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The Dr Hemagglutinin, Afimbrial Adhesins AFA-I and AFA-III, and F1845 Fimbriae of Uropathogenic and Diarrhea-Associated *Escherichia coli* Belong to a Family of Hemagglutinins with Dr Receptor Recognition

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The receptor specificities of four *Escherichia coli* cloned hemagglutinins, AFA-I, AFA-III, F1845 fimbriae, and the Dr hemagglutinin were studied. Evidence is provided that all four hemagglutinins recognize as their receptor the Dr blood group antigen. However, results of experiments using enzyme-treated erythrocytes and monoclonal antibodies indicate that the four adhesins recognize different epitopes on the Dr antigen and thus constitute a family of Dr receptor-recognizing bacterial adhesins. Furthermore, the same results suggest that the Dr antigen itself may be divided into subcomponents on the basis of bacterial adhesins.

In recent years, attempts have been made to study non-P, or so-called X, adhesins from uropathogenic *Escherichia coli* (6, 21). One such adhesin of unknown receptor specificity, O75X, has been found to recognize a receptor on the Dr^a blood group antigen (14). According to the nomenclature used for P, M, G, S, and, recently, F fimbriae, the O75X adhesin has been renamed the Dr hemagglutinin (6, 10, 17, 19). Mannose-resistant hemagglutination (MRHA) caused by the Dr hemagglutinin is inhibitable by chloramphenicol and modified tyrosine. Our observations suggest that the Dr blood group receptor for the Dr hemagglutinin is located on the decay-accelerating factor (DAF), one of the cell membrane proteins that regulate the complement cascade and protect erythrocytes from being lysed by autologous complement (13). A recent study by Westerlund et al. (22) indicated that the Dr hemagglutinin is also capable of binding to type IV collagen and that this binding is likewise chloramphenicol sensitive. We recently proposed that Dr tissue receptors lining colonic glands and the urinary tract from the bladder to the kidney and pelvis may mediate colonization of the colon, followed by ascending infection of the urinary tract (16). Hybridization with *dra* genes, recently cloned and characterized, showed that *draD*-positive *E. coli* strains are common in girls with their first documented bladder infection and that the Dr hemagglutinin might be the first virulence factor shown to be associated with cystitis (15).

The afimbrial adhesins AFA-I and AFA-III of uropathogenic *E. coli*, the recently cloned fimbrial adhesin F1845 from a diarrhea-associated strain of *E. coli* (S. S. Bilge, C. R. Clausen, W. Lau, and S. L. Moseley, submitted for publication), and the Dr hemagglutinin exhibit similarities in both the organization and size of their gene products (8, 9, 15). Furthermore, AFA-III, the F1845 fimbriae, and the Dr hemagglutinin were all cloned from strains belonging to the O75 serogroup. This communication reports a comparative analysis of the receptor specificities for AFA-I, AFA-III, F1845, and the Dr hemagglutinin, providing evidence that all four hemagglutinins belong to a family of hemagglutinins recognizing the Dr receptor.

The bacterial strains used were EC901(pBJN406), HB101 (pILL22), HB101(pILL115), and HB101(pSSS1), which encode the Dr hemagglutinin, AFA-I, AFA-III, and the F1845 fimbriae, respectively (8, 15; S. S. Bilge et al., submitted). Bacteria were grown on Luria agar supplemented with ampicillin (100 μg/ml) or chloramphenicol (20 μg/ml) if required (9, 15).

To study the receptor specificities of the four adhesins, MRHA tests with different human erythrocytes were performed as described previously (12). Strains expressing the Dr, AFA-I, AFA-III, and F1845 hemagglutinins were suspended in phosphate-buffered saline to a concentration that gave an identical minimal hemagglutination titer of 1:128 with a 3% (vol/vol) suspension of normal, untreated erythrocytes. Undiluted suspensions were used for MRHA experiments, except for antibody-treated erythrocytes. Erythrocytes lacking high-incidence receptors of the IFC-related blood group complex and those from patients with paroxysmal nocturnal hemoglobinuria (PNH), composed of 98% PNH type III (PNHIII) (DAF^-) and 2% PNHI (DAF^+) cells, have been previously described (4, 13). All four recombinant bacterial strains reacted with erythrocytes of all phenotypes except the IFC-negative, Dr^a-negative, and PNHIII erythrocytes (Table 1). Dr^a-positive erythrocytes lacking other components of the IFC complex reacted strongly. These results indicate that the receptor for all four adhesins is located on the Dr antigen, yet unlike the Dr hemagglutinin, MRHA by F1845, AFA-I, and AFA-III was not inhibitable by chloramphenicol (Table 2).

To study the possibility that different epitopes of the Dr antigen were recognized by the different adhesins, the MRHA capacity of each strain was tested with enzyme-treated normal O blood group erythrocytes, prepared as previously described (4), and with erythrocytes preincubated with monoclonal antibodies against DAF. Enzyme-treated erythrocytes were tested with a panel of antibodies that are known to react or to not react with properly

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TABLE 1. Recognition of erythrocytes from individuals lacking high-frequency antigens of the IFC complex by the Dr, AFA-I, AFA-III, and F1845 hemagglutinins

Patient or donor	Phenotype	MRHA by *E. coli* harboring recombinant plasmid[a]:			
		pBJN406 (Dr^+)	pILL115 (AFA-III)	pILL22 (AFA-I)	pSSS1 (F1845)
1	Dr (a−)	−	−	−	−
2	Dr (a−)	−	−	−	−
3	IFC−[b]	−	−	−	−
4	Tc (a−b−c+)	+++	+++	+++	+++
5	Cr (a−)	+++	+++	+++	+++
6	Es (a−)	+++	+++	+++	+++
7	Wes (b−)	+++	+++	+++	+++
8	PNHIII[b]	−	−	−	(−)

[a] −, No reaction; +++, strong reaction; (−), weak reaction (can be observed after 5 min).
[b] Erythrocytes lacking all five antigens of the complex.

enzyme-treated erythrocytes (4). This procedure, routinely used in blood serology, allows standardization and reproducibility of experimental conditions for enzyme-treated erythrocyte membranes. Inhibition of MRHA by monoclonal antibodies BRIC110 and BRIC128 (kindly provided by D. J. Anstee, Southwestern Regional Blood Transfusion Center, Southmead, Bristol, United Kingdom) was performed by incubating 20 μl of each antibody with 100 μl of a 3% suspension of erythrocytes for 30 min on ice, followed by three washes with phosphate-buffered saline, pH 7.1 (18). Bacterial suspensions of minimal hemagglutination titer (1:128) were diluted 1:64 and used in hemagglutination tests with an equal volume of a 3% (vol/vol) suspension of erythrocytes.

Pronase and chymotrypsin treatment of erythrocytes alters the Dr^a antigen so that treated erythrocytes are no longer agglutinated by alloantibody to this blood group antigen (2, 3). In contrast, trypsin does not affect Dr^a in a similar manner. As expected, therefore, none of the four hemagglutinins of recombinant strains agglutinated the chymotrypsin-treated erythrocytes (Table 2). Pronase-treated erythrocytes were not agglutinated by AFA-I, AFA-III, or the Dr hemagglutinin. However, F1845 gave very weak MRHA, being negative at first but exhibiting a weak, granular reaction if incubated longer than 5 min. AFA-III, F1845,

TABLE 2. Hemagglutination of normal human erythrocytes treated with enzymes, antibody, or receptor analog by the Dr, AFA-III, AFA-I, and F1845 hemagglutinins

Treatment of erythrocytes	MRHA by *E. coli* harboring recombinant plasmid[a]:			
	pBJN406 (Dr^+)	pILL115 (AFA-III)	pILL22 (AFA-I)	pSSS1 (F1845)
Untreated	+++	+++	+++	+++
Chymotrypsin	−	−	−	−
Pronase	−	−	−	(−)
Trypsin	+++	+++	(−)	+++
Papain	+++	+++	+++	+++
Chloramphenicol	−	+++	+++	+++
Anti-DAF, BRIC110	−[b]	++	++	+
Anti-DAF, BRIC128	+++	+	++	+++

[a] +++, Strong reaction; −, no reaction; (−), weak reaction (can be observed after 5 min).
[b] Weak positive reaction can be observed if increased concentrations of bacteria are used.

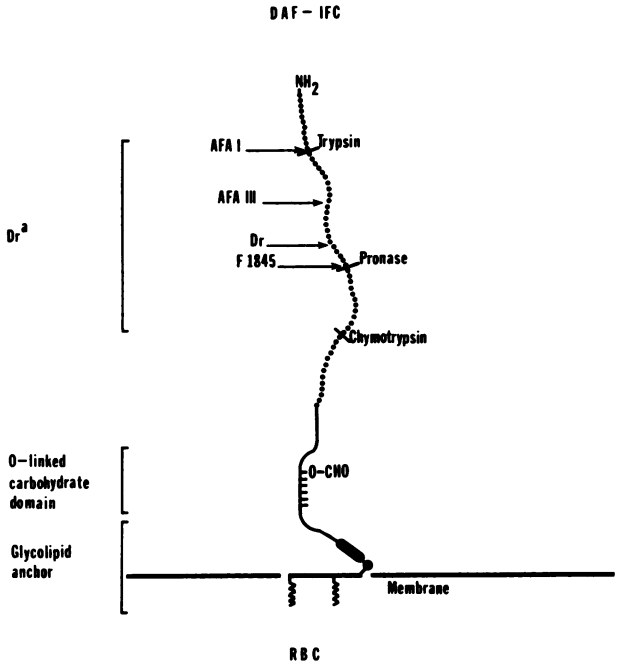

FIG. 1. A hypothetical simplified model of the DAF molecule proposing Dr blood group antigen and receptor binding sites for the Dr, AFA-I, AFA-III, and F1845 hemagglutinins. RBC, erythrocyte.

and Dr adhesins strongly agglutinated the trypsin-treated erythrocytes, but AFA-I exhibited a weak, delayed hemagglutination. Furthermore, only the Dr hemagglutination was inhibited by chloramphenicol. A hypothetical model of the DAF molecule is shown in Fig. 1 (11). The Dr blood group antigen, thought to be a portion of the DAF-IFC glycoprotein, and proposed cleavage sites for tested proteases are indicated on the simplified model for clarity. The various proteases cut the Dr peptide chain at different sites and release different portions from the membrane. Chymotrypsin may cleave close to the membrane, because chymotrypsin-treated erythrocytes were not agglutinated by any of the four bacterial clones. This is consistent with the finding that chymotrypsin-treated erythrocytes are not agglutinated by antibodies to the Dr blood group antigen (2, 3). Pronase may cut the Dr antigen further out from the membrane and remove the part of the peptide chain recognized by AFAs and Dr but not that recognized by F1845. The result with trypsin suggests that the receptor site for AFA-I recognition is further out from the membrane than the sites for AFA-III, Dr, and F1845. Other hypothetical models including those requiring multiple forms of the Dr blood group antigen are also consistent with the data presented. These results suggest that the four hemagglutinins may recognize different receptor sites on the Dr blood group antigen.

Results of inhibition of MRHA by the monoclonal antibodies confirmed the differences in receptor specificities observed with enzyme-treated cells and, furthermore, seem to distinguish between AFA-III and F1845 (Table 2). Erythrocytes preincubated with anti-DAF monoclonal antibody BRIC110 were not agglutinated by the Dr^+ strain at a minimal hemagglutinin titer of 1:64, but were agglutinated weakly if the concentration of bacteria was doubled. BRIC110 was less effective in inhibiting MRHA by the other three strains. The anti-DAF monoclonal antibody BRIC128 was unable to completely inhibit any of the four strains but

was the most effective against AFA-III. This study has provided evidence that the four adhesins, AFA-I, AFA-III, the Dr hemagglutinin, and F1845 fimbriae, recognize the Dr blood group antigen but also that the four adhesins recognize different epitopes of that antigen, and thus each probably represents a unique adhesin belonging to a family of Dr receptor-recognizing hemagglutinins.

Uropathogenic *E. coli* strains express types of P fimbriae and associated adhesins that differ antigenically and, as recently described, in their receptor specificities (5, 7, 10, 18). This study indicates that an analogous situation exists among hemagglutinins recognizing the Dr blood group antigen. The F1845 structural protein exhibits only limited homology with the Dr hemagglutinin structural protein deduced from the Dr hemagglutinin gene sequence, and the two adhesins differ in antigenic and binding properties (T. Swanson, S. Bilge, B. Nowicki, S. Hull and S. Moseley, manuscript in preparation). The genes for the Dr hemagglutinin, AFA-I, and AFA-III were all cloned from uropathogenic *E. coli* strains, and strains containing DNA sequences homologous to those of the Dr hemagglutinin gene were recently shown to be associated with cystitis (8, 15). Genes for the F1845 fimbriae were cloned from *E. coli* isolated from a child with protracted diarrhea. Archambaud et al. also showed that AFA-related sequences were present in 7.6% of enteropathogenic *E. coli* (1). The high density of Dr receptor binding sites in the kidney interstitium and in colonic glands may facilitate colonization of the urinary tract and lead to chronic interstitial nephritis (16). Colonization of a digestive tract rich in Dr receptors may be important for the development of protracted diarrhea. Additional studies with appropriate tissues may determine if differences in receptor density or in binding specificities could account for the different locations of infection and clinical origins of the strains, e.g., diarrhea versus urinary tract infections.

The use of bacterial hemagglutinins has also provided additional information regarding the Dr blood group antigen. The finding that anti-DAF monoclonal antibody BRIC110 inhibited MRHA by the Dr hemagglutinin supports an earlier study indicating that the Dr receptor may be a portion of DAF (13). Differences in agglutination of enzyme-treated erythrocytes by the four adhesins suggests that there is either one Dr^a antigen with separate binding sites for each adhesin or more than one form of Dr blood group antigen that may offer binding sites. The Dr^a blood group antigen, heretofore considered serologically indivisible, may be divided into subcomponents on the basis of bacterial adhesins.

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