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Localization of Binding Sites for Purified *Escherichia coli* P Fimbriae in the Human Kidney

TIMO K. KORHONEN,[1]* RITVA VIRKOLA,[1] AND HARRY HOLTHÖFER[2]

Department of General Microbiology[1] and Department of Bacteriology and Immunology,[2] University of Helsinki, SF-00280 Helsinki, Finland

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Binding sites in the human kidney for purified P fimbriae of pyelonephritogenic *Escherichia coli* were determined. The purified KS71A ($F7_1$) fimbriae bound only to epithelial elements of the kidney, i.e., to the apical aspect of proximal and distal tubular cells, as well as to the apical and cytoplasmic sites of collecting ducts. In addition, binding was seen at the vascular endothelium throughout the kidney and at the parietal epithelium of the glomeruli. The binding was specifically inhibited by the receptor analog of *E. coli* P fimbriae, globotriose. The binding sites identified suggested a possible pathogenetic mechanism for the invasion of P-fimbriated bacteria into the renal parenchyma, as well as for their subsequent spread into the circulatory system.

P fimbriae are a major bacterial virulence factor in human urinary tract infections. They are known to mediate the adhesion of *Escherichia coli* to exfoliated epithelial cells in human urine in vitro (10). Fimbriae are also thought to be important for the bacteria in vivo by helping to colonize the urinary tract and to resist the cleansing action of urine flow. Indeed, after the first studies by Svanborg-Edén et al. (26), demonstrating the capacity of pyelonephritogenic *E. coli* strains to adhere to human uroepithelium, a number of studies have confirmed the association of P fimbriae with bacterial virulence in human pyelonephritis (6, 25, 30).

At present, the P fimbriae-mediated adhesion of *E. coli* is perhaps the best-known example of specific bacterial adhesion. P fimbriae recognize blood group P-active glycosphingolipids on human erythrocytes and epithelial cells (7, 15). The minimum receptor-active structure for P-fimbriated bacteria, the disaccharide sequence α-D-Gal(1-4)-β-D-Gal (Gal, galactose) (7), has been chemically synthesized for diagnostic purposes (28). P fimbriae from a number of *E. coli* strains have been purified and characterized immunologically (20), and a number of P fimbriae gene clusters have been cloned into *E. coli* K-12 and characterized in detail (2, 16, 22, 31). The *E. coli* strains carrying P fimbriae have been further analyzed for a number of properties, including serotypes, outer membrane proteins, and possession of other putative virulence factors and adhesin types (14, 30). These studies have shown that the P-fimbriated and pyelonephritogenic *E. coli* strains share several other independent phenotypic characteristics which have provided the basis for assigning these bacteria to clonal groups and which probably reflect their evolutionary relationships.

The binding specificity of the P fimbriae has been well characterized at the macromolecular (3) and the receptor epitope (7) level. However, currently only little and partially contradictory (17, 18, 27) information is available about the tissue level distribution of the receptor molecules. As this issue is of crucial importance for the understanding of the pathogenesis of human pyelonephritis, we wanted to test whether purified P fimbriae of *E. coli* can be used to localize the receptor-active domains in the human kidney.

MATERIALS AND METHODS

Fimbriae and antisera. The purified P fimbriae, termed the KS71A fimbriae, and a hyperimmune serum raised against it in rabbits were available from previous work (22). The fimbriae had been purified from an *E. coli* K-12 strain that carried the recombinant plasmid pKTH3024 encoding the KS71A fimbriae (22). Before use in the binding assays, the antiserum was absorbed overnight at 4°C with ground kidney tissue.

Kidney samples. Four kidney samples obtained from the macroscopically normal pole of kidneys carrying renal adenocarcinoma at the opposite pole were used (5). Two of the kidneys were from males and two from females of blood groups BRh^-, B, ORh^+, and O. Frozen sections of the tissues studied were cut in an LKB (Bromma, Sweden) cryostat, and sections (4 μm 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, Federal Republic of Germany) in phosphate-buffered saline (PBS) and washed three times in 50 ml of PBS.

Binding assay. The binding of purified KS71A fimbriae to the tissue sections was assayed by indirect immunofluorescence microscopy. The fimbriae were diluted, in most cases to 400 μg/ml, in PBS containing either methyl-α-D-mannoside (for a control [Sigma Chemical Co., St. Louis, Mo.]) or globotriose (for inhibition [Biocarb Chemical Co., Lund, Sweden]), both at 15 mM. The suspensions were kept for 15 min in an ice bath. Then 50 μl of the suspension was pipetted onto tissue sections on glass slides, which were incubated at 4°C overnight. The slides were washed in 50 ml of ice-cold PBS three times for 5 min and fixed for 10 min with ice-cold paraformaldehyde. After being washed with PBS as above, the slides were incubated with an anti-KS71A fimbriae serum (diluted 1:20 in PBS) at room temperature for 1 h, washed, and incubated with tetramethyl rhodamine B isothiocyanate-conjugated swine anti-rabbit immunoglobulin G (Dakopatts a/s, Glostrup, Denmark) diluted 1:40 in PBS.

* Corresponding author.

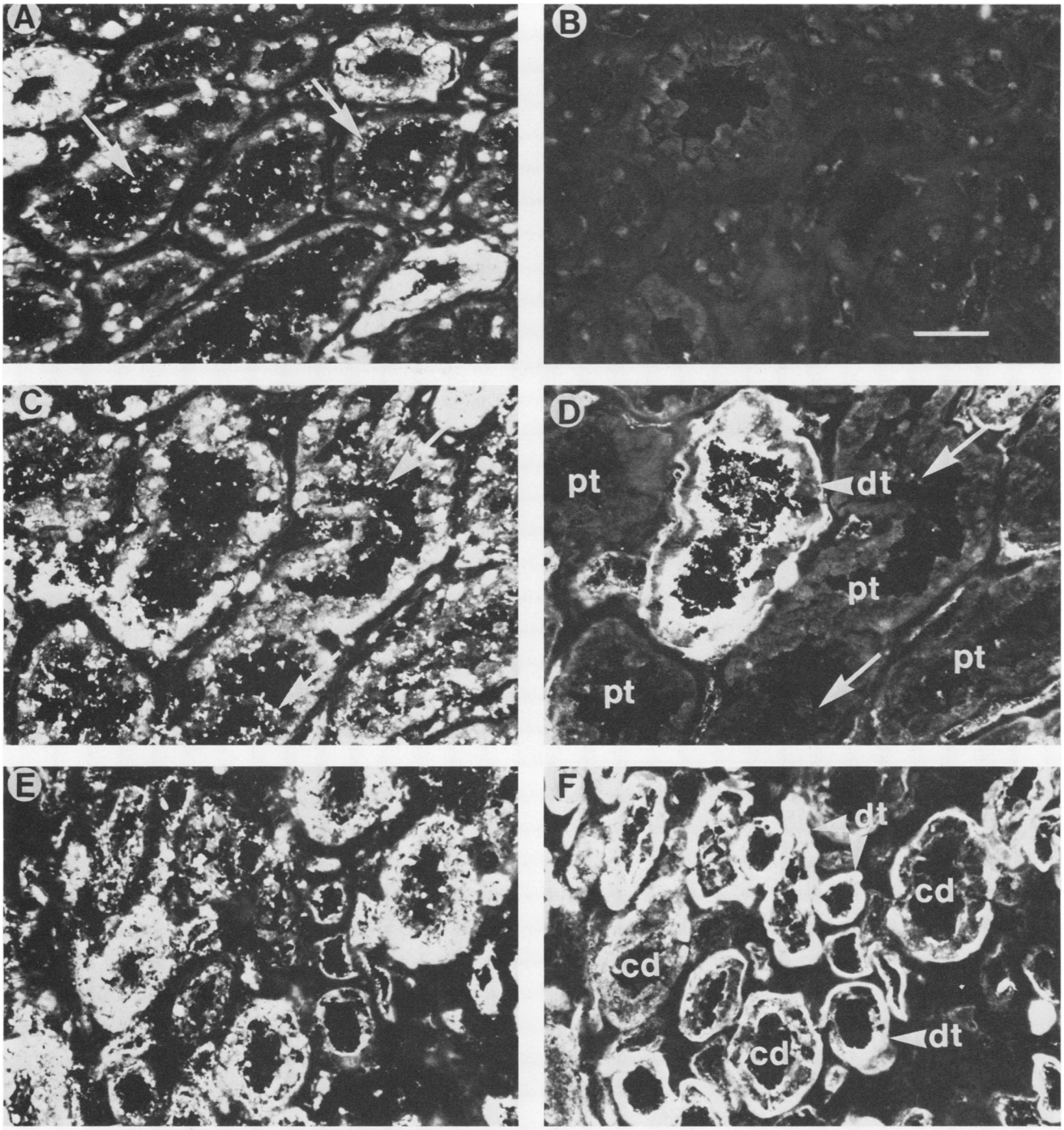

FIG. 1. Binding of the purified KS71A fimbriae to frozen sections of human kidney. In the presence of methyl-α-D-mannoside (A), the fimbriae bound to the luminal aspect of tubular cells (arrows). With globotriose (B), there was a complete lack of binding. Double stainings were performed with the fimbriae (C, E) and FITC-PNA (D, F). The fimbriae bound to the luminal aspect of proximal (pt; arrows in panels C and D) and distal (dt) tubules, as well as to the luminal and cytoplasmic aspects of collecting ducts (cd). Bar, 10 μm.

The slides were then washed with PBS, mounted with nicethamide (5), and examined in a standard microscope (Carl Zeiss, Oberkochen, Federal Republic of Germany) equipped with an epi-illuminator and filter systems for tetramethyl rhodamine B isothiocyanate and fluorescein isothiocyanate (FITC).

Tissue markers. For the identification of various segments of the distal nephron and vascular endothelium (4, 5), the tissue sections with the bound adhesin were double stained with FITC-conjugated *Arachis hypogaea* (PNA) or *Ulex europaeus* (UEAI) lectins (E-Y Laboratories, San Mateo, Calif.) as recently described (5, 17). The tissue sections were

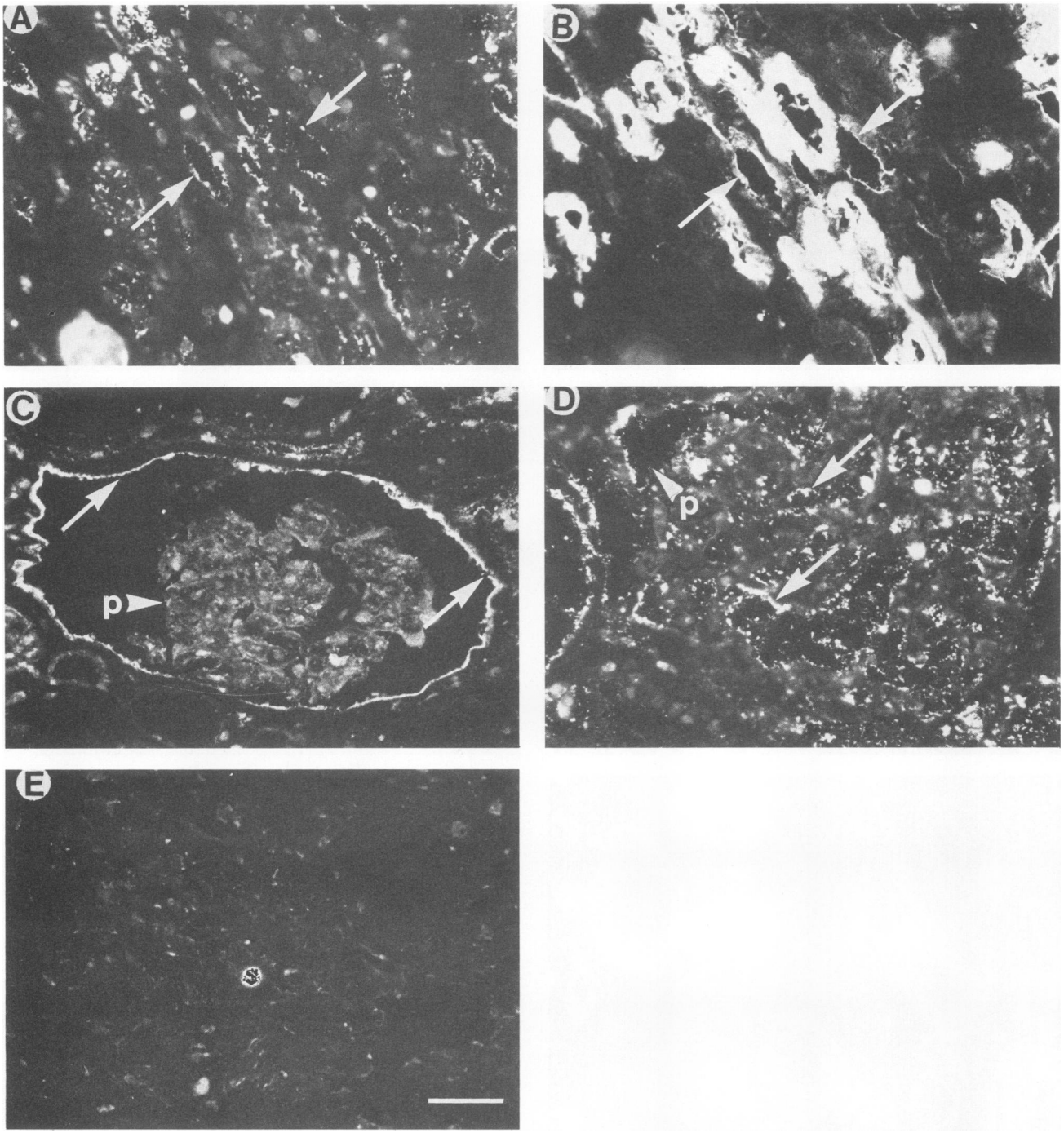

FIG. 2. Binding of the purified KS71A fimbriae to vascular endothelium and glomeruli. The vascular endothelium was double stained with FITC-UEAI (B). (A) Strong binding (arrows) to the lumen of capillaries (positive for UEAI) can be seen. Binding of the fimbriae to glomeruli in the presence of methyl-α-D-mannoside (C and D) or globotriose (E; note complete inhibition) was observed. The fimbriae were used at concentrations of 150 (C) and 400 (A, D, and E) μg/ml. Strong binding to the parietal glomerular epithelium (arrows in panel C) and to capillaries (arrows in panel D) can be seen. In contrast, no binding is seen at the visceral glomerular epithelium (podocytes, p). Bar, 10 μm.

incubated with the lectins (50 to 100 μg/ml in PBS containing Ca^{2+} and Mg^{2+}; Dulbecco solution, Orion Corp. Ltd., Helsinki, Finland) at room temperature for 40 min and were then washed three times with PBS before being mounted.

RESULTS

The purified KS71A fimbriae bound strongly and selectively to frozen sections of human kidney (Fig. 1A). In most instances, a granular binding pattern could be observed,

probably because of the aggregation of fimbrial filaments known to take place in detergent-free suspensions (1). The molecular specificity of binding was demonstrated by the complete inhibition of the fimbrial binding by globotriose (Fig. 1B), a receptor analog for P fimbriae (7), whereas methyl-α-D-mannoside, a receptor analog for type 1 fimbriae (19), had no effect on the binding (Fig. 1A). It was, however, necessary to absorb the antifimbriae serum with ground kidney tissue to remove the unspecific reactions of the serum with the tissue. Binding assays at various fimbrial concentrations showed that a concentration of 150 to 400 μg/ml was optimal for localization studies.

The KS71A fimbriae bound to the apical but not to the cytoplasmic or basolateral aspect of both proximal and distal tubular cells (Fig. 1A, C and D), as identified by morphological criteria (29) and by the binding of FITC-PNA (5). In contrast to proximal and distal tubular cells, the fimbriae bound also to the cytoplasmic aspect of the cells of collecting ducts (Fig. 1E and F). The fimbriae bound strongly also to the vascular endothelium in the renal interstitium, as shown by double staining with FITC-UEAI (Fig. 2A and B).

In the glomeruli, the fimbriae bound strongly to the parietal epithelium (Fig. 2C), whereas the visceral epithelium of the glomeruli (podocytes) (Fig. 2C and D) were virtually lacking any stain. In addition, the purified fimbriae bound to the walls of glomerular capillaries (Fig. 2D). Also, this binding was completely inhibited by globotriose (Fig. 2E) but not affected by methyl-α-D-mannoside (Fig. 2D). No difference in the binding patterns of the KS71A fimbriae were observed between the four tissue samples studied. We were not able to have the P blood group types of the kidney donors. However, in view of their uniform reactions with the P fimbriae and of the extremely low frequency of the blood group $\bar{p}$ (21), it is unlikely that any of the kidney donors were of this blood group.

DISCUSSION

Our results demonstrate that purified *E. coli* P fimbriae can be used to localize bacterial receptors on mammalian tissues. This possibility has been questioned after genetic studies demonstrated that the adhesin (responsible for the binding specificity) and the fimbrillin (subunit of the filament) are encoded by separate genes in the P fimbrial gene cluster (16), indicating that there are two proteins involved. However, we (10, 11) and also other investigators (16) have been able to demonstrate that P fimbriae purified from wild-type *E. coli* strains retain the ability to recognize the α-D-Gal(1-4)-β-D-Gal disaccharide target and to bind to the epithelial cells of human urine. These results indicate that the fimbrillin and adhesin proteins are physically associated in wild-type strains. This view is also supported by recent genetic studies (23, 32).

The purified KS71A fimbria, which is very similar to the so-called $F7_1$ antigen (24), bound to vascular endothelium both in the renal interstitium (Fig. 2A and B) and in glomeruli (Fig. 2D). The fimbriae bound also to the luminal aspect of proximal and distal tubules (Fig. 1C to F) and to the cytoplasm and luminal aspect of collecting ducts (Fig. 1E and F). Finally, the fimbriae bound strongly to the parietal epithelium in the glomerulus (Fig. 2C and D). Thus, the binding of KS71A fimbriae was restricted to epithelial elements and the vascular endothelium of the kidney.

Interestingly, P fimbriae did not bind to the visceral epithelium of the glomeruli (podocytes) (Fig. 2C and D). Podocytes are known to be coated with sialic acids (9), which explains the lack of P-fimbrial binding to these sites. On the other hand, podocyes are recognized by the S fimbriae of *E. coli*, reacting with sialyl(α2-3)galactosides (12), but otherwise the pattern of binding sites in the human kidney is similar for P and S fimbriae of *E. coli* (10a).

Our results on the localization of P-fimbrial receptors in the kidneys are not in line with those of O'Hanley et al. (18). These investigators located P-fimbrial binding sites of murine kidneys to the cells of proximal tubules, of the loop of Henle, and of collecting ducts but could not find any receptor activity in the glomeruli. In the present study, the fimbriae bound strongly to the glomeruli, especially to the parietal epithelium (Fig. 2C). These differences in results can probably be explained, in addition to the species differences, by the methodologies used in the two studies. We used highly purified P fimbriae, the binding of which was detected by specific antibodies, whereas O'Hanley et al. (18) used antibodies raised against the α-D-Gal(1-4)-β-D-Gal disaccharide, the minimal receptor structure for P fimbriae (7). The blood group P system is based on three antigens: the P^k, P, and P^1 glycosphingolipids (21), of which the P^k and P^1 antigens have the α-D-Gal(1-4)-β-D-Gal moiety in a terminal position but globoside (P antigen) in a subterminal position. The antiserum used by O'Hanley et al. (18) did not react with globoside, which is readily recognized, however, by P-fimbria bacteria (15) and by purified P fimbriae (11).

P fimbriae are a major virulence factor in human childhood pyelonephritis (26, 30) and in septic or meningitic infections resulting from pyelonephritis (13). In this respect, binding of the P fimbriae to the luminal aspect of collecting ducts and of distal and proximal tubules may be important, because this would offer a natural route to the glomeruli for the bacteria.

Adhesion assays with whole bacteria have shown that exfoliated epithelial cells in the urine of persons suffering from repeated urinary tract infections more readily bind P-fimbriated *E. coli* than do epithelial cells from healthy persons (8). This may imply that the receptor density for P-fimbriated *E. coli* varies between individuals. The receptor density may thus determine the susceptibility of the individuals to the disease. Determination of the receptor density and of the localization of the receptor-active tissue domains might be an important step in the identification of individuals prone to urinary tract infections before these people are subjected to the disease. We consider that purified bacterial adhesins offer suitable tools for such studies, as they retain the binding properties of the intact bacteria while being easy to use in precise localization studies.

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