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A Hemagglutinin of Uropathogenic *Escherichia coli* Recognizes the Dr Blood Group Antigen

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A receptor moiety and blood group substance recognized by the O75X adhesin was studied. Well-defined erythrocytes representing different blood group systems and bacterial derivatives carrying plasmid pBJN406 encoding the adhesin were used in a direct hemagglutination assay. We showed that Dr blood group antigen, a component of the IFC blood group complex, is the receptor for the O75X fimbrialike adhesin (Dr hemagglutinin) of uropathogenic *Escherichia coli*. The molecule recognized by the Dr hemagglutinin on Dr blood group substance is a chloramphenicol-like structure. The inhibitory effect of the active compounds indicates that a tyrosine-containing molecule could be a natural receptor for the Dr hemagglutinin. Dr blood group substance was found in tubular basement membrane and Bowman's capsule of the human kidney. Specific attachment of a Dr hemagglutinin-positive bacterial strain to the kidney substructures was inhibited by chloramphenicol.

Adhesive capacity for epithelial tissue is a virulence factor for *Escherichia coli* which causes intestinal and extraintestinal infections (2, 3, 17, 18). The attachment results from the interaction of host cell receptors with bacterial surface structures known as fimbriae. Bacterial fimbriae and associated proteins may facilitate specific attachment to erythrocytes, as well as to various tissues, and are often called hemagglutinins. Although the importance of hemagglutinins in pathogenicity is well documented, the receptor structures and blood group antigens recognized by various adhesin types are known in only a few cases. P-fimbriae, a major virulence factor in childhood pyelonephritis, recognize the α-D-Gal(1-4)β-D-Gal (Gal, galactose) carbohydrate moiety of the P blood group antigen (5, 7). S fimbriae, associated with meningitis, recognize O-linked sialyloligosaccharides of glycophorin A (14). A few *E. coli* strains show blood group M-specific hemagglutinating activity (21). Other mannose-resistant hemagglutinins constitute a heterogeneous group of unknown receptor specificity and are termed X. About 20% of the pyelonephritis-associated *E. coli* strains possess X hemagglutinins (4, 20).

In this study, we used a systematic approach with erythrocytes representing different blood group systems to identify the receptor for one such X hemagglutinin associated with serotype O75 *E. coli*.

We show that the Dr blood group antigen, a component of the IFC blood group complex, is the receptor for the O75X fimbrialike adhesin. The molecule recognized by the Dr hemagglutinin is a chloramphenicol-like structure.

MATERIALS AND METHODS

Bacteria. *E. coli* BN406 and BN407 are recombinant strains carrying the plasmid pBJN406 and exhibit mannose-resistant hemagglutination associated with the O75X adhesin and specific adherence to basement membrane and Bowman's capsule as described previously (9, 10). *E. coli* BN53, IH11128, HU1005, and HU824 were clinical isolates from patients with pyelonephritis (9, 10, 19). For hemagglutination (HA) assays, each strain was subcultured twice on L-agar, supplemented with chloramphenicol (20 μg/ml) if required.

Patients lacking high-frequency antigens of the IFC complex. Patient 3, a 27-year-old man, had a protein-losing enteropathy and ileocecal tumor; a hemicolectomy was performed which cured the protein-losing enteropathy. An alloantibody detecting a high-frequency blood group antigen was found in his serum (1).

Patient 4, an 18-year-old man, had a protein-losing enteropathy, and a hemicolectomy was performed. An alloantibody of a specificity identical to that of patient 3 was detected in his serum. This agglutinin was designated IFC and defines the overall IFC blood group complex (L. Walthers, M. Salem, J. Tessel, B. Laird-Fryer, and J. J. Moulds, Transfusion **23**:423).

Patient 1, a 48-year-old woman, was found to have an alloantibody to a high-frequency blood group antigen before hysterectomy. Her erythrocytes carried IFC blood group complex receptors in modified form, and her serum contained an antibody of unique specificity designated anti-Dr^a (8).

Erythrocytes. Erythrocytes of the following phenotypes, representing different blood group systems, such as ABO and structurally related systems, blood group systems with highly alloimmunogenic antigens, other identified blood group systems, public antigens, and so-called silent phenotypes (16), were used for the HA tests: O_h, U^-, $En(a^-)$, $\bar{p}$, P_1^k, Rh_{null}, $LW(a^-b^+)$, $Lu(a^+b^-)$, $Lu(a^-b^-)$, K^+k^-, $Js(a^+b^-)$, K_o, $Fy(a^-b^-)$, $Jk(a^-b^-)$, $Di(a^+b^-)$, $Yt(a^-b^+)$, $Co(a^-b^+)$, $Co(a^-b^-)$, $At(a^-)$, $Jo(a^-)$, $Jr(a^-)$, $Kn(a^-)$, $Sd(a^-)$, $Cr(a^-)$, $Tc(a^-b^+)$, $Tc(a^-b^-c^+)$, $Dr(a^-)$, $Es(a^-)$, IFC^-, and WES_b. They were washed with phosphate-buffered saline and suspended to 3% in phosphate-buffered saline for HA tests.

HA assay. HA tests were performed over crushed ice in the presence or absence of inhibitors, as described previously (9, 10). Of the inhibitors used (see Table 2), thiamphenicol was kindly donated by A. E. Soria from Sterling Drug Inc., and chloramphenicol sodium succinate was donated by G. Neri from Farmitalia Carlo Erba.

Adhesion assay. Adhesion assays with BN406 or purified

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TABLE 1. Reaction of erythrocytes from individuals lacking high-frequency antigens of the IFC complex with selected *E. coli* strains

Patient or donor no.	Phenotype	HA by *E. coli*			
		BN406 O75X$^+$	BN53 O75X$^+$	HU824 P$^+$	HU1005 X$^+$
1	Dr(a$^-$)	−	−	+	+
2	Dr(a$^-$)	−	−	+	+
3	IFC$^-$	−	−	+	+
4	IFC$^-$	−	−	+	+
5	Tc(a$^-$b$^-$c$^+$)	+	+	+	+
6	Cr(a$^-$)	+	+	+	+
7	Es(a$^-$)	+	+	+	+
8	WES$_b$	+	+	+	+

[a] The erythrocytes of patients 3 and 4 lacked all high-incidence receptors of the IFC-related blood group complex (e.g., Tc^a, Cr^a, Dr^a, Es^a, and WES$_b$).

O75X adhesin and frozen sections of human kidney were performed as described previously (9, 10). Tests were done in the presence or absence of 2 mM chloramphenicol. Samples were stained by the immunofluorescence method (10, 11).

RESULTS

Blood group specificity of the O75X adhesin. To study the blood group specificity of O75X-positive *E. coli* adherence, we examined its HA capacity with human erythrocytes representing different blood group systems. Six *E. coli* strains differing in hemagglutinin types were used. Recombinant strains BN406 and BN407 and clinical isolates BN53 and IH11128 were positive for O75X adhesin, HU824 exhibited P fimbriae, and HU1005 exhibited an X hemagglutinin with unknown receptor specificity. The O75X-positive strains reacted with all erythrocyte phenotypes except IFC-negative and Dr-negative erythrocytes; Dr-positive erythrocytes lacking other components of the IFC complex, such as Tc, Cr, Es, and WES$_b$ blood group antigens, reacted strongly (Table 1). Strains with P or X hemagglutinins reacted with all of the above-mentioned erythrocytes. A positive reaction of BN406 with Dr-positive erythrocytes and no reaction with IFC- and Dr-negative erythrocytes indicates that the Dr blood group antigen is an erythrocyte receptor for the O75X hemagglutinin.

Binding specificity of O75X-positive *E. coli* to Dr-positive erythrocytes. The receptor moiety in Dr blood group antigen recognized by the O75X adhesin was studied by comparing the inhibitory effects of several potential receptor analogs. We initially observed that a commercial reagent erythrocyte diluent inhibited HA caused by the O75X-positive *E. coli* BN53 but not by P-fimbriated and other X-fimbriated strains. The reagent components were tested for MIC. We found that only one component of the reagent, chloramphenicol, inhibited HA, and the MIC was 2 μM. Moreover, the agglutinate was disaggregated in a few seconds into the homogeneous erythrocyte-bacterial mixture upon addition of chloramphenicol, indicating that bacterial cells were eluted from the erythrocyte receptor (12). Adherence of HU824 (P$^+$), HU 1005 (X$^+$), and several S$^+$ fimbriated bacteria was not inhibited by chloramphenicol.

To study which of the chemical groups in the chloramphenicol structure contribute to the binding of IH11128 and BN407, the inhibitory activity of chloramphenicol analogs was tested (Table 2). Thiamphenicol, which has a SO_2CH_3 substituent added at the *para* position on the benzene ring, and also chloramphenicol base, which lacks the acetylamide group, showed no inhibitory activity. The MIC of chloramphenicol sodium succinate, with a substitution at the C-3 position, was 9 μM. These results suggest that the tested groups on the chloramphenicol molecule are important for expression of full inhibitory activity.

The next group to be tested included compounds which contain in their structure a *p*-nitrophenyl group, like chloramphenicol, but which differ in the aliphatic part (C-1 to C-3). Analogs of *p*-nitrophenyl in which the aliphatic chain is substituted with glucopyranoside, glucuronide, glucosaminide, or phosphorylcholine were not active. However, *p*-nitrophenylglycerol inhibited HA of IH11128 at a 60 mM concentration, indicating that a three-carbon aliphatic structure bound to the benzene ring is required for the compound to be active.

The next group analyzed included chemicals containing a hydroxyl group at the *para* position on the benzene ring. The MICs of *p*-hydroxyphenylpropionic acid and *p*-hydroxyphenylacetic acid were both 100 mM; α-phenylpyruvic acid lacking the hydroxyl group on the benzene ring was inactive. Hence, compounds with a three-carbon chain and a hydroxyl group on the benzene ring showed weak activity.

Natural compounds of human biochemical pathways with a hydroxyl group at the *para* position on the benzene ring, a three-carbon aliphatic chain, and an amine or amide group at C-2 were the next to be tested (Table 2). Tyrosine and *N*-acetyltyrosineamide showed no activity. However *t*-butoxycarbonyl-*O*-benzyl-L-tyrosine, which has three methyl groups at positions similar to those of the chlorine molecules in chloramphenicol, inhibited HA at a 0.3 mM concentration. *N*-Acetyl-L-tyrosine, α-methyl-L-tyrosine methylester, and tri-tyrosine showed weak inhibitory activity. Other peptides, amino acids, sugars, and chemicals tested, including L-seryl-L-tyrosine, L-leucyl-L-tyrosine, L-tyrosylglycine, glycyl-L-tyrosine, D-tyrosyl-L-valylglycine, L-phenylalanine, L-alanine, leucine, serine, glycine, glucose, sucrose, mannose, D-ribose, *p*-aminosalicylic acid, *p*-anisidine, dinitro-

TABLE 2. Inhibition of HA caused by *E. coli* IH11128 and BN407

Inhibitor	MIC (mM) for *E. coli*	
	IH11128	BN407
Chloramphenicol	0.002	0.009
Chloramphenicol succinate	0.009	0.018
Thiamphenicol	>100	>100
Chloramphenicol base	>100	>100
α-*p*-Nitrophenylglycerin	60	100
p-Nitrophenyl-β-D-glucopyranoside	>100	>100
p-Nitrophenyl-β-D-glucuronide	>100	>100
p-Nitrophenylphosphorylcholine	>100	>100
p-Nitrophenyl-*N*-acetyl-β-D-glucosaminide	>100	>100
p-Nitrophenyl	>100	>100
3-(*p*-Hydroxyphenyl)-propionic acid	100	100
p-Hydroxyphenylacetic acid	100	100
β-Phenylpyruvic acid	>100	>100
p-Hydroxyphenyl	>100	>100
t-Butoxycarbonyl-*O*-benzyl-L-tyrosine	0.3	0.6
N-Acetyl-L-tyrosine	60	100
α-Methyl-DL-*p*-tyrosine methylester	100	>100
L-Tyrosyl-L-tyrosyl-L-tyrosine	100	>100
L-Tyrosine	>100	>100

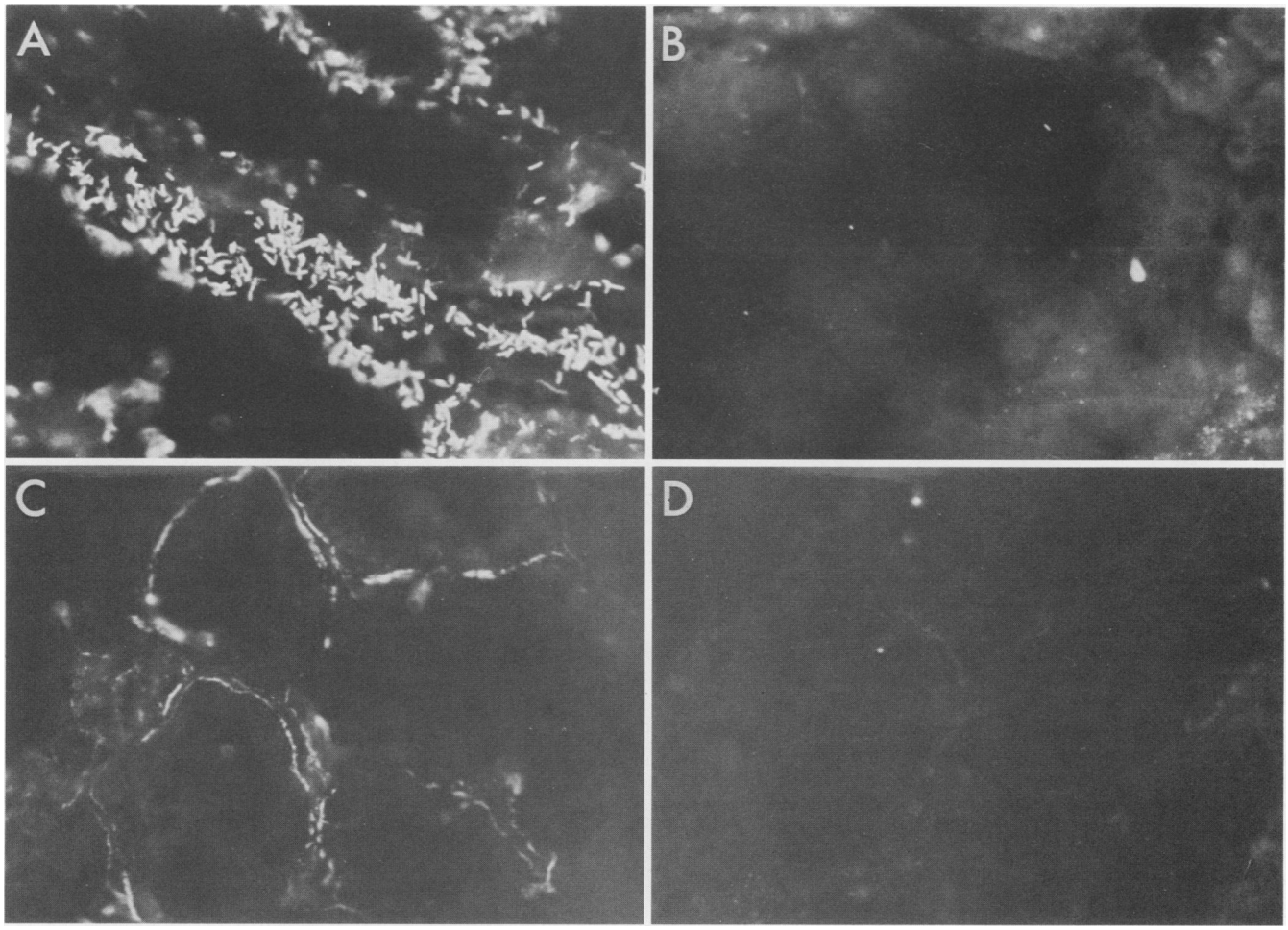

FIG. 1. Binding specificity of *E. coli* BN406 and purified O75X adhesin to human kidney. Note very strong binding of bacterial cells to interstitial tissue of human kidney in A and no attachment in the presence of chloramphenicol in B. C shows binding of purified O75X adhesin to basement membrane, and D shows no binding in the presence of chloramphenicol.

salicylic acid, pyruvic acid, acetamide, glycerol, propanol, gentamicin, and neomycin showed no activity.

Binding specificity of BN406 and the O75X adhesin to human kidney. The specificity of attachment to the Dr antigen in the kidney recognized by the O75X adhesin was studied by testing the inhibitory effect of choramphenicol on attachment. Chloramphenicol inhibited specific attachment of *E. coli* BN406 and the purified O75X adhesin isolated from BN406 to the basement membrane and Bowman's capsule (Fig. 1). Thus, the receptor molecules on the Dr antigen in erythrocytes and kidney tissue seem to be similar.

DISCUSSION

In vitro recombinant DNA techniques have permitted us to construct a recombinant molecule that encodes the O75X adhesin (9). The results presented in this report indicate that the Dr blood group antigen, a component of the IFC blood group complex, is the receptor for an adhesin of uropathogenic *E. coli*. We propose the term Dr hemagglutinin for the O75X fimbrialike adhesin. Studies with *E. coli* BN53, the recombinant *E. coli* BN406, and the purified adhesin from BN406 revealed that the structures recognized by the adhesin were present in tubular basement membrane and Bowman's capsule; hence, Dr antigen expressed in the kidney substructures serves as a receptor for some uropathogenic *E. coli* (6, 9, 10).

Although the molecular structure of the Dr antigen is not defined, the results presented in this paper indicate that the receptor moiety in the Dr blood group antigen recognized by the Dr hemagglutinin is a chloramphenicol-like molecule. Attachment of Dr^+ bacteria and purified Dr hemagglutinin to human tissue was inhibited by chloramphenicol. Chloramphenicol analogs were less active than chloramphenicol, which suggests that all structures on the benzene ring and at the C-2 and C-3 positions contribute to binding of the Dr hemagglutinin.

Since chloramphenicol is not known to be a natural product of human biochemical pathways, we analyzed several natural compounds and the influence of a variety of substitutions or modifications on their activity. In our study, some compounds with a hydroxyl group on the benzene ring were weakly active. The amino acid tyrosine is similar to chloramphenicol, with replacement of the nitro group on the benzene ring by a hydroxyl group (Fig. 2). However, tyrosine was not inhibitory. Among tyrosine-containing compounds, *t*-butoxycarbonyl-*O*-benzyl-L-tyrosine showed relatively high activity, though it was still 100-fold less than that of chloramphenicol. Our results indicate that a tyrosine-

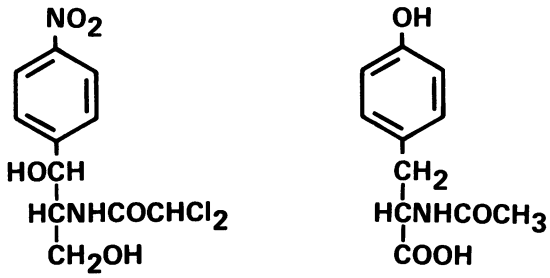

FIG. 2. Chemical structures of chloramphenicol and *N*-acetyl-L-tyrosine. Note the similarity of structure between the two compounds.

containing molecule could be a part of the receptor moiety for the Dr hemagglutinin; *N*-acetyl-L-tyrosine also showed some inhibitory activity. Thus, the Dr hemagglutinin, like the M hemagglutinin (21), may recognize a protein-containing receptor. On the other hand, chloramphenicol is dramatically more active than other receptor analogs known for *E. coli* hemagglutinins; for example, the MIC of the inhibitor for S fimbriae is 2.3 mM, that of the inhibitor for P fimbriae is 0.4 mM, and that of the inhibitor for type 1 fimbriae is 0.5 mM (5, 7, 13, 14). This indicates that the receptor portion of the Dr antigen is very similar to chloramphenicol.

The density and accessibility of bacterial receptors on host epithelial cells are believed to affect host susceptibility to certain infectious diseases (15). An important implication of the present work is that the basement membrane and Bowman's capsule are the kidney substructures with the highest density of Dr blood group antigen. A high density of Dr antigen in Bowman's capsule may permit Dr hemagglutinin-positive strains to colonize the upper urinary tract and then, after invasion, interact with Dr-rich interstitial tissue elements, leading to chronic nephritis. The efficiency of chloramphenicol in blocking attachment of Dr hemagglutinin-positive *E. coli* to Dr receptor in the tissue gives us a very attractive tool for studying protection against and/or treatment of the infection caused by Dr hemagglutinin-positive *E. coli*.

The molecular nature of the receptor on target tissue, its possible role in pathogenicity, and applications of Dr hemagglutinin for tissue staining are currently under study.

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