connective tissue areas in the human kidney (17), and some *E. coli* strains are also known to bind to basement membrane or connective tissue proteins, e.g., laminin (24) and fibronectin (3). Our present results indicate that S fimbriae bind specifically to human epithelia.

S-fimbriated bacteria and the purified S fimbriae bound similarly to vascular endothelium in both large vessels of kidney tissue (Fig. 2D and 3E) and capillary endothelium in the interstitium (Fig. 3C) and in the glomerulus (Fig. 3F). S fimbriae bound also to tubular epithelium, i.e., to the luminal and cytoplasmic aspects of proximal and distal tubules and of collecting ducts (Fig. 1A through D and 3A through C), but not to tubular basement membranes. In addition to tubular epithelium, S fimbriae bound strongly to visceral and parietal glomerular epithelium (Fig. 3F). Thus, the binding of S fimbriae was restricted to the various epithelial elements of the kidney. The molecular specificity of the adhesion was shown by the facts that the binding of bacterial cells and of purified fimbriae were inhibited specifically by the receptor analog of S fimbriae, sialyl( $\alpha$ 2-3)lactose (14, 20) and that the bacteria and the fimbriae bound to the same tissue domains.

Of interest is the binding of S fimbriae to the visceral epithelium of the glomerulus (podocytes; Fig. 3F). Podocytes are known to have a sialic acid coating (2, 9) that is essential for maintenance of the normal epithelial organization and presumably also for an intact filtration function at these sites (15). Kerjaschaki et al. (9) have recently characterized the major sialoprotein of the rat renal glomerular epithelium, podocalyxin, as a 140-kilodalton protein containing 4.5% sialic acid. It is thus interesting to speculate that in vivo binding of S-fimbriated bacteria to podocytes in association with an ascending urinary tract infection could result in impairment of renal filtration function. Indeed, our recent experiments have shown that bacteria expressing cloned S fimbriae colonize and damage renal tissue in experimentally infected rats more readily than do recombinant strains lacking S fimbriae (R. Marre, J. Hacker, W. Henkel, and W. Goebel, submitted for publication). In this respect the binding of S fimbriae to the lumen of collecting ducts and tubules may be important, as these structures provide a natural entry for the bacteria to invade the kidneys.

It is of interest that P-fimbriated *E. coli* strains, known to be associated with human childhood pyelonephritis, bind to approximately the same sites in the kidney as do the S-fimbriated strains (17). S fimbriae do occur on some pyelonephritogenic *E. coli* strains but are mainly associated with strains causing neonatal sepsis and meningitis (13). Their loose association with human urinary tract infection

could be due to the presence of specific sialyl oligosaccharides and sialoglycoproteins in human urine (19), which may inhibit S fimbrial binding in the urinary tract.

S fimbriae are known to undergo phase variation (18) and are expressed in vivo during a systemic *E. coli* infection in the mouse (18a). On the basis of the present results, we suggest that the biological function of S fimbriae is to mediate bacterial adhesion to human epithelial tissue, a preinvasive phase of the disease, and to the vascular endothelium during the spread and invasion to secondary tissue targets, such as meninges, which rapidly takes place after peritoneal infection with S-fimbriated *E. coli* in the rat (T. K. Korhonen, J. Parkkinen, M. Leinonen, K. Saukkonen, P. H. Mäkelä, unpublished data). Sialyl( $\alpha$ 2-3)galactose-containing glycoconjugates are known to occur in many tissues, and Kerjaschki et al. (9) found podocalyxinlike proteins in endothelia of all rat organs they tested. Thus, it is likely that receptor-active structures for S fimbriae are not restricted to the kidney epithelium.

The density and accessibility of bacterial receptors on host epithelial cells are believed to affect the susceptibility of humans and animals to certain infectious diseases (8, 23). An important implication of the present work is that purified bacterial adhesins can be used in determining, for example by immunofluorescent techniques, the occurrence and distribution of bacterial receptors in host tissues. This has been in doubt since genetic studies showed that in some fimbrial gene clusters, like those of S (5) and P (16) fimbriae, the binding property (or the adhesin) and the fibrillin are encoded by separate genes. However, recent genetic studies (21; F. Lindberg, B. Lund, and S. Normark, work presented at a meeting on Molecular Biology of Microbial Pathogenicity, in Luleå, Sweden, 1985) have given evidence suggesting that in wild-type *E. coli* strains the adhesin is physically associated with the fimbrial filament, which is in accordance with our present and previous (10, 11, 14) results showing binding specificities in purified fimbrial filaments.

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