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RAPID COMMUNICATION

Presence of the Dr Receptor in Normal Human Tissues and Its Possible Role in the Pathogenesis of Ascending Urinary Tract Infection

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The Dr hemagglutinin of uropathogenic *Escherichia coli* recognizes the Dr^a blood group antigen, a component of the IFC or Cromer-related blood group complex. The present report used the Dr hemagglutinin to demonstrate location of the Dr receptor in selected human tissues and to evaluate the possible use of this lectin as a tissue marker recognizing sites sensitive for bacterial colonization. It was found that the Dr receptor was expressed in different parts of the digestive, urinary, genital, and respiratory tracts, and skin. Intense staining by Dr hemagglutinin was shown in colonic, bronchial, and endometrial glands, and skin eccrine sweat glands. Structures of the urinary tract showing strong fluorescence were renal tubular basement membrane, Bowmans' capsule, and transitional epithelium. The role of Dr^a antigen as receptor for adhesion for Dr-positive *E. coli* in ascending colonization of urinary tract and the possible importance of Dr^a in human pathology is discussed. (Am J Pathol 1988, 133:1–4)

THE Dr HEMAGGLUTININ is a fimbrialike structure present on the cell surface of uropathogenic *Escherichia coli.*[1] These fimbriae have been shown to have lectinlike specificity and to recognize the Dr^a blood group antigen on the surface of human red cells.[2,3] Dr^a is a factor of the IFC or Cromer-related complex, which also includes Cr^a, Tc^a, Es^a, and WES^b blood group antigens.[4,5] Recently isolated and characterized as a 70,000 molecular weight (mol wt) glycoprotein,[6] the IFC complex is found on cell membrane of erythrocytes, leucocytes, and platelets. Although the molecular structure of Dr^a antigen has not been defined, a recent study indicated that the receptor on the Dr^a antigen recognized by Dr hemagglutinin could be a tyrosine-containing molecule.[3] The attachment of the Dr hemagglutinin to this tissue receptor could be inhibited by modified tyrosine or chloramphenicol, both showing similarity in their chemical structures. tures.

The first case of erythrocytic Dr^a deficiency was described in 1984.[4] Patients with this phenotype have been diagnosed to have several diseases including carcinoma of the rectum, ileocecal tumor, or protein-losing enteropathy.[4,5] The frequency of Dr^a negative individuals in any population, and the distribution and location of Dr^a antigen in human tissues, as well as its significance in pathologic conditions, have not been analyzed, however. For example, the urinary tract (UT) and other target tissues may contain Dr^a antigen that serves as the receptor for Dr hemagglutinin and thus facilitate colonization by *E. coli,* which is the first step in an infectious process.

The present report used the Dr hemagglutinin isolated from a recombinant bacterial strain to demonstrate location of the Dr receptor in selected human tissue samples and to evaluate the possible use of this lectin as a tissue marker recognizing sites sensitive for bacterial colonization.

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Materials and Methods

Bacteria, Proteins, and Antibodies

The Dr hemagglutinin-positive recombinant strain *E. coli* BN406 carrying plasmid pBJN406 was grown on Luria agar supplemented with chloramphenicol (20 μg/ml).[2] The Dr hemagglutinin was isolated and purified from *E. coli* BN406 by using deoxycholate and urea as described previously.[2,7] Specific rabbit anti-Dr hemagglutinin antibody and fluorescein isothiocyanate conjugated goat anti-rabbit immunoglobulins were used in an indirect immunofluorescence (IF) stain to detect tissue binding sites.[2]

Adhesion Assay

Tissue specimens obtained from autopsy and surgical specimens were snap frozen in liquid nitrogen. After screening a variety of tissues, the following tissue types were chosen for final study: kidney, urinary tract including pelvis, ureter, bladder and urethra, lung, uterus, fallopian tube, colon, and skin. These were chosen because several of them are known to be the site of *E. coli* infection. Four-micron tissue sections were cut and mounted on glass slides, fixed with 3.5% (wt/vol) paraformaldehyde in PBS for 10 minutes, and washed three times (5 minutes each) with 50 ml PBS.[2] Forty microliters of purified Dr hemagglutinin (500 μg/ml) was pipetted onto tissue sections and incubated in a moist chamber for 30 minutes at room temperature. After washing, the binding of Dr hemagglutinin to tissue sections was detected by indirect IF. Control samples stained by replacing the primary antibody with buffer or preimmune rabbit serum showed no staining.

Results

High affinity binding sites in human tissues, potentially available for colonization by uropathogenic *E. coli* strains carrying Dr hemagglutinin, were studied by indirect IF staining with Dr-specific lectin. This study documented that the Dr blood group antigen was expressed in different parts of the digestive, respiratory, urinary, and genital tracts, and skin. Intense staining by Dr hemagglutinin was shown in colonic, bronchial, and endometrial glands (Figures 1A, C, E), eccrine sweat glands (picture not shown) and epithelium of fallopian tube also showed similar staining (Figure 1F). Although a cell membrane staining pattern was identified, the strongest staining was seen at the luminal portions of the glands, correlating with the presence of well-developed microvilli covered by glycocalyx at this location (Figure 1A). Basement membrane of bronchial glands but not of those in the skin, ureter, or colon also showed some staining (Figure 1C). The staining pattern of the kidney is shown in Figure 1B. Structures showing strong fluoresence were renal tubular basement membranes and Bowman's capsule. The ureter showed strong staining of transitional epithelial cells, where both cytoplasmic and cell membrane staining were present (Figure 1D). Aside from strong staining of urothelium, weak but definite staining was also noted focally in the subepithelial tissue, which might represent blood vessels or collagen bundles. Other portions of the urinary tract, including the renal pelvis, bladder, and urethra showed similar staining, except that the staining of the renal pelvic epithelium was weaker focally. Intensive staining was also noted in the nuclei of the keratinocytes in the epidermis (Figure 1G) and tracheal epithelial cell nuclei (picture not shown).

Discussion

Bacterial adhesion to tissues of the mammalian host is important in the initiation of various infectious diseases.[8,9] This adhesion is mediated by bacterial structures, generally called adhesins. Receptors on red cells for some bacterial adhesins are currently known at the molecular level.[3,10–13] Little is known about the distribution of receptors and adhesion sites for bacteria in mammalian tissues, however.[14,15] Recent studies have shown high-affinity binding sites for Dr-positive and P-fimbriated *E. coli* in human kidney tissue.[14] These studies also have demonstrated different tissue tropism for *E. coli* carrying different lectinlike specificity. P-fimbriated *E. coli* showed adherence to glomeruli and to lumens of proximal and distal tubules but not to collecting ducts and peritubular sites. Dr hemagglutinin-positive strains, however, showed adherence to Bowman's capsule and renal interstitium. Recent studies have successfully isolated Dr hemagglutinin from recombinant strain of *E. coli* BN406 and have proven that this lectinlike adhesin has a specificity for Dr^a blood group antigen.[2,3] The present report shows that various types of normal human tissues do express the Dr receptor and such expression can be easily demonstrated by the Dr hemagglutinin.

The density and accessibility of receptors for bacteria on host cells are believed to influence host susceptibility to certain infectious diseases.[16] It is also believed that the prerequisite step in ascending urinary tract infection is a colonization of the colon by an endogenous strain and subsequent colonization of the periurethral area. To develop ascending colonization and infection, bacteria may use potential binding sites on

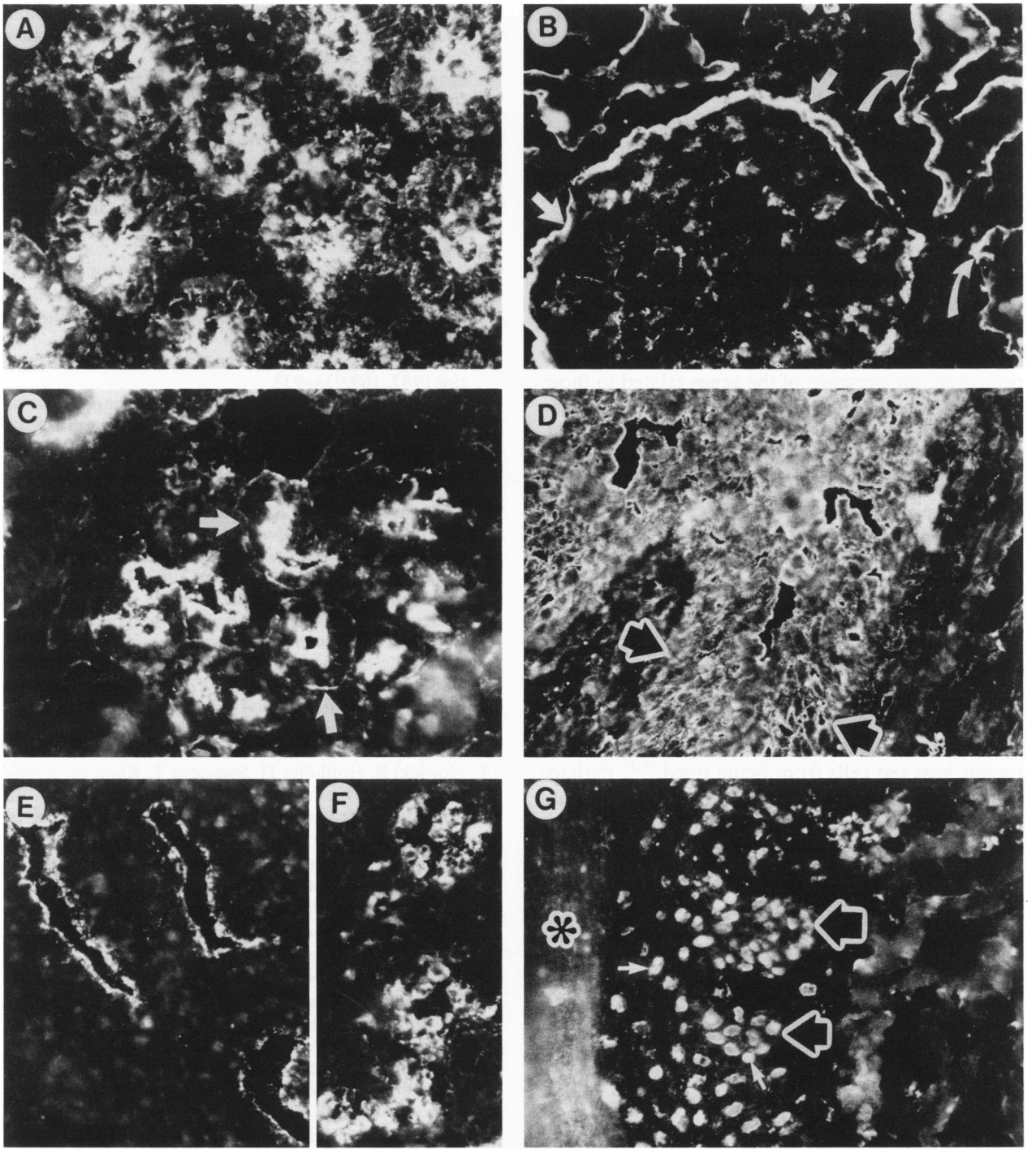

Figure 1—Binding specificity of purified the Dr hemagglutinin to human tissues. **A**—colonic glands; **B**—renal tubular basement membrane (curved arrows) and Bowman's capsule (straight arrows); **C**—bronchial glands, arrows show basement membrane; **D**—ureter, transitional epithelial cells (area between arrows); **E**—endometrial glands; **F**—fallopian tube; **G**—skin, nuclei (thin arrows), asterisk and thick arrows show layers of skin, stratum corneum, and malpighian layer, respectively.

transitional epithelium lining urethra, bladder, and ureters and then reach the kidney.

The present study revealed the presence of the Dr-rich structures in the colon and urinary tract. High density of Dr receptor in the colon may permit its colonization by Dr hemagglutinin-positive *E. coli*. Colonization of the lower urinary tract may occur due to attachment of bacterial cells to Dr-rich transitional epithelium in the urethra, bladder, ureter, and renal pelvis. High density of Dr receptor in the Bowman's capsule may facilitate colonization of the glomerulus. Perhaps at this step bacteria could

use other virulence mechanisms,[8] such as invasiveness, to reach and interact with Dr-containing basement membrane, lidding to chronic, interstitial nephritis.

Although the distribution of the Dr hemagglutinin on uropathogenic *E. coli* is not well known, several lines of evidence are available to suggest the pathogenetic importance of the Dr receptor. Dootson and coworkers[17] showed that *E. coli* serotype 075, a serotype often associated with Dr hemagglutinin, was significantly more frequently found in urine of patients with urinary tract infection than in the normal fecal flora (20.9 *vs.* 6.6%). Another line of evidence comes from the authors' laboratory, where, using DNA/DNA hybridization, the presence of the genes related to those encoding Dr hemagglutinin among *E. coli* strains isolated from urinary tract infections (UTI) was studied. These data indicate that Dr positive strains are associated with symptomatic UTI (28% of 700 strains tested) (Nowicki B, Svanborg-Edén C, Hull R, Hull S, unpublished observation). Together these studies indicate that Dr-positive *E. coli* are relatively common in UTI and that the Dr hemagglutinin is probably important in pathogenesis of UTI.

The Dr receptor in structures other than the colon and urinary tract may not be accessible to serve as a potential receptor for colonization. Recent studies revealed that Dr^a is a portion of decay accelerating factor (DAF) that regulates the complement cascade and protects host red cells from being lysed.[18,19] Perhaps the presence of Dr^a or DAF in human organs could protect them from lytic activity of autologous complement. The role of Dr^a blood group antigen and Dr hemagglutinin in human pathology are currently under study.

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