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Virulence Factors in *Escherichia coli* Urinary Tract Infection

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INTRODUCTION

Escherichia coli, the most prevalent facultative gram-negative bacillus in the human fecal flora, usually inhabits the colon as an innocuous commensal. According to the special pathogenicity theory (411), special properties enabling *E. coli* to overcome host defenses in a new environment are necessary in order for it to escape the limitations of the colonic mileu and move into new niches devoid of competition from other bacterial species (111, 411). Virulence (from the Latin word for poisonous) is defined as the ability of an organism to cause disease in a particular host. In *E. coli*, virulence results from the cumulative impact of one or several special properties, or virulence factors (VFs), which serve to distinguish potential pathogens from harmless intestinal strains (Fig. 1).

The practical goal of investigations into the virulence properties of any pathogen is the development of specific anti-VF interventions (such as vaccines) to prevent infection (191). Urinary tract infection (UTI) is the most common form of extraintestinal *E. coli* infection, and *E. coli* is the most common cause of UTI. At some point during their lives, at least 12% of men and 10 to 20% of women experience an acute symptomatic UTI (231, 312) and an even greater number develop asymptomatic bacteriuria (ABU). More than 100,000 patients are hospitalized annually in the United States because of renal infection (231), with its attendant risk of gram-negative sepsis and death. In the past decade there has been a virtual explosion of information regarding the VFs of *E. coli* associated with UTI (111, 191, 411, 516). This review summarizes the current state of knowledge regarding the genetics, mechanisms of action, and clinical significance of the VFs thought to be most important in *E. coli* UTI.

INVESTIGATION OF VFs

At the most basic epidemiological level, potential urovirulence factors (uro-VFs) are identified by comparing the prevalence of the bacterial property of interest among urinary isolates with that among fecal strains from healthy control subjects. More refinement is possible if the urinary isolates can be categorized according to the severity of the clinical syndrome, i.e., acute pyelonephritis (most severe; associated with fever, chills, and flank pain from renal inflammation), cystitis (moderately severe; associated with burning and pain on voiding plus, possibly, suprapubic pain or tenderness from bladder inflammation), or ABU (least severe; characterized by the total absence of symptoms) (149, 231). Comparisons of prevalence of the property of interest can then be made between the various UTI syndromes. Renal scarring has also been used as an endpoint in epidemiological studies; however, as described below, little has been learned about specific VFs contributing to renal scarring. Determination of the upper (kidney and ureter) or lower (bladder and urethra) urinary tract source of UTI isolates by localization methods (bladder washout, ureteral catheterization, or the antibody-coated-bacteria test) (231) allows more-precise definition of the anatomical significance

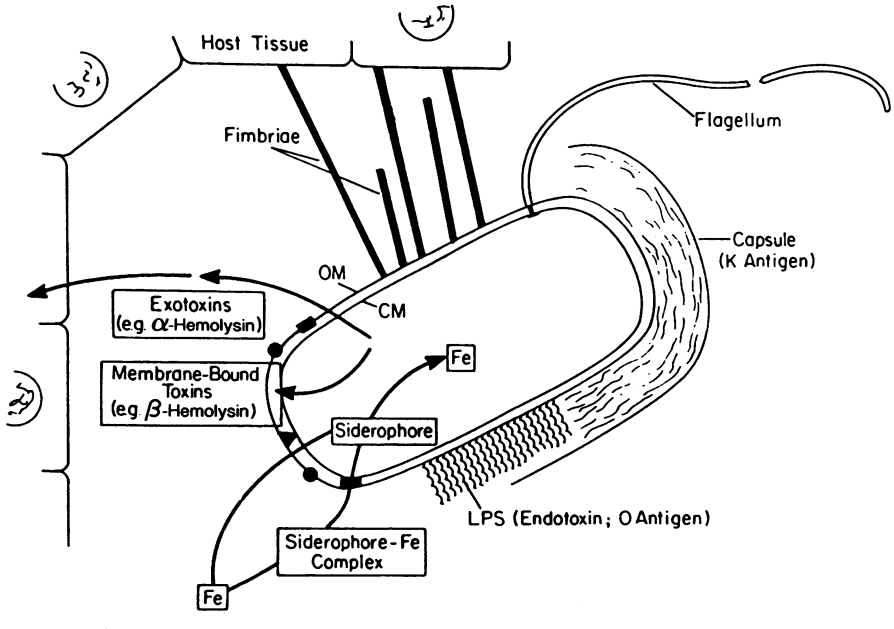

FIG. 1. Schematic representation of an *E. coli* cell interacting with host tissue, highlighting features relevant to bacterial pathogenicity. Membrane proteins involved in transport, serum resistance, etc., are indicated by solid black circles, triangles, and rectangles. OM, Outer membrane; CM, cytoplasmic membrane; LPS, lipopolysaccharide. Adapted from reference 111, with permission from the publisher.

of suspected VFs. In addition, comparisons between different host populations (boys versus girls, pregnant versus nonpregnant women, patients with normal versus abnormal or instrumented urinary tracts, etc.) clarify the host-parasite interactions related to a particular VF. Combined results from multiple different epidemiological studies (as compiled for this review) may better reflect the true prevalence of a VF among uroisolates associated with a particular UTI syndrome or patient group than can the results of a single study alone. However, such totaled results can also conceal what may be important differences between individual studies (resulting from different methods and definitions, different study designs, and different populations studied) and so must be interpreted with caution.

Human epidemiological studies can identify associations between certain bacterial properties and UTI, but direct assessment of the contribution to virulence of these properties requires the use of animal models. Here, selection of an appropriate animal model and of appropriate bacterial strains is crucial. Models involving nonphysiological manipulations of the urinary tract (renal trauma, ureteral ligation, direct intrarenal injection, etc.) or intravenous injection of bacteria do not faithfully reproduce human UTI (242). Human infection most commonly occurs in patients with anatomically and functionally normal urinary tracts and involves spontaneous ascent of bacteria from the urethra to the bladder and (in a minority of patients) to the kidney and bloodstream. The animal species used must have in common with humans those aspects of the urinary tract that are important in the function of the VFs being studied, e.g., cell surface receptors for adhesins (see P fimbriae below). Comparisons of the virulence of wild-type strains that differ in the property of interest are the simplest way to study that property's contribution to virulence, but as in epidemiological studies in humans, this approach leaves unanswered the question of whether the property itself or associated factors are responsible for observed differences in virulence. Differences can be attributed to the property in question with greater confidence when strains that are genetically identical except for the factor of interest are compared. The more precise the methods used to derive such isogenic strains (e.g., site-directed mutagenesis), the greater the likelihood that the factor of interest is the only variable affecting virulence.

Finally, mechanisms of action of possible VFs identified through epidemiological or animal studies are commonly determined in vitro at the cellular or subcellular level. Such information often strengthens the case that a particular property plays a role in virulence and may suggest ways to interfere with its function. Some of the purported VFs of *E. coli* are discrete bacterial structures or products (e.g., fimbriae and hemolysin), in which case the laboratory is helpful in clarifying their functional significance. In contrast, other VFs are functionally defined properties (e.g., serum resistance), in which case the laboratory is needed to determine the responsible bacterial structures or products.

ADHERENCE

Adherence to solid substrates is a property common to many pathogenic microorganisms, including viruses, gram-positive and gram-negative bacteria, yeasts, and protozoa (17). By attaching to host structures, microbial pathogens avoid being swept along by the normal flow of body fluids (blood, urine, intestinal contents) and eliminated (439), although host cells with adherent bacteria can be shed, thereby eliminating the organisms despite attachment (400). Attachment is considered a necessary first step in the colonization of host mucosal surfaces and a precedent to invasive infection in many situations (439, 513, 522, 612).

Uroepithelial-Cell Adherence and Hemagglutination

In the late 1970s it was recognized for the first time that strains of *E. coli* causing UTI typically agglutinate human erythrocytes despite the presence of mannose (mannose-resistant hemagglutination [MRHA]) (102, 122, 162) and adhere to human uroepithelial cells (512, 517, 518, 526). Also, adherence to uroepithelial cells is usually unaffected by mannose (mannose-resistant adherence) and is more common among strains exhibiting MRHA than among those exhibiting only mannose-sensitive hemagglutination (64, 181, 440, 516). The close association observed in individual strains between epithelial-cell adherence and MRHA (198, 248, 252, 597) was explained by the discovery that among most urinary isolates, both properties are mediated by fimbriae (see below) (224, 248, 278, 524, 597).

Fimbriae as Mediators of Uroepithelial-Cell Adherence and MRHA

The observation that both MRHA and epithelial-cell adherence are mediated by fimbriae is consistent with the results of studies by Duguid et al. (101, 102, 105). They established that the agglutination of erythrocytes by clinical isolates of *E. coli* is due to bacterial attachment to and cross-linking of erythrocytes via thin fiberlike appendages (Fig. 2), which these investigators termed fimbriae (from the Latin word for threads or fringe). Brinton later named these structures pili (from the Latin word for hairs) and showed that they retained their hemagglutinating capacity when sheared from bacteria and purified (45). Fimbriated strains also bind to leukocytes, platelets, spermatozoa, yeast cells, pollen, latex beads, and spores (46, 105), demonstrating that hemagglutination is one example of the general phenomenon of bacterial attachment rather than a unique interaction of bacteria with erythrocytes (105).

Fimbriae are morphologically and functionally distinct both from flagella, which are thicker, longer, more flexible appearing, and responsible for motility but not for attachment (105), and from sex pili, which are thicker and function in conjugation but not in attachment to other surfaces (45, 46). According to Brinton's structural analysis, type 1 fimbriae (which can also serve as a model for other fimbrial types) have a diameter of 7 nm, a length of 0.5 to 2 μm, and a 0.2- to 0.25-nm-diameter central axial hole (Fig. 3) (46). They are composed of repeating subunits polymerized in a helix, with 3 and 1/8 subunits per turn (46). Whether the term fimbriae or pili is preferable for these adherence organelles is controversial (46, 82, 101, 104, 463). Favoring the term fimbriae are its priority, the simple adjectival form (fimbrial), and the distinction it emphasizes between adhesive appendages and sex pili. On the other hand, pili is simpler, there is a readily understood singular form (pilus), and there is a simple term for structural subunits (pilin). The dispute over which term should take precedence is moot, however, since both are in common use and are generally understood to be synonymous.

Adherence Assays

Bacterial adherence to epithelial cells or cell monolayers is studied by incubating cells and bacteria together to allow

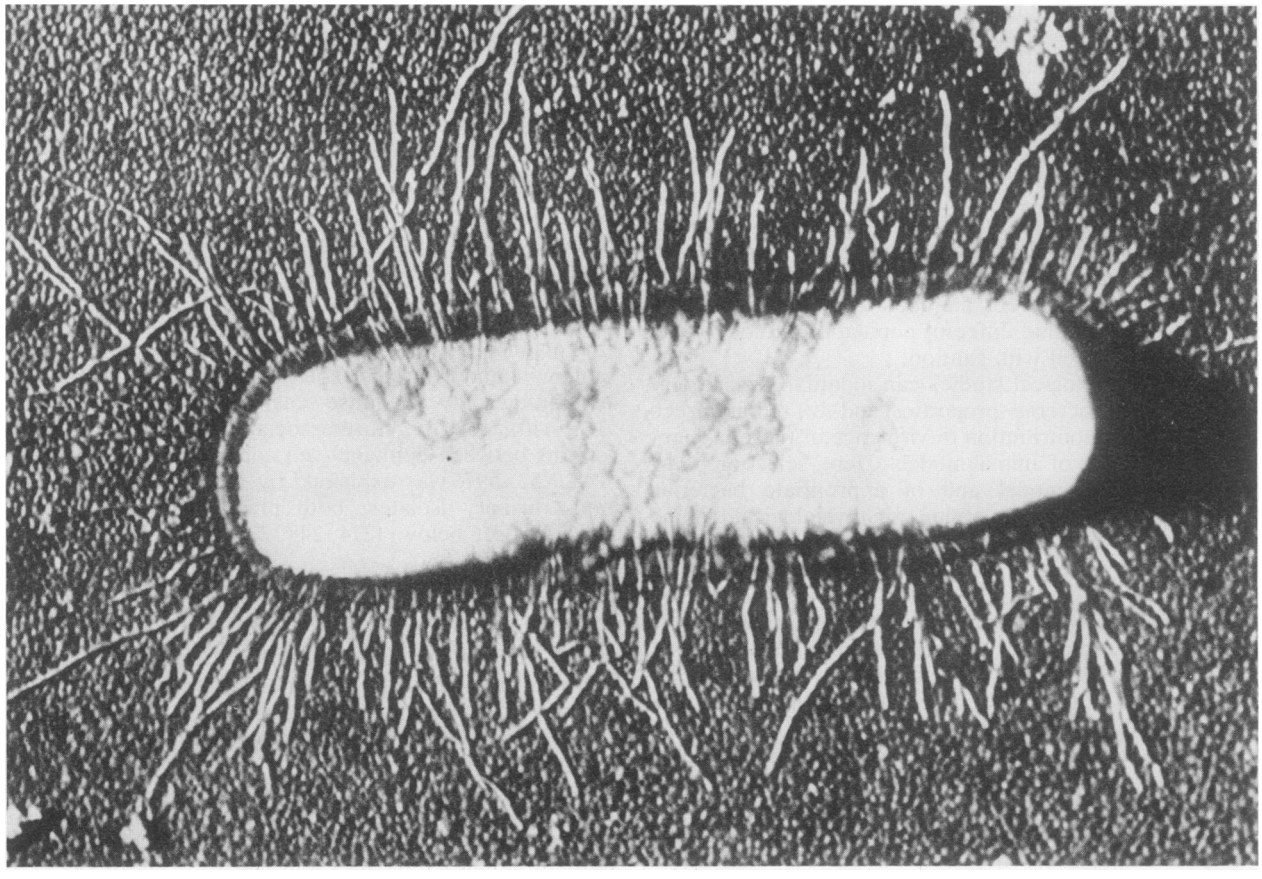

FIG. 2. An early electron photomicrograph of a fimbriated *E. coli* cell, showing protoplast, cell wall, about 200 fimbriae, and no flagella. Fixed with formaldehyde and shadow-cast at 15°. ×45,000. From reference 105, with permission from *The Journal of Pathology*.

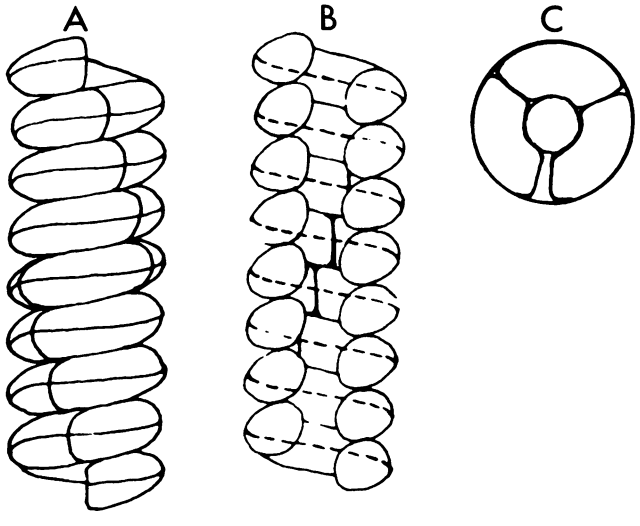

FIG. 3. Model of type 1 pili (fimbriae): side view (A), cross section (B), and end view (C). Outer diameter, 7 nm; inner diameter, 2 to 2.5 nm; pitch distance of helix, 2.32 nm; 3 and 1/8 subunits per turn; M_W of subunit, 17,000. From reference 46, with permission from the publisher.

adherence, removing unattached bacteria by repeated washings (512) or filtration (64, 425, 474), and determining the number of adherent bacteria remaining by using microscopy (64, 485, 512) or radiometric techniques (425, 474, 475). A number of technical considerations influence the reliability of such assays (64, 463), including the growth conditions used to prepare bacteria (224, 517), the origin of the epithelial cell (62, 224, 524), the cell type (i.e., squamous or transitional) (263, 438, 512, 517, 527), and, possibly, the viability of the cell (64, 252, 438, 463, 512). Adherence of some strains to Tamm-Horsfall protein (THP) (uromucoid, urinary slime) complicates the interpretation of adherence tests, as THP often coats uroepithelial cells (64, 463) (see Type 1 fimbriae below).

Effect of Antimicrobial Agents on Adherence

The ability of bacteria to mediate MRHA or to adhere to epithelial cells is influenced by prior exposure to subinhibitory concentrations of a variety of antimicrobial agents. Ampicillin, sulfonamides, trimethoprim, and tetracycline decrease but nalidixic acid increases hemagglutination and epithelial-cell adherence (183, 471, 508, 530, 555, 582, 583).

Association of Adherence With Other VFs

MRHA or type VI hemagglutination (123) and uroepithelial-cell adherence are more prevalent among strains of certain O serogroups, especially O1, O2, O4, O6, O7, O8,

and O18 (102, 122, 123, 162, 512, 518, 566). These adherence properties are also associated with the *E. coli* K1 (77, 123) or K12 (518) capsular antigen, although the O-group association may be primary (518). MRHA and fimbriation are common among hemolysin-producing strains (77, 105, 122, 162, 178, 198), and $MRHA^+$ hemolytic strains release more histamine from mast cells than do $MRHA^-$ hemolytic strains (168); these observations suggest that mannose-resistant adhesins and other VFs may function in an additive or synergistic fashion and be jointly selected for in uropathogenic strains.

Host-Pathogen Interactions in Adherence

Adherence of bacteria to epithelial cells is a function of the host as well of the pathogen. Isolates taken during UTI episodes in patients with recurrent UTI (RUTI) adhere no better to control vaginal epithelial cells than do other strains (134), but vaginal (or buccal, uroepithelial, or periurethral) cells from such patients have a greater adherence capacity for standard *E. coli* strains than do comparable cells from control patients (50, 133, 252, 256, 425, 440, 475, 476, 512, 525). Uroepithelial cells from UTI-free patients may even possess an antibacterial property that is diminished in cells from patients with RUTI (485). Uroepithelial cells collected near the middle of the menstrual cycle may have an increased bacterial adherence capacity (438, 474, 527), although this is not a consistent finding (133, 463, 475, 527). Estrogen treatment increases the bacterial adherence capacity of rat bladders (501) and HeLa cells (511). However, whether hormonal fluxes account for cyclic changes in bacterial adherence capacity in women is uncertain, since there is no apparent increase in adherence capacity associated with the use of oral contraceptives (475) or with estrogen replacement therapy after menopause (132). Increasing age is not consistently associated with an increased epithelial-cell adherence capacity (440, 475, 502).

Epidemiology and Animal Studies

Because fimbriation, hemagglutination, and epithelial-cell adherence properties were absent from organisms collected directly from the urine of some infected patients (190), several investigators have concluded that fimbriation and adherence are not relevant in vivo phenomena (190, 191, 463). However, using more-sensitive methods and more-careful patient selection, others have found evidence of fimbriation and adherence in bacteria in a substantial proportion of urine specimens from infected patients (313, 390, 427), demonstrating that fimbriae are expressed in vivo and do mediate adherence during infection.

The prevalence and degree of bacterial adherence to uroepithelial cells are closely associated with the clinical category of UTI. Of isolates from patients with pyelonephritis or bacteremia, 70 to 100% adhere to uroepithelial cells (with a mean of approximately 30 bacteria per cell), compared with 50 to 60% of strains (with about 20 bacteria per cell) among cystitis patient isolates, 22 to 36% of strains (with about 10 bacteria per cell) among ABU patient isolates, and 10 to 36% of fecal strains (with about seven bacteria per cell) (181, 198, 338, 469, 470, 472, 509, 512, 518, 527). Hemagglutination testing yields similar results, with the highest proportion of MRHA strains among pyelonephritis patient isolates (50 to 81%) and progressively lower proportions among cystitis patient isolates (17 to 52%), ABU patient isolates (11 to 19%), and fecal isolates (2 to 29%) (48, 49, 102, 139, 162, 181, 315, 316, 354, 399, 423, 509, 566). This evidence indicates that mannose-resistant uroepithelial-cell adherence and MRHA are characteristic of strains with an increased ability to cause UTI (especially the more clinically severe forms) and suggests that these properties may contribute directly to urovirulence. Strains mediating MRHA were more virulent than non-MRHA strains in animal models in some (198, 264, 566, 567) but not all (360) studies. Confirmation that *E. coli* adhesins mediating MRHA and mannose-resistant uroepithelial-cell adherence contribute to uropathogenicity followed further elucidation of the binding specificities, structure, and genetics of these adhesins (see below) (Table 1).

MANNOSE-RESISTANT ADHESINS

The mannose-resistant adhesins of *E. coli* strains exhibiting MRHA are diverse, as indicated by the variety of patterns in which they agglutinate the erythrocytes of different species and blood groups (105, 123, 584). On the basis of receptor specificity, these adhesins can be considered two groups: (i) those recognizing P blood group antigens (P fimbriae) and (ii) others, termed X adhesins or X fimbriae. As receptor specificity has been determined for additional mannose-resistant adhesins (e.g., S, M, F, Dr [Table 1]), the proportion of strains properly included in the X (or unknown specificity) category has progressively decreased.

P FIMBRIAE

Receptors

Gal(α1-4)Gal specificity. The search for uroepithelial-cell receptors to which urinary bacteria adhere in the presence of mannose led to the discovery that most adhering strains agglutinate human erythrocytes of the P_1, P_2, and P_1^k but not the p blood group (250, 251, 254, 298) (Table 2), evidence suggesting that most of these strains bind to P blood group antigens (Table 2). Purified fimbriae from these strains agglutinate human erythrocytes with the same binding specificity as the bacteria from which they are derived, leading to the designation P fimbriae (278, 281, 388).

The P blood group antigens (Table 3) are a family of oligosaccharides containing a terminal or internal Gal(α1-4)Galβ moiety (Gal-Gal) (Fig. 4) that are present on certain mammalian cells primarily as the carbohydrate component of glycosphingolipids (210, 337). The P_1 antigen is also present in glycoproteins in humans (598) and is found in pigeon and dove eggs (135) and hydatid cysts (47) and on certain enteric bacteria (461).

Several lines of evidence confirm that glycolipids containing the Gal-Gal moiety are receptors for adhering *E. coli* strains and that this moiety is the major determinant of binding. First, strains expressing P-antigen-specific adherence bind to surfaces on which the Gal-Gal moiety is present either naturally (i.e., erythrocytes or uroepithelial cells from individuals of the P_1, P_2, or P_1^k blood group) or by the application of Gal-Gal-containing substances (e.g., cell membrane glycolipid fractions, purified glycolipids, or Gal-Gal-containing synthetic compounds) to structures otherwise not receptive to P-antigen-specific bacterial adherence (e.g., erythrocytes or uroepithelial cells from individuals of the p blood group, guinea pig erythrocytes, and latex beads) (88, 255, 287, 298, 299, 314, 516, 528, 531, 534). Second, agglutination of (or adherence to) structures with surface Gal-Gal epitopes is blocked by Gal-Gal-containing substances (250, 251, 255, 298, 299, 516, 531). Finally, P-anti-

TABLE 1. Adhesins of uropathogenic *E. coli*

Adhesin type	Synonym(s)	Fimbriae present	Receptor	Role in UTI[a]
Mannose resistant[b]				
P related				
P	Gal-Gal, Pap	+	Gal(α1-4)Gal	+++
F	Prs, Pap-2	+	Forssman antigen	++
ONAP		+	Gal(α1-4)Gal + A blood group trisaccharide	+/−
X				
Dr related				
Dr	O75-X	−	Dr blood group antigen[c]	++
AFA-I, AFA-III		−	Dr blood group antigen[c]	+
S		+	NeuNAc(α2-3)Gal	+/−
M		−	M blood group antigen (alpha-glycophorin)	+/−
G		+	GlcNAc	+/−
NFA-1, NFA-2		−	?	+/−
Mannose sensitive[d]				
Type 1 fimbriae (common pili)		+	Mannosides ± hydrophobic component	++
Miscellaneous		−	Mannosides	+/−
Other[e]				
F1C	Pseudo type 1	+	?	+/−

[a] Scale is +/− to +++ in order of increasing importance in UTI (based on human and animal model studies).
[b] Mannose-resistant agglutination of human erythrocytes.
[c] Chloramphenicol inhibits adherence mediated by the Dr hemagglutinin (O75-X) but not by AFA-I or AFA-III.
[d] Mannose-sensitive agglutination of guinea pig erythrocytes.
[e] Nonhemagglutinating.

gen-recognizing bacteria adhere to all Gal-Gal-containing glycolipids but not to glycolipids lacking the Gal-Gal moiety (35). Conformational analysis using hard-shell sphere calculations shows a bend in the saccharide chains of Gal-Gal-containing glycolipids at this disaccharide, with a largely hydrophobic surface exposed on the convex side that probably represents a significant portion of the binding epitope (Fig. 5) (35, 531).

P-fimbriated strains differ slightly in their binding specificity, some showing greater adherence to globoside than to globotriaosylceramide and some recognizing globoside but not the Gal-Gal moiety alone (88). Only about one-third of P-fimbriated strains recognize the internal Gal-Gal moiety of the Forssman antigen (Table 3), the predominant glycolipid antigen of sheep erythrocytes (299). Studies of sheep erythrocyte agglutination by strains expressing cloned P fimbriae (Pap, for pilus associated with pyelonephritis) yield conflicting results (260, 309, 323, 327). In some cases, Forssman antigen recognition is due to the presence of a variant adhesin termed Prs (for P-related sequence) or Pap-2, which binds weakly to globoside or Gal-Gal-coated latex beads but strongly to the terminal GalNAc(α1-3)GalNAcβ moiety of the Forssman antigen (260, 309, 323, 327). Some P-antigen-recognizing strains, termed ONAP (for O-negative, A-positive) (487), adhere better to P_1 erythrocytes of the A blood group than to those of the O or B blood group (469). These strains require both the Gal-Gal moiety and the A blood group trisaccharide [GalNAc(α1-3)Fuc(α1-2)Galβ] for hemagglutination (487) and bind with high affinity to globo-A and to the Forssman antigen but not to other globoseries glycolipids (309).

TABLE 2. P blood group antigens and phenotypes[a]

P blood group phenotype	Antigen(s) on erythrocytes	Frequency in population
P_1	P_1, P, $(P^k)^b$	75%
P_2	P, $(P^k)^b$	25%
P_1^k	P_1, P^k	Very rare
P_2^k	P^k	Very rare
p	None	Very rare

[a] See references 131 and 337.
[b] Small amounts of the P^k antigen are present on P_1 erythrocytes; P_2 erythrocytes contain even smaller amounts.

Distribution and density of receptors for P fimbriae. Receptors for P fimbriae are present on erythrocytes from humans, pigs, pigeon, fowl, goats, and dogs but not on those from cows, guinea pigs, or horses (424). Human erythrocytes and uroepithelial cells from individuals of the P_1 and P_2 blood groups do not differ in P-fimbrial receptor density (314), despite the presence on P_1 erythrocytes of an additional P blood group antigen (P_1) (337) and of greater amounts of the P^k antigen (131). Uroepithelial cells from men and women have a similar receptor density for P-fimbriated strains, as do squamous and transitional uroepithelial cells (314, 532).

Human polymorphonuclear leukocytes (hPMNLs) express only trace amounts of Gal-Gal-containing glycolipids (330), and the species that is present is a poor receptor for P-fimbriated organisms (35). Thus, in contrast to type 1 fimbriae (see below), P fimbriae do not promote adherence of bacteria to hPMNLs (515) and may partially protect strains that also express type 1 fimbriae from adherence to and killing by hPMNLs (34). After incubation of hPMNLs with globoside, P-fimbriated strain adherence is increased, presumably because globoside is incorporated into the hPMNL membrane (515).

Although Gal-Gal-containing glycolipids are only a minor component of the membrane glycolipids of shed human uroepithelial cells (519), they are the predominant species of human renal glycolipids (333, 341, 342). In histologic sections of human kidney tissue, antigloboside antibodies bind to proximal tubular epithelial cells and to occasional mesangial or endothelial cells but not to the distal tubule, collecting ducts, or Bowman's capsule (336). In contrast, fluorescein-

TABLE 3. Structures and nomenclature of Gal(α1-4)Gal-containing oligosaccharides[a]

Antigen	Structure of oligosaccharide[b]	Trivial name	Symbol (short form)
P^k	Gal(α1-4)Gal(β1-4)Glc-	Globotriaose	$GbOse_3$ (Gb_3)
P	GalNac(β1-3)Gal(α1-4)Gal(β1-4)Glc-	Globotetraose (Globoside[c])	$GbOse_4$ (Gb_4)
P_1	Gal(α1-4)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc-		
Forssman	GalNAc(α1-3)GalNAc(β1-3)Gal(α1-4)Gal(β1-4)Glc-		

[a] See references 210 and 337.
[b] Gal(α1-4)Gal moiety is underlined in each oligosaccharide.
[c] The ceramide conjugate of globotetraose (i.e., globotetraosyl ceramide).

labeled purified P fimbriae or whole P-fimbriated bacteria adhere to all these structures (260, 282, 378, 580) (Table 4). P fimbriae also bind to the epithelial and muscular layers of the bladder (260, 579, 580) and to a loosely adherent surface-associated substance on human colonic cells, possibly contributing to *E. coli* colonization of the human intestinal tract (614).

Structure and Genetics

Morphologically, P fimbriae exhibit the *E. coli* fimbrial structure described by Brinton (46) (see above) (281, 412). Each is composed of approximately 10^3 helically polymerized subunits, with one major subunit species (PapA) constituting the bulk of the fimbria (Fig. 6) (304). Three minor adherence-related fimbrial subunits (PapE, PapF, and PapG) are present in minute amounts at the fimbrial tips (304, 323, 450) (Fig. 7). These fimbrial proteins, as well as a number of accessory proteins, are encoded by a chromosomal multicistronic gene cluster termed *pap* (18, 376) (Fig. 8); alternative designations (*fso*, *fst*) are sometimes used for different P-fimbrial variants (450).

PapA, the major structural subunit, is necessary for the formation of fimbriae but not for Gal-Gal adherence, since even in the absence of fimbriae the adhesin complex can be expressed on the cell surface (306, 375, 376, 450) (Fig. 8). *papA* mutants of rough strains are nonfimbriated but still mediate Gal-Gal-specific adherence; however, *papA* mutants of smooth wild-type strains do not mediate Gal-Gal adherence, suggesting that extension of the adhesin away from the cell surface by its attachment to fimbriae is impor-

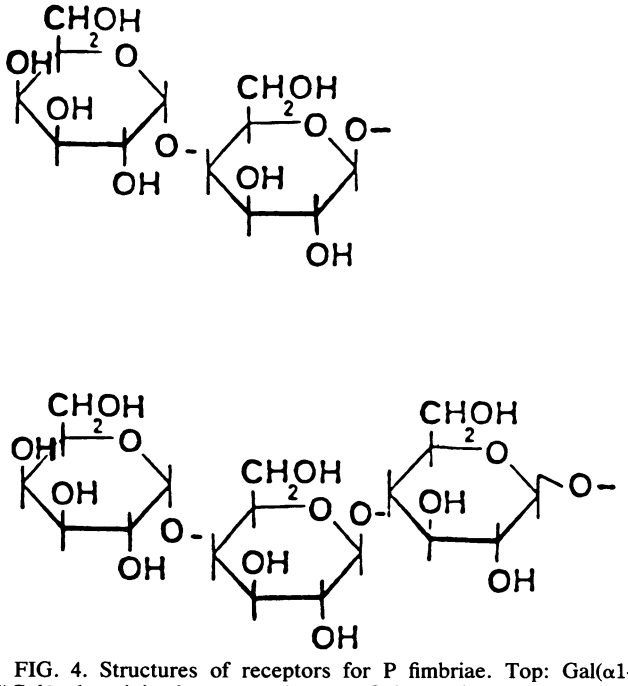

FIG. 4. Structures of receptors for P fimbriae. Top: Gal(α1-4)Galβ, the minimal receptor. Bottom: Gal(α1-4)Galβ(1-4)Glcβ, the P^k antigen(globotriaose), containing the minimal digalactoside receptor moiety as the terminal (leftward) disaccharide portion of the molecule. Adapted from reference 531, with permission from the publisher.

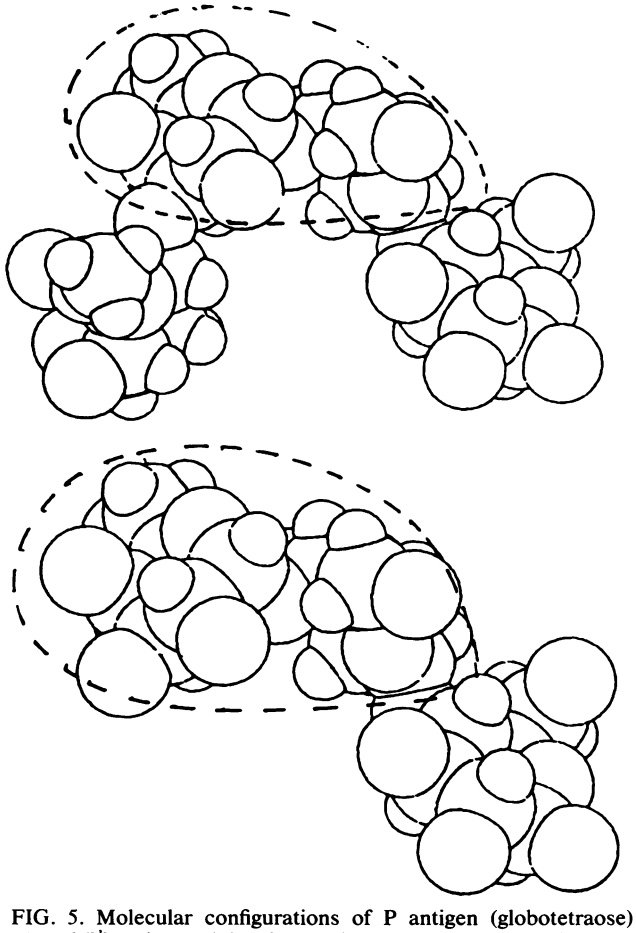

FIG. 5. Molecular configurations of P antigen (globotetraose) (top) and P^k antigen (globotriaose) (bottom). Dotted liens indicate the proposed P-fimbrial receptor binding areas. The conformational data are based on carbon-13 and proton nuclear magnetic resonance spectroscopy and hard-sphere atomic model calculations. From reference 531, with permission from the publisher.

TABLE 4. Binding of *E. coli* adhesins to human kidney and bladder sections and to epithelial cells in urine[a]

Tissue site	Adhesin binding[b]				
	S fimbriae	P fimbriae	Type 1 fimbriae	Type 1C fimbriae	O75X adhesin
Kidney[c]					
Bowman's capsule	+++	+++	–	–	+++[d]
Glomerulus	+++	+++	–	–	–
Proximal tubulus	++	++	+++	–	+++[d]
Distal tubulus	++	++	(+)	++	+++[d]
Collecting duct	++	+	(+)	++	+++[d]
Vessel walls	+++[e]	+++[e]	+++	+++[e]	–
Bladder					
Epithelium	++	+	–	–	+
Vessel walls	+++[e]	+++[e]	++	+++[e]	–
Muscular layer	+	+	+++	+	+
Connective tissue	++	–	–	–	+++
Urine					
Epithelial cells	+[f]	+[g]	–[g]	–[f]	+[g]

[a] Table is based on information from reference 580, used with the permission of the publisher.

[b] Symbols: –, +, ++, +++, undetectable, weak, moderate, and intense binding, respectively. Data are based on binding of purified adhesins and labeled bacteria.

[c] Data for S, P, and type 1 fimbriae and the O75X (Dr) adhesin are adapted from references 280, 282, 283, and 579.

[d] To basement membranes.

[e] Mainly to endothelial cells.

[f] Only positivity (+) and negativity (–) are indicated.

[g] Data for P and type 1 fimbriae and the O75X (Dr) adhesin are from references 278, 283, and 412.

tant for efficient adherence when O-polysaccharides coat the cell (304, 573).

In contrast to *papA* mutants, *papEFG* mutants produce normal-appearing fimbriae but lack Gal-Gal-specific adherence (304, 374). PapF-PapG constitutes the minimal adhesin complex, which is linked to fimbriae by PapE (82, 306, 321, 551). PapG is the actual adhesin molecule responsible for Gal-Gal specificity (195, 323). *trans* complementation of a *papG* mutant with the gene for an alternative adhesin (PrsG) yields fimbriated cells with Prs binding specificity (i.e., Forssman antigen instead of Gal-Gal), demonstrating substitution of the Prs adhesin for the Pap adhesin during fimbrial assembly (323). The primary and secondary structures of PapG (35 kDa) are not similar to those of PapA (19.5 kDa) and the structural subunits of other *E. coli* fimbriae (305, 323, 551) except at the C terminus. Interestingly, the amino acid sequence of PapG is related to that of the B subunit of Shiga toxin (214, 303, 323), which also has Gal-Gal binding specificity (albeit for terminal Gal-Gal units only). In addition to its adhesin function, PapG may play a role in the initiation of subunit polymerization in some P-fimbrial variants (450).

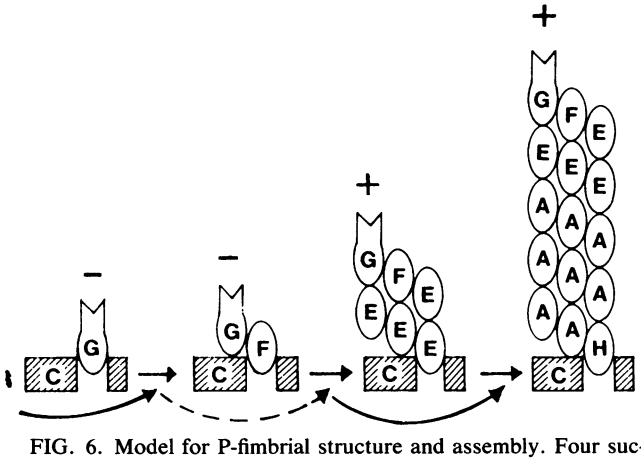

FIG. 6. Model for P-fimbrial structure and assembly. Four successive stages of assembly are shown from left to right. +, structure that can bind the digalactoside receptor; –, structure unable to bind the receptor; A, PapA; C, PapC; E, PapE; F, PapF; G, PapG. Reprinted by permission from *Nature* **328**:84–87 (304). Copyright 1987, Macmillan Magazines, Ltd.

PapF is also important in the initiation of subunit polymerization (324, 450). It is found in close physical association with PapG at the fimbrial tip (82, 304, 450), where it helps stabilize PapG or anchor it to underlying structures (304) (Fig. 6). PapE (16.5 kDa) and PapF (15 kDa) share structural homology with PapA and other *E. coli* structural fimbrial subunits (i.e., two cysteines in the amino-terminal half of the molecule, a penultimate tyrosine, a size of approximately 150 amino acids, and similar hydrophobicity plots) (18, 305).

Several other proteins are important in P-fimbrial synthesis (82, 304, 374, 551). PapD (27.5 kDa) is present in the periplasmic space and may complex with fimbrial subunits, stabilizing them during translocation across the periplasmic space to the outer membrane prior to assembly. PapC (80 kDa) assists in the transport of subunits out of the cell and in their assembly into complete fimbriae (Fig. 6). PapH terminates fimbrial assembly and helps anchor fimbriae.

Regulation of Expression

P-fimbrial expression is subject both to rapid, random phase variation (447) and to environmental influences. Individual cells express P fimbriae only, other fimbriae only, a mixture of fimbrial types, or no fimbriae, with the relative prevalence of each variant determined by growth conditions (145, 381).

With few exceptions (145), P fimbriation is favored by growth at 37°C and on agar plates and inhibited by growth at 18 to 22°C and in broth (2, 145, 158, 381). The temperature dependence of P-fimbrial expression operates at the transcriptional level through a regulatory determinant in or near *papB* (2, 158, 551). Shifts between 37°C and room temperature are associated with DNA rearrangements in this region that suggest a genetic switch (2). No such rearrangements are seen during the fimbrial phase shifts associated with growth in broth versus growth on plates (2), demonstrating that other control mechanisms are active. *papB* acts on *papA* in *cis*, whereas *papI* regulates *papA* in *trans* (448). Little is known regarding the regulation of *papCD* and *papEFG*, the other two transcriptional units of the P-fimbrial gene cluster (158).

Concentrations of trimethoprim below the MIC inhibit the expression of P fimbriae and of Gal-Gal-specific adherence (80, 555, 560). Whereas outer membrane protein profiles are unchanged in trimethoprim-treated cells, freeze-fracture electron microscopy shows disorganization of both bacterial membranes, suggesting that any fimbriae formed by these cells could be lost from the outer membrane (555).

Obstacles to an Effective Anti-P-Fimbrial Vaccine

P fimbriae purified from different clinical isolates often (185, 281, 395) but not always (93, 281) fail to cross-react

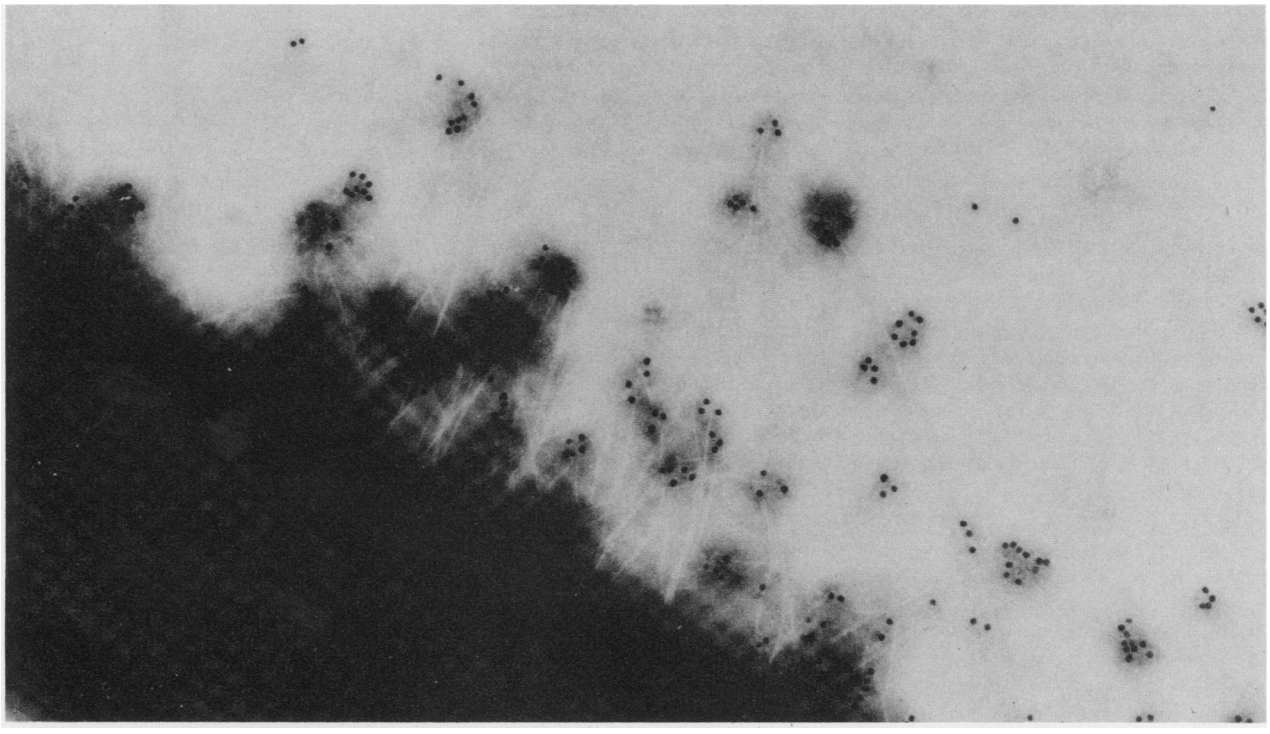

FIG. 7. Immunoelectron micrograph of an *E. coli* cell expressing F13 (Pap) P fimbriae. The bacteria were incubated with antiserum against purified PapD-PapE complexes. This serum does not react with *papE* mutant strains. Thus, the gold particles that appear as black dots on the micrograph indicate PapE (and, presumably, PapF and PapG) molecules located at the tips of the fimbriae. From reference 324, by permission of the publisher, Butterworth and Co. (Publishers) Ltd.

serologically. The F (fimbrial)-antigen system for cataloging serological variants of *E. coli* fimbriae uses designations 1 to 6 for previously described fimbriae (F1, type 1 fimbriae; F2, CFA/I; F3, CFA/II; F4, K88; F5, K99; and F6, 987P) and higher numbers for newly identified fimbrial types (410). To date, eight P-fimbrial serovariants ($F7_1$, $F7_2$, and F8 to F13) have received F designations (94, 410). In general, serological cross-reactivity between different P-fimbrial F types is observed only with polyclonal sera; monoclonal antibodies to purified P fimbriae exhibit only minimal intertype cross-reactivity or are F-type specific (1, 93, 94), and sometimes even identify variants within a particular F type (1). Sera collected from patients following an episode of pyelonephritis react with P fimbriae from the patients' strains (96, 467) but may (467) or may not (96) cross-react with other P-fimbriated strains. The degree of cross-reactivity between P fimbriae from different strains is greatest with denatured, purified fimbriae and least with whole fimbriated bacteria (468, 481). Immunoblots of fimbriae purified from strains of different F types reveal single or multiple fimbrial subunits of various sizes, ranging from 17 to 22 kDa, with no single band common to all strains (185, 274, 426, 428, 429, 468). Subunits of different molecular weights in different strains are sometimes precipitated by the same antiserum (429), demonstrating antigenic conservation among physically distinct proteins.

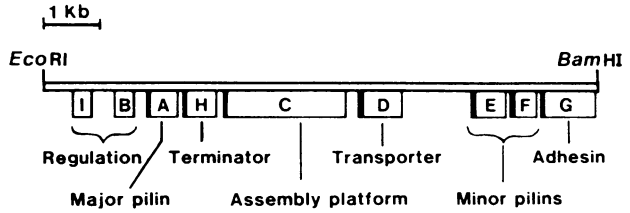

FIG. 8. Overview of the *pap* gene cluster. PapA is the major (structural) fimbrial subunit. PapH seems both to terminate fimbrial growth and to anchor the fully grown fimbriae to the cell surface. PapC is located in the outer membrane and forms the assembly platform for fimbrial growth. PapD is a periplasmic protein which forms complexes with the fimbrial subunits before assembly. PapE, PapF, and PapG are minor fimbrial components. PapG is the adhesin molecule conferring Gal(α1-4)Gal binding specificity, PapF complexes with PapG, and PapE attaches the PapF-PapG complex to fimbriae. Reprinted by permission from *Nature* **328**:84–87 (304). Copyright 1987, Macmillan Magazines, Ltd.

Whereas some monoclonal antibodies block adherence mediated by the homologous P-fimbrial type (95, 144) and by some but not all heterologous types (95), most monoclonal anti-P-fimbrial antibodies fail to inhibit agglutination or adherence of even the homologous fimbrial type (1, 95). Polyclonal anti-P-fimbrial antisera inhibit P-fimbrial adherence or agglutination in some instances (412, 446, 529), but exceptions occur (468). In addition, anti-P-fimbrial antibodies in patients' sera following pyelonephritis fail to block adherence mediated by the homologous P-fimbriated strain (96). From these observations it is clear that efforts to devise clinically effective vaccines to block P-fimbrial adherence in humans face a number of obstacles (395, 484). It remains to be determined whether a vaccine can stimulate adhesin-specific antibodies that are more broadly cross-reactive than those described thus far (95).

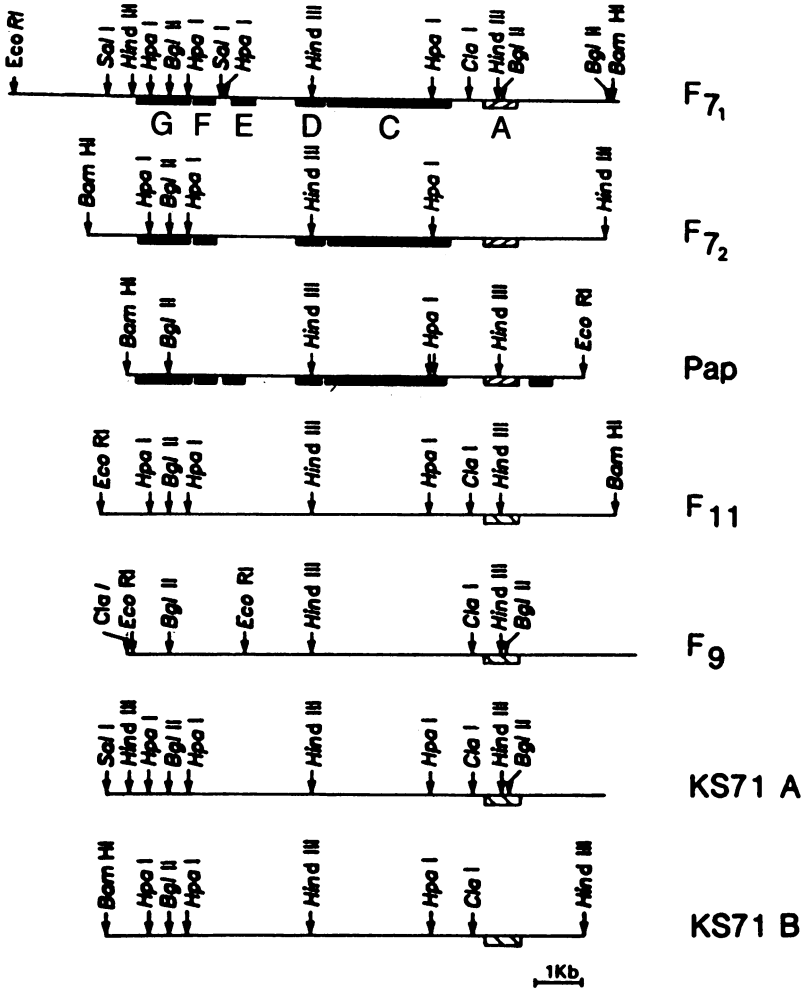

FIG. 9. Comparison of the physical maps of the gene clusters encoding different serovariants of P fimbriae, including F7 (570), $F7_2$ (572), Pap (F13) (375), F11 (92), F9 (91), KS71A (444), and KS71B (444). The boxes indicate genes involved in expression of fimbriae or MRHA; hatched boxes represent genes encoding the major (structural) fimbrial subunit. Letter designations for genes are those of Norgren et al. (375). Adapted from reference 571, with permission from the publisher.

Structural Comparisons between P-Fimbrial Serovariants

Despite their serologic heterogeneity, PapA molecules from different P-fimbrial serovariants exhibit a high degree of amino acid homology at the N and C termini (185, 269, 273, 274, 392, 468, 469, 568). The conserved amino acids are hydrophobic and are presumably oriented toward the interior of the mature protein, playing an essential structural role while avoiding immunological selective pressure (326, 568, 569). In contrast, there is great variation in the primary structure of the central portions of PapA, possibly because this area is hydrophilic, exposed, and subject to maximal selective pressure from the host immune system while being nonessential for fimbrial function (274, 326, 392, 569). In many instances, there is also substantial heterogeneity between different P-fimbrial types with respect to the amino acid sequences of the minor fimbrial subunits (PapE, PapF, and PapG) (195, 325, 326). Most P-fimbriated clinical isolates share *papG* sequence homology with F11 but not with F13 strains (113).

The physical map of the P-fimbrial gene cluster is similar among the various F types (Fig. 9) (68, 571). Accessory proteins from one F type can assemble subunits of a different F type to yield morphologically and functionally normal fimbriae (326, 571). Sequences encoding accessory proteins such as PapC and PapD are also highly conserved between different P-fimbrial serotypes (326). These observations demonstrate a close evolutionary relationship between the P fimbriae of different strains.

Clonal Expression of P Fimbriae

When clinical isolates are grouped according to O:K:H serotype, outer membrane protein pattern, biotype, multilocus enzyme electrophoresis pattern, or a combination of these characteristics, expression (or nonexpression) of P fimbriae is somewhat uniform within the clonal groups (42, 290, 432). Furthermore, certain P-fimbrial subtypes are found in association with specific *E. coli* clonal groups (274, 374, 410, 413, 414), suggesting that different fimbrial genetic variants have been transmitted vertically through different lineages of *E. coli*. That the relationship between P-fimbrial subtypes and *E. coli* genetic groups is more complex becomes apparent when fimbriae are classified by using immu-

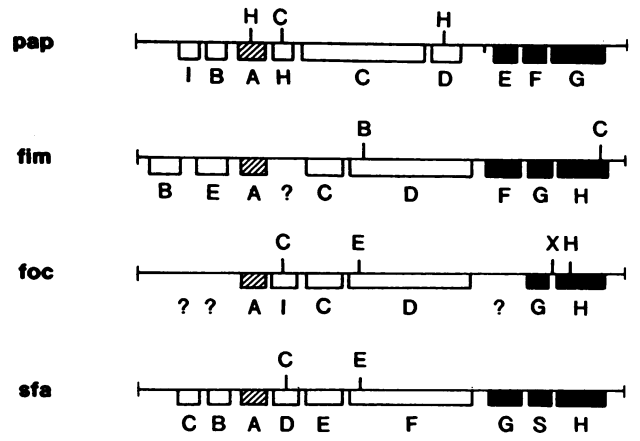

FIG. 10. Physical and genetic maps of gene clusters encoding P fimbriae (*pap*) (304), type 1 fimbriae (*fim*) (271), F1C fimbriae (*foc*) (449), and S fimbriae (*sfa*) (172a). Symbols for endonucleases are C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; and X, *Xho*I. Solid boxes indicate genes encoding minor proteins; hatched boxes indicate genes encoding major (structural) fimbrial subunits. Adapted from reference 449, with permission from the publisher.

noblotting or *pap* restriction fragment length polymorphism analysis. The "one clone, one fimbrial type" model seems to hold in some cases (e.g., within groups O4, O6, and O2), but multiple fimbrial variants are sometimes found within a single clonal group of *E. coli* and multiple clonal groups sometimes share a common fimbrial subtype (13, 429, 432). These observations suggest that while in many cases the presence of P fimbriae of a particular genetic or serologic type can be considered characteristic of a clonally related group of strains, there is more genetic and phenotypic diversity within the purported clonal groups than was previously recognized (432). Furthermore, acquisition of genes for P fimbriae may occur through horizontal transmission between genetically dissimilar groups of strains as well as through vertical transmission within a clonal lineage (13, 432).

Similarity to Other Fimbriae

PapA exhibits a moderate degree of amino acid homology at the amino terminus with the structural subunits of type 1 and other fimbriae of *E. coli* (269, 392, 428, 521). The overall arrangement of the P-fimbrial gene cluster is similar to, although distinct from, the arrangement of gene clusters encoding S, F1C, and type 1 fimbriae of *E. coli* (Fig. 10) (449). A modest degree of amino acid homology (28%) is even present between *E. coli* P fimbriae and *Haemophilus influenzae* fimbriae, and antisera raised to the fimbriae of one species agglutinate fimbriated bacteria of the other species (171, 344). However, among members of the family *Enterobacteriaceae*, only *E. coli* contains P-fimbrial determinants (203). These observations suggest that all *E. coli* fimbriae evolved from an ancestral fimbrial prototype common to other gram-negative bacilli, with P fimbriae representing a derivative unique to *E. coli*.

Linkage with Other VFs

Many P-fimbriated strains have two or three complete copies of the P-fimbrial determinants, and many have partial copies (14, 204, 229). Multiple copies of the *pap* gene cluster are common within certain electrophoretic types and certain O groups (e.g., O16 and O25) but are not necessarily found in all members of the particular group (13). In some strains, the *pap* gene cluster is genetically linked with determinants for other VFs, including P fimbriae of other F types, fimbriae with binding specificities other than Gal-Gal, and hemolysin (13, 21, 193, 201, 205, 322). P-fimbrial determinants are characteristically present among strains that also carry hemolysin determinants (13, 205). However, the copy numbers of P-fimbrial and hemolysin determinants are the same (as would be expected if they are genetically linked) in only half of such strains (205). The direct linkage of hemolysin and P-fimbrial determinants described for four O4 strains and one O6 strain (322) appears to be the result of independent recombinational events, since the DNA segments separating the hemolysin and P-fimbrial gene clusters in these strains differ with respect to base pair sequence and length (4 to 16 kbp). In one unusual strain, gene clusters for two P-fimbrial variants appear at either end of a 60- to 70-kbp block of VF genes coding for Prs fimbriae, hemolysin, and F1C fimbriae (193).

Animal Models

Experimental pyelonephritis is relatively easy to establish in rats because vesicoureteral reflux (VUR) (abnormal retrograde flow of urine from the bladder up the ureter to the kidney) occurs spontaneously (242). However, rat uroepithelial cells do not express receptors for P fimbriae (179, 279), and the form of globoside isolated from rat kidneys contains a Gal(α1-3)Galβ linkage in place of the critical Gal(α1-4)Galβ linkage present in human globoside (365). Thus, the rat is a species of questionable value for evaluating the pathogenic role of P fimbriae in UTI (179).

In contrast to rat tissues, mouse renal tissues contain appreciable concentrations of Gal(α1-4)Galβ-containing glycolipids (7, 328). The concentration of total glycolipids, and of each globoseries glycolipid, differs between different strains of mice and between male and female mice (7, 328). In female BALB/c mice, antiglobotriaose antibodies identify receptors for P fimbriae in the vagina, bladder, ureter, renal pelvis, collecting duct, and tubular cells, with no staining of the glomerulus or loop of Henle (391).

Whether they are live or killed, P-fimbriated strains elicit a greater degree of pyuria in mice following inoculation into the bladder than do non-P-fimbriated strains; this inflammatory response is blocked by the coadministration of a Gal-Gal-containing compound (308). Aggregates of purified P fimbriae and endotoxin exhibit a similar Gal-Gal-dependent stimulation of pyuria, suggesting that P fimbriae promote local inflammation by attaching a source of endotoxin to urinary tract mucosa (308). In mice, P fimbriae are a major determinant of bacterial colonization or invasion both of the upper urinary tract and, in the absence of type 1 fimbriae, of the bladder (97, 180, 264, 391). P-fimbriated organisms produce disseminated infection when administered intravenously to mice (566), suggesting that P fimbriae may also play an important role outside the urinary tract. Administration of globoside along with the bacterial inoculum protects mice from urinary tract colonization with P-fimbriated organisms (520), and immunization against P fimbriae from a wild-type strain confers protection against subsequent renal infection with the same wild-type strain (182, 391).

The erythrocytes and uroepithelial cells of nonhuman primates (*Macaca mulatta*, *Macaca fascicularis*, green monkeys, chimpanzees, and baboons) express receptors for P

fimbriae (457, 533). When inoculated into the ureter, P-fimbriated bacteria induce ureteritis in the macaque monkey, with resultant VUR and pyelotubular backflow (intrarenal reflux) (253, 457); coadministration of a Gal-Gal-containing solution moderates these changes (533). Vaccination with purified P fimbriae protects monkeys from histologic changes of pyelonephritis following ureteral injection of a P-fimbriated strain (456). In vaccinated animals, bacteria covered with antibodies lie free within the renal tubules, with no evidence of tubular cell damage, whereas in control animals, bacteria adhere to the tubules, with death of tubular cells (456). Infants of monkeys immunized with purified P fimbriae during the third trimester of pregnancy are protected against UTI following bladder challenge with the homologous P-fimbriated strain (235).

Taken together, these animal studies suggest that P fimbriae are important in localization of infection to the upper urinary tract, that they promote an increased local inflammatory response, and that they contribute to invasive renal infection. Administration of receptor analogs or induction of antifimbrial immunity by vaccination with homologous fimbriae protects against ascending infection with P-fimbriated bacteria. It remains to be demonstrated that immunity to P fimbriae from one strain protects against infection with other P-fimbriated strains.

Epidemiology

Expression during UTI. In most clinical studies, urinary isolates are tested for P fimbriae after culture on agar, sometimes with multiple passages to induce P-fimbrial expression (see below) (230, 393). However, agglutination of receptor-coated latex beads and immunofluorescence have also been used to document the presence of P fimbriae directly on organisms in voided urine (267, 427, 534). Urinary bacterial populations are heterogeneous with respect to P-fimbrial expression (427), with P-fimbriated organisms often adherent to cells and non-P-fimbriated organisms free in the urine (427). P fimbriation is probably even more prevalent among strains adherent to the uroepithelium at the site of infection than among organisms shed in the urine (227). Further studies using sensitive techniques to detect P-fimbriated organisms in situ are needed to adequately characterize the expression of P fimbriae during acute UTI.

Clinical studies. The proportion of strains expressing P fimbriae declines progressively from a high of 70% among isolates from patients with pyelonephritis to 36% among cystitis patient isolates, 24% among ABU patient isolates, and 19% among fecal strains (Table 5). The prevalence of P fimbriation in isolates from patients with bacteremia arising from a UTI (urosepsis) is as high (177 of 248 [71%]) (13, 42–44, 230) as in isolates from pyelonephritis patients (70%) (Table 5), whereas in isolates from patients with bacteremia from other sources, it is much lower (28 of 99 [28%]) (42–44). These observations suggest that P fimbriae contribute to the ability of *E. coli* strains to cause UTI, especially the more clinically severe forms, and that strains lacking P fimbriae are at a disadvantage in the urinary tract. Testing for P fimbrial expression has been proposed as a way to help confirm the diagnosis of pyelonephritis (38, 534).

In contrast to the low incidence of P-fimbriated strains in fecal samples from healthy controls (Table 5), fecal carriage of P-fimbriated strains is common among patients with pyelonephritis (20, 249). In at least some cases the P-fimbriated fecal strain is the same as the urinary isolate by O:K serotyping (249), supporting the fecal-perineal-urethral hypothesis of UTI pathogenesis (505). Nosocomially transmitted P-fimbriated strains have caused outbreaks of pyelonephritis in infants, with some evidence of secondary transmission to patient household contacts (548, 549). Screening for fecal carriage of P-fimbriated strains in family members of patients with pyelonephritis and treatment of asymptomatic carriers of P-fimbriated strains has been advocated (455), although the clinical utility of screening for P-fimbrial carriage, even in high-risk epidemiological settings, is unconfirmed (219, 247).

Role in determining clinical manifestations of UTI. Taken together, the abundance of receptors for P fimbriae in human renal tissue, the importance of P fimbriae in upper urinary tract colonization in mice, and the association of P fimbriae with acute pyelonephritis in humans suggest that P fimbriae are required for colonization and invasion of the human upper urinary tract. However, determinations of the site of human infection (upper versus lower urinary tract) based on the clinical manifestations of UTI are often inaccurate (231). Studies of women with UTI in which the site of infection is determined by localization techniques (bladder washout, ureteral catheterization, or the antibody-coated-bacteria test) demonstrate that the proportion of strains expressing P fimbriae is greatest with acute pyelonephritis, lower with cystitis, and lowest with ABU, as would be expected from the results of other studies (Table 5) (146, 295, 372). Surprisingly, however, the proportion of strains expressing P fimbriae is similar between upper and lower urinary tract isolates within each clinical category (146, 295, 372). This seemingly paradoxical dissociation of anatomical location and clinical manifestations of infection would be explained if P fimbriae are necessary not for upper tract infection per se but for the local and systemic signs of inflammation commonly used to define acute pyelonephritis. P-fimbriated strains stimulate a greater inflammatory response in children with UTI, even when strains from within the same clinical category of UTI are compared (90, 338). P fimbriae also contribute to an enhanced local inflammatory response in the mouse model of UTI (see Animal Models above). Thus, either the ability to adhere specifically to tissues within the upper urinary tract or the ability to promote more severe inflammation (whatever the site of attachment), or both, may underlie the association of P fimbriae with more clinically severe forms of UTI.

Compromised hosts and patients with increased susceptibility to UTI. The incidence of P-fimbriated strains is lower among isolates from girls with recurrent pyelonephritis (315, 316) and boys with first-episode pyelonephritis (89) who have VUR than among those from similar patients without VUR (Table 6). In contrast, there is no decrease in the incidence of P-fimbriated strains associated with VUR in girls with predominantly first-episode pyelonephritis (15, 114) (Table 6). Most studies evaluating the microbiological significance of impaired host defenses in adults with pyelonephritis (98, 99, 470) (Table 6), *E. coli* urosepsis (43, 230), or asymptomatic renal infection (226, 372) document a decreased requirement for P fimbriae in strains from patients with one or more compromising conditions. Underlying medical illnesses, urinary tract abnormalities, and urinary tract instrumentation independently predict a decreased requirement for P fimbriae in *E. coli* strains from adults from urosepsis (43, 230).

These findings suggest that urinary tract abnormalities or instrumentation may nullify normal anatomic and functional defense mechanisms, allowing non-P-fimbriated organisms access to the kidneys or other deep tissue sites. Because of

TABLE 5. Association of P fimbriae with clinical source of isolate

Reference	Proportion (%) of strains expressing P fimbriae[a]				
	PN	CY	ABU	Urine[b]	Fecal
13				16/20 (80)[c]	
15	39/49 (73)[d]	15/48 (31)		51/97 (53)[d]	7/40 (18)
42				35/58 (60)[c]	10/98 (10)
43				53/75 (71)[c,d]	
44				27/37 (73)[c]	
89	79/137 (58)[d]	4/23 (17)		94/195 (48)[d]	
98	38/40 (95)[d]	17/50 (34)	27/71 (38)	82/161 (51)[d]	
99	22/38 (56)[d]	30/111 (27)		52/149 (35)[d]	
114	57/74 (77)[d]	14/61 (23)	12/61 (20)	83/196 (42)[d]	8/50 (16)
117	107/135 (79)	50/21 (41)	30/119 (25)	187/375 (50)	16/120 (13)
146	24/29 (83)	17/28 (61)	72/139 (52)	123/211 (58)[d]	3/19 (16)
177				68/93 (73)	50/96 (52)
209				30/43 (70)	15/43 (36)
217	170/210 (81)	36/113 (32)		206/323 (64)	
220	27/30 (90)			27/30 (90)	7/41 (17)
222	98/122 (80)			98/122 (80)	
230				36/58 (62)[c,d]	
249	33/35 (91)	5/26 (19)	5/36 (14)	43/97 (44)	6/82 (7)
295	13/23 (57)	22/116 (19)	6/40 (15)	41/179 (33)	
301		32/66 (48)		32/66 (48)	
315	58/131 (44)[d]			58/131 (44)[d]	
316	103/183 (56)[d]	13/57 (23)	36/274 (13)	227/514 (44)[d]	
317	72/122 (59)[d]			72/122 (59)[d]	
338	45/52 (86)		11/41 (27)	56/93 (60)	
372			2/25 (23)[d]	2/25 (23)[d]	
393	12/12 (100)	17/26 (65)	1/6 (17)	30/44 (68)	2/73 (29)
470	78/120 (65)[d]	13/35 (37)		91/155 (59)[d]	
509	12/24 (50)[d]	2/16 (13)	5/37 (14)	19/77 (26)[d]	
553	14/17 (82)[d]			14/17 (82)[d]	
557	51/67 (76)	14/60 (23)	11/60 (18)	76/187 (41)	
607	48/57 (84)	20/43 (47)		68/100 (68)	
Total[e]	1,200/1,701 (70)	321/900 (36)	218/909 (24)	2,097/4,050 (52)	124/662 (19)

[a] PN, Pyelonephritis or febrile UTI; CY, cystitis; ABU, asymptomatic bacteriuria.
[b] Preceding three columns plus any other urinary isolates of unspecified clinical category.
[c] Urosepsis patient isolates.
[d] Includes patients with known compromising conditions.
[e] Because of differences in study design, methods, definitions, and populations in different studies, totaled results must be interpreted with caution.

this, some have proposed that the absence of P fimbriae in a pyelonephritis isolate from an adult could be used as an indicator of a possible underlying anatomic abnormality or medical illness, warranting further investigation of the patient (99); however, this approach is not well supported by the available data (228). Another possible consequence of the decreased importance of P fimbriae in the setting of host compromise is impaired effectiveness of an anti-P-fimbrial vaccine in compromised hosts (230, 289). However, in contrast to the situation among patients with urinary tract abnormalities or instrumentation, neither the upper tract localization model nor the attachment-inflammation model of P-fimbrial function adequately explains the decreased importance of P fimbriae in pyelonephritis or urosepsis occurring in adults with underlying medical illnesses. It is clear that more remains to be learned regarding the interactions of P fimbriae with host defense systems in determining the site and clinical manifestations of UTI.

Renal scarring and RUTI. The relationships between an individual's P blood group phenotype or secretor status, the uroepithelial-cell receptor density for P-fimbriated organisms, and the development of RUTI, renal scarring, and renal dysfunction are complex and poorly understood. Although the P_1 blood group is not associated with an in-

TABLE 6. Association of compromising host conditions[a] with prevalence of P-fimbriated strains in patients with pyelonephritis

Patient population studied	Proportion (%) of strains expressing P fimbriae		Reference
	Noncompromised host	Compromised host	
Girls and boys	27/37 (73)	9/12 (75)	15
Boys	52/63 (83)	19/33 (56)	89
Adults	25/25 (100)	13/15 (87)	98
Adults	17/21 (81)	5/17 (29)	99
Girls	25/31 (81)	15/17 (88)	114
Girls	37/57 (65)	21/74 (28)	315
Girls	75/105 (71)	28/78 (36)	316
Girls	57/77 (74)	15/45 (33)	317
Women	39/49 (80)	39/71 (55)	470
Girls	12/14 (86)	2/3 (67)	553
Total[b]	366/479 (76)	166/365 (45)	

[a] Compromising conditions (as defined by different investigators) include underlying anatomic abnormalities of the urinary tract, urinary tract instrumentation, medical illnesses, and pregnancy.
[b] Because of differences in study design, methods, definitions, and populations in different studies, totaled results must be interpreted with caution.

creased uroepithelial-cell receptor density for P-fimbriated bacteria (216, 221, 314) or with RUTI in women (221, 363, 488), it may be associated (for unclear reasons) with RUTI and recurrent pyelonephritis in girls (297, 315, 316, 319). Women with RUTI are more likely than infection-free controls to be nonsecretors of blood group substances (266, 488), probably because uroepithelial cells from nonsecretors have an increased binding capacity for P-fimbriated organisms (314) and because P fimbrial binding is as important in RUTI as in first-episode UTI (505). The decreased availability of receptors for P fimbriae in secretors, which is independent of P blood group and A/B/O or Rh status (314), may be due to a shielding of the smaller P blood group antigens by the larger overhanging A/B/O or Le^b oligosaccharides, much as trees shade bushes in a forest (483). In nonsecretors, the absence of fucosyl transferase prevents cell surface expression of A, B, and H blood group antigens; thus, P fimbrial receptors may be more exposed and accessible to bacterial adhesins (483). Adults with renal scarring presumably resulting from infection also have uroepithelial cells with an increased binding capacity for P-fimbriated bacteria, independent of the P blood group (215, 216, 218). It seems unlikely, however, that this increased binding capacity contributes to the development of renal scarring by promoting infection with P-fimbriated organisms, since infection with P-fimbriated bacteria does not appear to be responsible for renal scarring in children (89, 317, 318, 320) (see also Renal Scarring below).

Summary

P fimbriae are important in the pathogenesis of UTI, primarily because they mediate Gal-Gal-specific bacterial adherence to epithelial cells within the human urinary tract, thereby permitting bacterial colonization and stimulating inflammation. In compromised hosts the requirement for P fimbriae in initiating serious UTI is decreased, suggesting that P fimbriae are necessary for *E. coli* to overcome certain components of the normal host defense system. Although receptor analog therapy is effective in animals, several technical difficulties need to be overcome before it will be practical for human use (182). Anti-P-fimbrial immunity protects animals against renal infection with homologous P-fimbriated strains, but the serological diversity of P fimbriae and the limited impact of anti-P-fimbrial antibodies on adherence complicate efforts to develop anti-P-fimbrial vaccines for human use. There is currently no proven clinical role for P fimbrial testing.

X ADHESINS: THE Dr FAMILY, S FIMBRIAE, AND OTHERS

Dr Family of Adhesins

Most urinary isolates that express MRHA in the absence of P fimbriae (X adherence) hybridize with DNA probes specific for nonfimbrial adhesins that bind to various portions of the Dr blood group antigen (15, 291, 379, 384). This family of adhesins includes the O75X adhesin (556) and the afimbrial adhesin I (AFA-I) and AFA-III adhesins (291, 293), all first identified in urinary strains (Table 1). These adhesins are structurally distinct from other *E. coli* fimbrial adhesins in that they appear as a fine mesh (15), a coillike structure (556), or a filamentous capsular coating (409) on the cell surface or are not visible by electron microscopy (293).

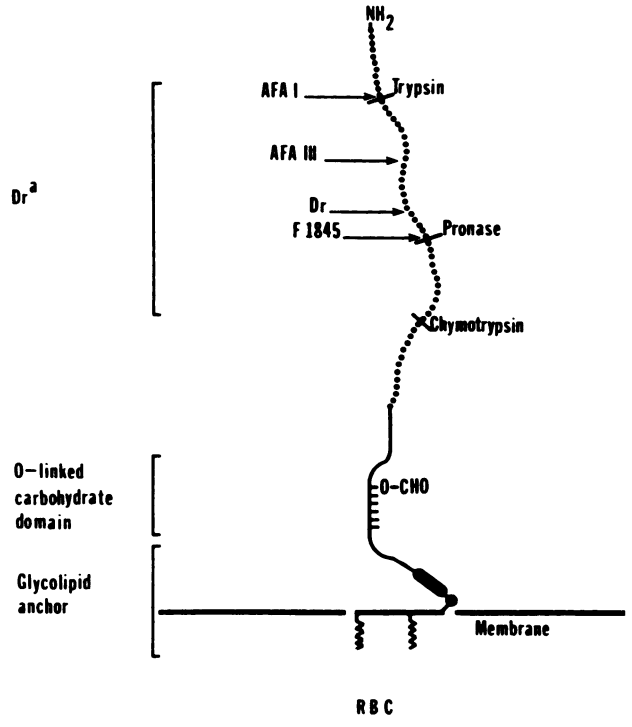

FIG. 11. Hypothetical simplified model of the decay-accelerating factor molecule, proposing Dr blood group antigen- and receptor-binding sites for the Dr, AFA-1, AFA-III, and F1845 hemagglutinins. RBC, Erythrocyte. From reference 379, with permission from the publisher.

Receptors. Different strains in the Dr adhesin family appear to recognize different portions of the Dr antigen (Fig. 11); i.e., chloramphenicol inhibits the binding of those strains previously designated O75X (380) but not other Dr-recognizing strains (379). In addition, for different Dr-recognizing strains, Dr^+ erythrocyte agglutination is affected differently by pretreatment of the erythrocytes with each of several proteases (379). The Dr antigen is located on the decay-accelerating factor, a cell membrane protein that helps regulate the complement cascade and prevents erythrocyte lysis by complement (384). Dr hemagglutinins bind to numerous sites within the urinary tract, including the renal interstitium, Bowman's capsule, tubular basement membranes (but not epithelial cells), ureteral transitional epithelial cells, and exfoliated epithelial cells in urine (Table 4) (283, 385).

Genetics. The adhesin gene clusters of different members of the Dr family are organized similarly, with five closely spaced genes, including one for the structural hemagglutinin (292, 377, 384). The amino acid sequence of this 16-kDa protein differs between different members of the Dr family (291) and is not similar to that of other *E. coli* fimbrial subunits (292). Some strains have multiple copies of the Dr gene cluster (291), but a single copy is most common (15, 291).

Epidemiology. In contrast to P fimbriae, hemagglutinins of the Dr family are associated with cystitis. Dr-related sequences are present in 26 to 50% of cystitis patient isolates but in only 6 to 26% of pyelonephritis and 6% of ABU patient isolates and in 15 to 18% of fecal isolates (15, 384). The Dr family constitutes 78% of X-adhering strains among cystitis

patient isolates, 46% among pyelonephritis patient isolates, and 75% among fecal strains (15). The relative contribution of the several Dr variants is unknown, since most epidemiological studies have not used adhesin-specific probes (15, 291, 384), and strains with different adhesin specificities and serological reactions often share sequence homology for Dr-related accessory genes (291). The variant originally designated AFA-I appears to be relatively uncommon among urinary isolates (291).

S Fimbriae and F1C Fimbriae

S fimbriae and F1C fimbriae are closely related adhesins expressed by some urinary strains (Table 1) (417, 449). S fimbriae, so named because their binding is specific for terminal sialyl-galactoside residues (420, 421), mediate X-type MRHA of human erythrocytes, a property that can be exploited to separate S-fimbriated cells from a mixed population (382). Expression of S fimbriae exhibits phase variation (383). Binding sites for S fimbriae are found on epithelial cells of the proximal and distal tubules, collecting ducts, and glomerulus; in the renal interstitium; and on renal vascular endothelium (Table 4) (280). S fimbriae contribute to virulence in a number of animal models of infection, including UTI (173, 340); anti-S-fimbrial antibodies are protective (386). In humans, S-fimbriated strains are more closely associated with meningitis and bacteremia than with UTI (417).

F1C fimbriae do not mediate hemagglutination or uroepithelial cell adherence, but they do bind to buccal epithelial cells and to some renal tissues, albeit with an unknown (non-mannose-sensitive) receptor specificity (Tables 1 and 4) (411, 449). They are expressed by 20% of urinary isolates after subculture (427), but their role in vivo is questionable in view of the failure to detect F1C fimbriae on strains studied directly in urine specimens from 20 patients with UTI (427).

Genetics and serological associations. S fimbriae and F1C fimbriae share a common genetic structure, with genes for a structural subunit, an adhesin, and various accessory proteins located together on the chromosome (Fig. 10) (176, 449). The S-fimbrial adhesin is located at the fimbrial tips (356) and, as with P and type 1 fimbriae, has a different amino acid sequence than the structural subunit (176, 356). The structural subunit shares N- and C-terminal amino acid sequence homology with the PapA protein of P fimbriae and, like PapA and other *E. coli* fimbrial subunits, contains an internal disulfide loop (482). Genetically and serologically, F1C fimbriae are more closely related to *E. coli* type 1 fimbriae than are S fimbriae, whereas the relationship of both F1C and S fimbriae to P fimbriae is more distant (418). S fimbriae are associated with O6:K15:H3, O18:K1:H7, and O83:K1 strains (176, 417) and are sometimes genetically linked with other VF determinants (197).

M Adhesin and G Fimbriae

Even less common than strains expressing other defined X adhesins are urinary strains with the M adhesin (three strains reported) (445, 554, 558) or G fimbriae (one strain reported) (445, 558) (Table 1). The M adhesin's binding specificity is for the terminal amino acid sequence of the M blood group antigen found on glycophorin A (554). This nonfimbrial adhesin's 19.5-kDa subunit lacks serological cross-reactivity and sequence homology with other *E. coli* adhesins (445).

G fimbriae bind to terminal *N*-acetylglucosamine moieties, agglutinating erythrocytes after treatment with endo-β-galactosidase to expose internal GlcNAc residues (558). Whether G fimbriae attach to urinary tract tissues is unknown, but a plant lectin (wheat germ agglutinin) with a similar binding specificity does bind to kidney structures (445). The N terminus of the G-fimbrial subunit is structurally similar to that of K99 fimbriae of enteric *E. coli* and P fimbriae from strain KS71A but shares little serological cross-reactivity with these or other *E. coli* fimbriae (445).

Other X Adhesins

Two other urinary strains have been identified in which X-pattern MRHA is mediated by surface protein adhesins of undefined binding specificity (Table 1) (156, 184). The nonfimbrial protein adhesins from these strains, called NFA-1 and NFA-2 by the investigators, are unrelated to AFA-I and the M adhesin (156, 184). They cross-react serologically with one another but have different-size subunits and different appearances when present on adhering cells (encapsulated, NFA-1; unencapsulated, NFA-2) (156).

Adherence by nonspecific hydrophobic interactions (332) may explain non-P-fimbrial MRHA and adherence to epithelial cells in some cases (140, 313). Hydrophobic interactions may contribute to adherence in fimbriated strains as well (313). It is not clear, however, that hydrophobicity per se is a significant determinant of adherence among urinary strains (269).

MANNOSE-SENSITIVE ADHESINS

With rare exceptions (118, 119, 121), mannose-sensitive adherence mediated by *E. coli* strains is due to type 1 fimbriae (103, 104) (Table 1). In clinical studies, mannose-sensitive hemagglutination of guinea pig erythrocytes is generally interpreted to indicate the presence of type 1 fimbriae.

TYPE 1 FIMBRIAE

Receptors

Specificity. Adherence mediated by type 1 fimbriae is blocked by solutions of D-mannose or α-methylmannoside and by concanavalin A (a lectin that binds to mannoside residues) but not by solutions of other monosaccharides or their derivatives (103, 389, 465, 466), leading to the speculation that the receptor for type 1 fimbriae includes mannose residues (104). The type 1-fimbrial receptor is probably an extended structure, since mannosides with an α-linked aromatic group, trisaccharides, and branched oligosaccharides that contain (α1-3)-linked mannosides are better inhibitors of type 1-fimbrial binding than D-mannose or α-methylmannoside alone (130, 366). Nitrophenol and its derivatives inhibit type 1-fimbrial adherence to epithelial cells but not type 1-fimbrial hemagglutination of guinea pig or human erythrocytes, suggesting that the type 1-fimbrial receptor on epithelial cells but not on erythrocytes includes an important hydrophobic component (124).

Distribution of receptors. Receptors for type 1 fimbriae are present on erythrocytes from many species (102). Type 1-fimbriated bacteria adhere to human buccal epithelial cells (389), intestinal cells (224, 366, 614), and vaginal cells (124), suggesting a possible role for type 1 fimbriae in *E. coli* colonization of the mouth, gut, and vagina. Most, but not all (474, 567), investigators report little contribution of type 1 fimbriae to *E. coli* uroepithelial-cell adherence. The adher-

ence of type 1 fimbriae to THP when it is present in high concentrations (64, 106, 412, 422) may contribute to discrepant findings regarding the role of type 1 fimbriae in uroepithelial-cell adherence, since THP often coats uroepithelial cells. In addition, by binding type 1-fimbriated bacteria, THP may prevent the bacteria from adhering to the urinary mucosa and allow them to be expelled in voided urine, thus acting as a nonspecific defense mechanism in the urinary tract (412). Paradoxically, at low concentrations, THP promotes type 1-fimbrial adherence to uroepithelial cells by an unknown mechanism (106).

Receptors for type 1 fimbriae are present in blood vessel walls and in the muscular layers but not the epithelium of the human bladder (Table 4) (580). Type 1-fimbriated bacteria bind to ureteral epithelium (138) and to human, monkey, rat, and porcine kidney cell lines (224, 279, 414, 466). However, the scanty binding sites for type 1-fimbriated cells within the human kidney (559) are limited to the vascular connective tissue layers of vessels and the cytoplasm and lumenal surfaces of proximal tubular cells (579); the distal tubules, collecting ducts, glomeruli, and vascular endothelium are devoid of receptors (Table 4) (579).

Interactions With Phagocytes

The role of type 1 fimbriae in bacterial interactions with hPMNLs is complex. Type 1 fimbriae promote adherence to hPMNLs (34, 277, 394, 515, 547) and phagocytosis by hPMNLs (494, 600); antifimbrial antibodies and solutions of mannose or α-methylmannoside block these interactions (334, 335, 494, 600). When present on unopsonized or hydrophilic bacteria (which interact minimally with phagocytes in the absence of type 1 fimbriae), type 1 fimbriae play a more important role in adherence to phagocytes (155, 515), granule release (506), and phagocytosis (394) than when they are present on opsonized or hydrophobic organisms (which readily interact with phagocytes even when nonfimbriated). Whereas purified type 1 fimbriae agglutinate phagocytes (334), only aggregated type 1 fimbriae (i.e., intact fimbriated bacteria or latex beads coated with purified type 1 fimbriae) stimulate chemiluminescence (155, 334), demonstrating that monovalent binding is insufficient to stimulate the oxidative burst. In contrast, the binding of purified type 1 fimbriae does prime hPMNLs to give a greater chemiluminescent response when subsequently stimulated by a variety of nonspecific stimuli or by latex-bound type 1 fimbriae (153, 335). The receptor for type 1 fimbriae on hPMNLs is a 150-kDa glycoprotein which may be the same as CR3, the C_3bi receptor (153, 460, 514). This receptor may mediate "priming," with the Fc receptor mediating stimulation of the respiratory burst (153).

The fate of bacteria adhering to hPMNLs via type 1 fimbriae is variable, depending on bacterial hydrophobicity and state of opsonization. Some strains escape phagocytosis but are killed all the same (34, 394), presumably by the contents of phagocytic granules that are released following bacterial binding (506) or by toxic products of the respiratory burst triggered by bacterial binding. Remarkably, type 1-fimbriated bacteria that are phagocytosed can survive within lysosomal vacuoles as long as they are unopsonized (154), possibly because of subnormal levels of myeloperoxidase activity in lysosomes containing unopsonized bacteria (154). Thus, type 1 fimbriae may not in all cases represent a liability for bacteria in their encounters with phagocytes.

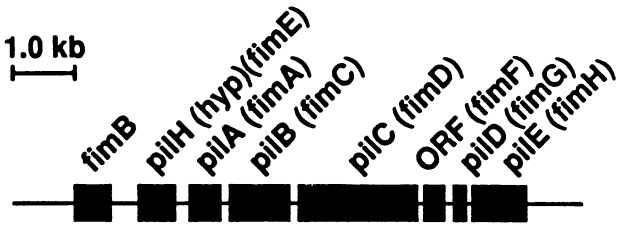

FIG. 12. Physical and genetic organization of the *pil-fim* regions encoding type 1 fimbriae labeled with the mnemonics both of Maurer and Orndorff (345) and of Klemm and Christianson (in parentheses) (271). PilA-FimA is the structural subunit; PilB-FimC and PilC-FimD are important in fimbrial assembly and in anchoring assembled fimbriae; *pilH-fimE* and *fimB* are regulatory genes; and PilD-FimG and PilE-FimH are the adhesin complex, with PilE-FimH constituting the actual adhesin molecule. Adapted from reference 33, with permission from the publisher.

Genetics and Structure

Like other *E. coli* fimbriae, type 1 fimbriae are encoded by a gene cluster that includes genes for a structural subunit, an adhesin, several accessory proteins (involved in subunit transport and assembly and in anchoring assembled fimbriae), and regulatory proteins (Fig. 12) (5, 6, 202, 270–272, 345, 346, 352, 401, 402). The adhesin (PilE-FimH) and the structural subunit (PilA-FimA) can be expressed independently from one another (352). There are three transcriptional units, one for the structural subunit and two for the adhesin and the remaining accessory proteins (270). Clinical isolates typically contain a single copy of the type 1 fimbrial gene cluster (15, 229).

Type 1 fimbriae exhibit the structure described by Brinton (46) (Fig. 3). They are quite stable, requiring extreme physicochemical conditions for depolymerization (46, 120, 392, 465). Disassembled type 1-fimbrial subunits can spontaneously reassemble under the appropriate conditions to form rodlike aggregates reminiscent of intact fimbriae (46, 120). Synthetic peptides derived from amino acids 22 to 35 of the N terminus of PilA-FimA also spontaneously self-assemble to form rodlike aggregates (4); these aggregates, but not the peptides alone, cross-react serologically with intact type 1 fimbriae, indicating that the quaternary structure of the peptide aggregates includes epitopes mimicking those of native fimbriae (4). In bacteria, fimbriae are assembled by the addition of new subunits to the base of growing fimbriae at the cell surface (109).

Type 1 fimbriae are hydrophobic (5, 45, 46, 224, 269), comprising predominantly nonpolar amino acids (46, 465). Brinton originally proposed that they mediate hemagglutination through nonspecific hydrophobic interactions (46), a view later challenged because of the mannose-sensitive nature of type 1-fimbrial binding and the observation that type 1 fimbriae do not agglutinate inside-out erythrocyte ghosts or liposomes (535). More recent evidence (see above) suggests that on some cell types the type 1-fimbrial receptor has both a hydrophobic component and a mannoside-binding region (124). The marked hydrophobicity of the adhesin protein (5, 188, 189) lends additional support to the concept that hydrophobic interactions are important in type 1-fimbrial binding.

The precise position of the type 1-fimbrial adhesin is controversial, with various lines of indirect evidence interpreted as indicating a tip (46, 188, 189) or a lateral (466, 535) location. Immunoelectron microscopy using antisera raised

to synthetic peptides derived from the amino acid sequence of PilE-FimH (the adhesin) identifies adhesin molecules both at the fimbrial tips and distributed sparsely along the length of the fimbriae (6), suggesting that both the "tip" hypothesis and the "lateral" hypothesis are correct.

Regulation of Expression

Expression of type 1 fimbriae is typically an all-or-none phenomenon, with most strains either fully fimbriated or devoid of fimbriae (45, 145, 208, 381), although exceptions have been described (207). In most clinical isolates, expression of type 1 fimbriae is controlled by environmental conditions, with growth on agar inhibiting fimbrial production and growth in broth promoting it (208, 269, 469). In some strains the reverse is true (145, 469); the pattern followed may depend in part on the clinical source of the strain (469). In contrast to P fimbriae, type 1 fimbriae are expressed at 20°C as well as at 37°C (269, 410). However, fimbriated phase variants have a higher growth rate at higher temperatures and a lower growth rate at lower temperatures than do nonfimbriated cells, leading to a predominantly fimbriated population at higher temperatures and a predominantly nonfimbriated population at lower temperatures (45). Type 1-fimbrial expression is promoted by growth at limiting oxygen concentrations (45). Subinhibitory concentrations of most cephalosporins increase type 1-fimbrial expression; cefuroxime, cephalexin, and imipenem have little effect; vancomycin, the aminoglycosides, spectinomycin, tetracycline, and chloramphenicol decrease expression; and penicillin and its derivatives either increase (268) or decrease (387) expression (110, 268, 387).

Random phase variation. Some type 1-fimbriated strains undergo random phase variation independent of growth conditions, with a small number of cells in the population switching from nonfimbriated to fimbriated or vice versa every generation (2, 45, 108, 283, 381). Different rates have been described for this switch (10^{-4} to 10^{-2} per generation, or even faster) (2, 45, 108, 283). The rate of switching is temperature dependent (46). Phase variation is controlled at the transcriptional level (108) both in *E. coli* K-12 and in clinical strains by a 314-bp invertable DNA segment located upstream of the structural subunit (112). The two genes immediately upstream from the subunit gene direct the inversion of this "switch" segment, switching it to the on (*fimB*) or off (*pilH-fimE*) position (270) by combining with integration host factor. Thereby the integration host factor is allowed to bind to a recognition site adjacent to the switch segment to mediate site-specific recombination (112). *pilH-fimE* also down-regulates the expression of type 1 fimbriae even when the switch is in the on position (404). Deletions in another regulatory region outside the bounds of the recognized fimbrial gene cluster but near the adhesin gene yield hyperadhesive, hyperfimbriated mutants (5).

Serology

Type 1 fimbriae of different *E. coli* strains share common antigens (102, 279, 410) but can be differentiated on the basis of strain- or group-specific antigens (208, 410, 427). In many cases, antifimbrial antibodies block fimbrial adherence (279, 466, 529). The adhesin protein, PilE-FimH, is antigenically conserved among strains with serologically different type 1 fimbriae (188). Type 1 fimbriae are no more common among strains from UTI-associated serotypes than among other strains (102).

Relationship to Other Fimbriae

Type 1 fimbriae of *E. coli* bear a closer serological and genetic relationship to type 1 fimbriae of *Shigella* and *Klebsiella* species than to type 1 fimbriae of other genera of *Enterobacteriaceae* (51, 66, 67, 69, 279). In *E. coli*, the organization of the type 1-fimbrial region is more like that of K88 fimbriae than that of P fimbriae (401). A minor degree of amino acid homology is present with P fimbriae (171, 386, 403) and even with *H. influenzae* fimbriae (171). However, type 1 fimbriae do not cross-react serologically with P fimbriae or *H. influenzae* fimbriae (171, 386). The F1B and F1C (see above) fimbriae of *E. coli* are closely related to type 1 fimbriae (F1A) serologically and structurally but not functionally (273).

Animal Models

In the mouse, antimannoside antibodies identify receptors for type 1 fimbriae in the vagina, bladder, ureter, renal pelvis, collecting ducts, and tubular epithelium (391). In most studies, type 1 fimbriae have been found to be particularly important in bladder colonization (180, 211, 213, 262, 477), although exceptions have been reported (265), and to be a more important determinant of bladder colonization than are P fimbriae (180). Because fimbriated organisms adhere to the bladder, urine cultures can be negative despite the presence of adherent bladder organisms (207). Only in the absence of P fimbriae do type 1 fimbriae contribute to upper urinary tract colonization (180). A role for type 1 fimbriae in frank renal invasion is not as well documented (97, 391, 567).

Administration of α-methyl mannoside (12), but not D-mannose (520), along with the bacterial inoculum protects mice from bladder colonization with type 1-fimbriated organisms. Antibodies directed against the adhesin or against mannoside (but not against the fimbrial subunit) protect against subsequent bacterial challenge (3). Antibodies directed against intact fimbriae are protective if mixed with the bacterial suspension at the time of bladder inoculation (207) but not if stimulated by active immunization of the experimental animal prior to bacterial inoculation (391). These observations suggest that interventions that block adherence can prevent UTI due to type 1-fimbriated strains.

Type 1 fimbriae also contribute to bladder colonization in rats (339). The receptor in the rat bladder may be a glycolipid, since adherence is diminished after treatment with lipase (79). The degree of renal scarring developing in rats after direct intrarenal injection of *E. coli*, as well as the degree of chemiluminescence and neutral protease release after incubation of bacteria with hPMNLs, is greatest among type 1-fimbriated strains (547). This evidence suggests that type 1 fimbriae may play a role in renal scarring by stimulating phagocyte-induced renal injury. Type 1 fimbriae contribute to oropharyngeal but not ileal colonization in rats (33, 170). Active or passive antifimbrial immunization protects rats from UTI (493) by blocking epithelial cell adherence (495).

Epidemiology

Expression during UTI. In the first study of type 1-fimbrial expression by urine organisms, mannose-sensitive hemagglutination activity was exhibited by only 2 of 24 infected catheter urine specimens (388, 390), even though 11 of the 24 isolates expressed type 1 fimbriae after subculture. These data

TABLE 7. Association of type 1 fimbriae with clinical source of isolate

Reference	Proportion (%) of strains expressing type 1 fimbriae[a]				
	PN	CY	ABU	Urine[b]	Fecal
13				3/20 (59)[c]	
15	10/49 (20)[d]	12/48 (25)		22/97 (23)[d]	23/40 (58)
48				95/112 (85)	23/40 (58)
98	24/25 (96)	17/17 (100)	26/27 (96)	67/69 (97)	
117	24/135 (18)	64/121 (53)	21/119 (18)	109/375 (29)	36/120 (30)
146	17/29 (58)	20/28 (71)	82/139 (59)	131/211 (62)[c]	8/19 (42)
177				71/93 (76)	54/96 (56)
181	95/111 (86)	94/103 (91)	82/119 (69)	271/333 (81)	77/120 (64)
209				19/43 (44)	
230				51/58 (88)[c,d]	
295	14/23 (61)	81/116 (70)	24/40 (60)	119/179 (66)	
393	12/12 (100)	26/26 (100)	6/6 (100)	44/44 (100)	73/73 (100)
388				75/135 (55)	
399				61/100 (61)	33/50 (66)
423		90/131 (69)	26/31 (84)	213/335 (64)	
557	61/67 (91)	50/60 (83)	49/60 (82)	160/187 (86)	38/50 (76)
607	47/57 (82)	35/43 (81)		82/100 (82)	
Total[e]	304/508 (60)	489/693 (71)	316/541 (58)	1,593/2,491 (64)	365/608 (60)

[a] PN, Pyelonephritis or febrile UTI; CY, cystitis; ABU, asymptomatic bacteriuria.
[b] Preceding three columns plus any other urinary isolates of unspecified clinical category.
[c] Urosepsis patient isolates.
[d] Includes patients with known compromising conditions.
[e] Because of differences in study design, methods, definitions, and populations in different studies, totaled results must be interpreted with caution.

called into question the in vivo relevance of type 1-fimbrial expression. Subsequently, type 1-fimbriated organisms have been identified in 15 of 37 (313), 5 of 20 (428), and 31 of 41 (267) voided urine specimens from noncompromised patients with acute UTI. In one study (267), type 1-fimbrial phase variants were identified in specimens collected from different sites within the urinary tract. These more recent findings demonstrate that not only type 1-fimbrial expression but also phase variation is a relevant in vivo phenomenon.

Clinical studies. Type 1 fimbriae are expressed by a similar proportion of all urinary and fecal strains (Table 7), ranging from a high of 71% among cystitis patient isolates to a low of 58% among ABU patient isolates, with fecal strains in the midrange at 60%. In contrast, the level of expression of type 1 fimbriae among blood isolates (145 of 178 [81%]) is more convincingly different from that of fecal strains (13, 230, 399). Individual studies have been interpreted to show that type 1 fimbriae are associated with pyelonephritis patient isolates only (114), cystitis patient isolates only (117), both pyelonephritis and cystitis patient isolates (48, 181, 209), and fecal isolates only (14). In one study of infants with UTI, a lower level of type 1-fimbrial expression was found among strains from boys than from girls, regardless of the clinical syndrome (607). These discrepant results may be attributable to differences in patient selection and definition and possibly to the variability in type 1-fimbrial expression with respect to culture conditions. Investigators using serial passage in unshaken broth to promote fimbrial expression (230, 393) report higher levels of expression than do those using a single passage or passage on agar (15). There is little evidence that type 1-fimbrial production is less common among strains from compromised patients than among those from noncompromised patients (15, 146, 230).

Strains expressing mannose-sensitive adhesins alone (in the absence of mannose-resistant adhesins) are associated with clinical cystitis (181), with UTI episodes in which the antibody-coated-bacteria test is negative (146), and with isolates from asymptomatic UTI episodes (423), evidence suggesting that type 1 fimbriae may play a more important role in colonization or infection of the bladder than in invasive UTI. Additionally, type 1-fimbrial expression is slightly more common in episodes of UTI localized to the lower (127 of 195 [65%]) as opposed to the upper (194 of 267 [73%]) urinary tract (48, 146, 295). Type 1 fimbriae may also contribute to bacterial persistence in patients with *E. coli* UTI during long-term use of an indwelling bladder catheter (355).

Summary

Type 1 fimbriae are common among *E. coli* strains from all clinical categories of UTI and among fecal strains. The adherence of type 1-fimbriated strains to host cells in the urinary tract may promote the development of cystitis, their adherence to and stimulation of hPMNLs may promote bacterial killing but may also contribute to renal scarring, and their binding to THP may allow the host to eliminate them from the urinary tract before they can initiate colonization or infection. Passive immunization against type 1 fimbriae protects animals from UTI with type 1-fimbriated strains, but whether active immunization protects animals or will be effective in humans remains to be determined. Receptor analog therapy is also effective in animal models and holds promise for human use.

AEROBACTIN

Bacterial Siderophores and the Superiority of Aerobactin

Iron is needed by all living cells (599). *E. coli* uses iron for oxygen transport and storage, DNA synthesis, electron transport, and metabolism of peroxides (20, 367). Ferric iron is highly insoluble, giving a free-iron concentration of 10^{-18} M at pH 7 (367), or 10^3 free iron atoms per ml (165); in comparison, bacteria contain 10^5 to 10^6 iron atoms per cell

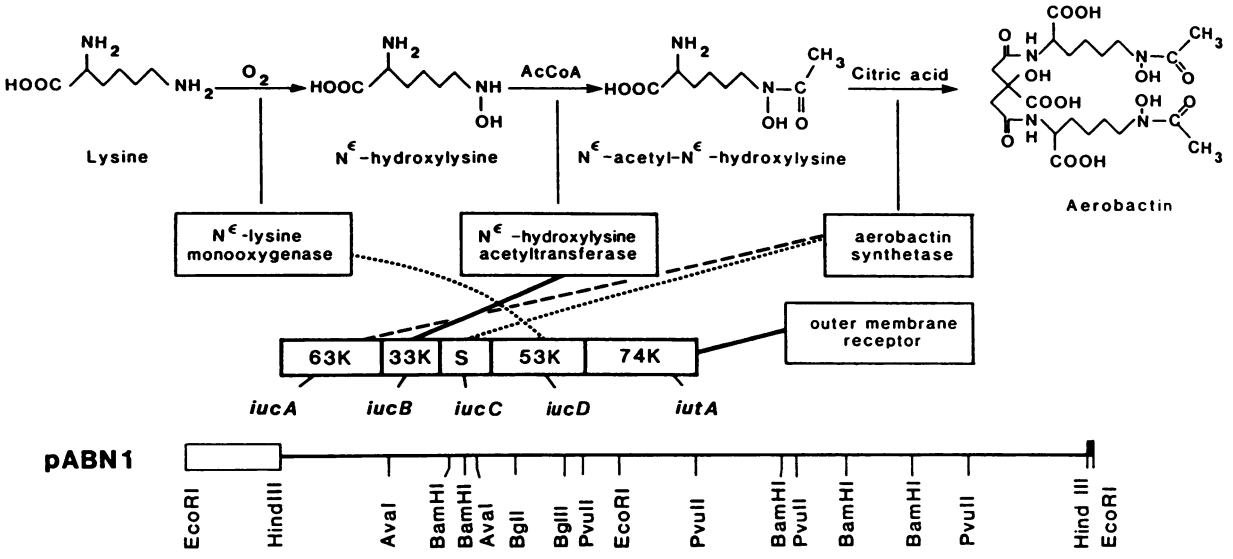

FIG. 13. Proposed pathway of aerobactin biosynthesis in pColV-K30. The sequence involves the hydroxylation of lysine (catalyzed by the product of *iucD*), the acetylation of hydroxylysine (catalyzed by the product of *iucB*), and the condensation of two acetylhydroxylysines (hydroxamic acids) with citric acid (catalyzed by the products of *iucA* and *iucC*). The 74-kDa product of *iutA* is the outer membrane receptor protein. Adapted from reference 84, with permission from the publisher.

(165). Although the total iron concentration in biological fluids is >20 μM (86), almost all of this iron is complexed with host iron proteins. Part of the host response to infection is to further reduce the amount of iron available to the invading pathogen by decreasing intestinal iron absorption, synthesizing additional iron proteins, and shifting iron from the plasma pool into intracellular storage (599). Thus, bacteria face a formidable challenge in meeting their iron needs during infection. In *E. coli*, the hydroxamate siderophore aerobactin is the most effective of the several iron chelation systems employed by enteric bacteria for iron acquisition (20, 28, 40, 86, 367, 594, 609). Aerobactin is a small molecule (M_W 616) formed from the condensation of two lysine molecules and one citrate (Fig. 13) (367). Following secretion by *E. coli* cells, aerobactin extracts Fe^{3+} from host iron-binding proteins and is taken up through a 74-kDa outer membrane receptor protein (56, 87) which is also the receptor for cloacin (a toxin produced by some *Enterobacter cloacae* strains) (26, 576). Strains with the aerobactin system have a growth advantage in low-iron conditions (40, 361, 608), including in serum and dilute urine.

The aerobactin system has many advantages over other siderophores. *E. coli* can use but does not synthesize ferrichrome (20) and cannot transport enough citrate to support growth in low-iron conditions in the absence of other siderophores (20, 367). Although enterobactin (enterochelin), the other major specialized siderophore of *E. coli*, has a higher affinity constant for iron (10^{52}) than does aerobactin (10^{23}) when deprotonated (86, 609), enterobactin's affinity constant at neutral pH is much lower (20). (In comparison, the affinity constant of transferrin for iron is $10^{22.7}$ [86] or 10^{30} [609]). Enterobactin deferrates transferrin more rapidly than does aerobactin in aqueous solutions; however, the reverse is true in serum or in the presence of albumin, presumably because enterobactin (but not aerobactin) binds to and is inactivated by proteins (20, 40, 86, 594, 609). Enterobactin is less soluble and less stable than aerobactin (86, 594). Release of iron from enterobactin requires hydrolysis of the siderophore (594), whereas aerobactin is continuously recycled without hydrolysis (40). In contrast to enterobactin, which leaves iron free in the cytosol, aerobactin delivers iron directly to bacterial iron centers (609). Neither enterobactin nor aerobactin is large enough alone to stimulate antibody production, but enterobactin (a catechol) binds to serum proteins in a haptenlike fashion, giving rise to antienterobactin antibodies that probably limit its usefulness in vivo (28). Finally, aerobactin production is stimulated by milder degrees of iron deprivation than are required to stimulate enterobactin production (86, 609).

Genetics

In almost all *E. coli* strains, the aerobactin system is encoded by a five-gene operon, with four genes encoding the enzymes needed for aerobactin synthesis and a fifth gene encoding the outer membrane receptor protein (Fig. 13) (27, 56, 84, 166, 288). The synthesis genes are termed *iuc*, for iron uptake: chelate, and the receptor gene is *iut*, for iron uptake: transport (84). Successive steps in the biosynthesis of aerobactin are catalyzed by the *iuc* genes in the sequence *DBAC* and involve (i) hydroxylation of lysine, (ii) acetylation of the hydroxyl group to give hydroxamic acid, and (iii) condensation of two successive hydroxamic acid molecules with citrate to give aerobactin (Fig. 13) (20, 84, 87). The first of these steps might be a good target for chemotherapy, since a comparable enzyme is not present in mammalian cells (20).

Regulation. Aerobactin production is regulated by the intracellular iron concentration through the *fur* (ferric uptake regulation) gene product (19, 27, 41, 562). When iron concentrations are high enough, the *fur* repressor complexes with iron (or other divalent cations) and binds to an "iron box" in the promoter region of the aerobactin operon (and other iron-regulated genes), blocking transcription (19, 20). In low-iron conditions, the *fur* repressor is released from the promoter region and transcription proceeds.

Plasmid versus chromosomal aerobactin. Aerobactin determinants are found both on plasmids and on the bacterial chromosome, with the chromosomal location predominating

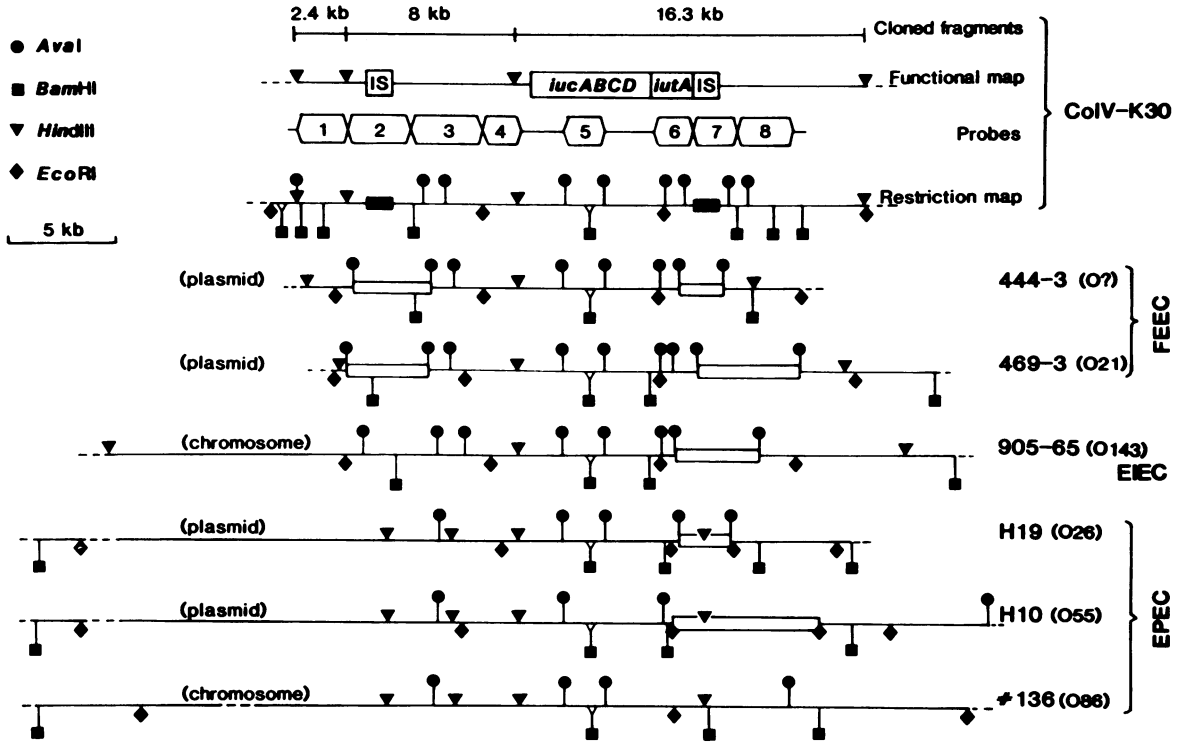

FIG. 14. Comparative restriction maps of the aerobactin system and its flanking regions in six clinical isolates of *E. coli*, including two strains with chromosomal aerobactin determinants (one enteroinvasive strain [EIEC] and one enteropathogenic strain [EPEC]) and four strains with plasmid aerobactin determinants (two extraintestinal isolates [FEEC] and two EPEC strains). Note high degree of conservation at the level of restriction sites within the aerobactin synthesis region (*iucABCD*) and substantial divergence within the upstream and downstream flanking sequences. IS*2*-related sequences were detected in the downstream regions of five of six strains but in the upstream regions of only two. Strain numbers, with respective O serogroups (in parentheses) and diagnostic classification, are shown at the right-hand side of the map, and the plasmid or chromosomal location of the aerobactin determinant in each strain is indicated on the left. The known positions of the IS*1* elements are indicated as black rectangles on the ColV-K30 map. Open rectangles on the other maps represent the smallest restriction fragments to which the IS*1*-containing probes of ColV-K30 (probes 2 and 7) hybridized. From reference 458, with permission from Elsevier Science Publishers (Biomedical Division).

among human clinical isolates (229, 563). The best-studied aerobactin system in *E. coli* is that of the large, conjugative colicin V plasmid pColV-K30 (27, 56, 608, 611). In pColV-K30 the aerobactin region is flanked by IS*1*-like insertion sequences, which in turn are bounded by replication sequences (430). Other plasmid-encoded aerobactin systems share strict homology with pColV-K30 within the aerobactin operon proper and have similar upstream flanking sequences (often including IS*1* and a replication sequence), whereas there is greater heterogeneity in the downstream flanking sequences (Fig. 14) (27, 458, 596). In strains with a chromosomal aerobactin system, the aerobactin-coding region is also identical to that found on plasmids (562). Here, however, flanking sequences diverge more widely from those of plasmid aerobactin systems (27, 458, 561), and IS*1* and replication sequences are absent (Fig. 14) (561). Consistent with the differences in upstream flanking sequences, plasmid and chromosomal aerobactin regions are regulated differently, albeit still through the *fur* repressor (562).

Transposition. Despite its superficial similarities to a transposon, the aerobactin region has not been shown to undergo transposition (85, 596). It seems unlikely that the aerobactin region has functioned as a transposon in recent evolutionary history (458) because the insertion sequences are not in the usual orientation, the region is too large, and the conserved region extends beyond the insertion sequences (596).

Association with Other VFs

The aerobactin system and P fimbriae are commonly found together in isolates from patients with UTI and urosepsis (217, 222, 229), although among urosepsis patient isolates this association holds only for chromosomally encoded aerobactin (229). Similarly, an association of chromosomally encoded aerobactin with hemolysin is apparent among urosepsis patient isolates (229), whereas there is no association of aerobactin with hemolysin among urosepsis or UTI patient isolates when strains with plasmid and chromosomal aerobactin systems are grouped together (229, 415). These observations suggest that plasmid and chromosomal aerobactin regions differ not only in their immediate genetic environment but also in their association with other VFs. Other virulence properties encountered more commonly among aerobactin-producing strains include K capsular antigens, resistance to phagocytosis, and survival in heat-inactivated serum (361).

Plasmids carrying the aerobactin region sometimes also carry antimicrobial agent resistance genes (70, 83, 229, 431). Among patients with urosepsis, strains carrying such plasmids are more common among compromised than among noncompromised hosts (229), possibly because of greater exposure to antimicrobial agents in the compromised patients. In England in 1986 to 1987, an *E. coli* strain carrying

an F1*me* plasmid that included the aerobactin system and multiple antimicrobial agent resistance genes was involved in an extensive outbreak of serious infections, including urosepsis, meningitis, and pneumonia (431). This plasmid was similar to the F1*me* plasmids that have been implicated in outbreaks of septicemic salmonellosis in the past decade in several parts of the world (70); like the epidemic plasmid from England, these salmonella plasmids also encoded aerobactin and resistance to multiple antimicrobial agents. In a separate report, an *E. coli* strain carrying a plasmid with aerobactin determinants and multiple antimicrobial agent resistance genes was isolated from a pancreatic abscess along with a strain of *Salmonella typhi* carrying the same plasmid (83). These observations suggest that aerobactin determinants may be passed between genera on plasmids in association with antimicrobial agent resistance genes, that selective pressure from antimicrobial agents may favor the acquisition of such plasmids, and that serious infections may result.

Other Hosts and Other Genera

In both humans and animals, enterotoxigenic *E. coli* rarely expresses the aerobactin system; in contrast, human enteropathogenic and enteroinvasive *E. coli* strains and isolates from domestic animals with sepsis and mastitis do (311, 610). Aerobactin is produced by >40% of *E. coli* and *Shigella* and *Enterobacter* strains and by a lower proportion of *Klebsiella*, *Citrobacter*, *Proteus*, *Morganella*, *Yersinia*, *Serratia*, and *Salmonella* strains (61, 86, 307, 441, 458). In some *E. coli*, *Enterobacter*, and *Shigella* strains, the aerobactin receptor protein differs slightly from the typical *E. coli* aerobactin receptor (73, 163).

Animal Studies

In a peritonitis model in mice, early studies (608, 611) showed that the enhanced virulence of an aerobactin-producing strain was attributable to the aerobactin system and not to colicin V, which was encoded on the same plasmid as the aerobactin system. In contrast, enterobactin (another *E. coli* siderophore) was not associated with enhanced virulence (362). Aerobactin-producing strains isolated from diseased poultry are more virulent in 1-day-old chicks than aerobactin-negative strains (294), and aerobactin-producing strains from patients with *E. coli* bacteremia are more lethal (but not more nephropathogenic) in a mouse model of ascending UTI than nonaerobactin-producing strains (361). There is no published experience with antiaerobactin interventions in animals.

Epidemiology

Phenotype-genotype comparisons. Aerobactin production by clinical isolates is commonly detected by using a bioassay system that relies on augmented growth in low-iron conditions of an aerobactin-requiring *E. coli* indicator strain around colonies of the test strain; biochemical assays are also available (57). Aerobactin use by a clinical isolate is usually identified by augmented growth of the strain in low-iron conditions when it is provided with exogenous aerobactin (57). Sensitivity to cloacin can also be used as an assay, since aerobactin and cloacin share the same outer membrane protein receptor (26, 27, 576).

DNA hybridization studies using aerobactin-specific probes and bioassays for aerobactin production or receptor expression generally give concordant results, although occasional exceptions occur (229, 294, 311, 416). The clinical category accounting for the greatest number of probe-positive, bioassay-negative strains is ABU (416); these strains presumably contain incomplete or nonfunctional copies of the aerobactin region. Rare probe-negative, bioassay-positive strains synthesize aerobactin by using genetic determinants other than the usual *E. coli* aerobactin biosynthesis genes (311), a situation similar to that in some *Enterobacter* strains (73, 597). The aerobactin system is common among strains of certain O:K:H serotypes, whereas strains of other serotypes seldom or never produce aerobactin (415). Most probe-positive, bioassay-negative strains belong to certain of the latter serotypes, including O1:K1:H^-, O6:K5:H1, and O75:K100:H^- (416).

Clinical studies. The aerobactin system is more common among *E. coli* strains isolated from patients with pyelonephritis (73%), cystitis (49%), or bacteremia (58%) than among ABU patient isolates (38%) or fecal strains (41%) (Table 8), evidence suggesting that aerobactin contributes to virulence both within and outside of the urinary tract. The association of aerobactin with more serious forms of UTI is seen specifically in infants (607), girls (217, 222), and women (217, 222). The markedly lower prevalence of aerobactin production among environmental isolates of *E. coli* (6%) (510) compared with human isolates (Table 8) suggests that aerobactin may facilitate colonization of the human gastrointestinal tract by commensal strains in addition to its role in infection. There is little evidence that the aerobactin system is any less prevalent among strains from compromised hosts than in those from noncompromised hosts (222, 229, 361).

Summary

The aerobactin system is associated with *E. coli* isolates from serious UTI and other serious infections in humans and animals, probably because it promotes bacterial growth in the limiting iron concentrations encountered during infection. The chromosomal aerobactin system is associated with other uropathogenic VF determinants, whereas the plasmid aerobactin system is often carried by plasmids encoding multiple antimicrobial agent resistance. The aerobactin receptor protein is a potential target for an antiaerobactin vaccine, and a unique enzymatic step involved in aerobactin biosynthesis could be the target of antiaerobactin chemotherapy.

HEMOLYSIN

The cytolytic protein toxin secreted by most hemolytic *E. coli* strains is known as alpha hemolysin (59). Although cell-bound (beta) hemolysins and secreted hemolysins other than alpha hemolysin have been described (22, 233, 497, 591, 593), their prevalence and clinical significance are unknown. This discussion is limited to alpha hemolysin.

Mechanism of Hemolysis

Alpha hemolysin lyses erythrocytes of all mammals and even of fish (442). The hemolytic substance present in culture supernatant is composed almost entirely of protein (442), with some evidence of a minor phospholipid (591) or lipopolysaccharide (37) component, and has an estimated M_W of 2×10^5 to 8×10^5 (233, 442, 489, 591). By protein gel electrophoresis the largest protein species is about 110 kDa (125, 126, 200, 233, 591), leading to the speculation that the

TABLE 8. Association of aerobactin production with clinical source of isolate

Reference	Proportion (%) of strains producing aerobactin[a]					
	PN	CY	ABU	Urine[b]	Fecal	Blood
55	53/71 (75)	125/209 (60)	48/76 (63)	226/356 (63)	23/67 (34)	64/93 (69)
61				83/177 (47)	25/61 (41)	49/108 (45)
217	151/210 (72)	47/113 (42)		19/323 (61)		
222	94/122 (77)			94/122 (77)[c]	53/120 (44)	89/159 (56)
229				45/58 (76)[c,d]		45/58 (76)
343					25/61 (41)	43/96 (45)
361				6/16 (38)[c]	8/19 (42)	24/32 (75)
415	97/139 (70)	48/119 (40)	24/112 (21)	169/370 (46)		
510					19/45 (42)	32/50 (64)
607	45/57 (79)	19/43 (44)		64/100 (64)		
Total[e]	440/599 (73)	239/484 (49)	72/188 (38)	885/1,522 (58)	153/373 (41)	346/596 (58)

[a] PN, Pyelonephritis or febrile UTI; CY, cystitis; ABU, asymptomatic bacteriuria.
[b] Preceding three columns plus any other urinary isolates of unspecified clinical category.
[c] Includes patients with known compromising conditions.
[d] Urosepsis patient isolates.
[e] Because of differences in study design, methods, definitions, and populations in different studies, totaled results must be interpreted with caution.

high-M_W substance in hemolytic supernatants is a complex of 110-kDa hemolysin protein monomers (591). Hemolysin complexes can be purified by a variety of methods (36).

Lysis of erythrocytes is generally described to require calcium, with a maximal effect at ≥10 mM (cf. physiological concentrations of 2 to 3 mM) (489), although lysis in the absence of calcium has been described (25). Calcium may modify hemolysin aggregates so as to make them hemolytically competent (442, 443). After a lag phase (the length of which is decreased with increasing hemolysin concentrations and by preincubation of hemolysin with Ca^{2+}) (442), lysis occurs rapidly (489).

Hemolysin molecules insert into lipid-containing membranes producing cation-selective channels of large conductance with a diameter of 2 nm (24, 349) that increase the permeability of erythrocyte membranes to Ca^{2+}, K^+, mannitol, and sucrose (24, 25, 232). The increase in permeability to Ca^{2+} is maximal after a single hit (232). At the membrane level, the toxin appears to be monomeric (24, 25, 348). At low hemolysin-to-erythrocyte ratios, subfractions of the erythrocyte population are lysed or bleached of hemoglobin (489), while the remaining erythrocytes appear normal. Erythrocyte ghosts act as a sink for hemolysin molecules (489).

Cytotoxicity

In addition to lysing erythrocytes, hemolysin is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury, and impaired host defenses. Exposure of hPMNLs to hemolysin stimulates chemiluminescence, degranulation, and release of leukotrienes and ATP; causes marked morphologic alterations; and impairs chemotaxis and phagocytosis. Lysis occurs at higher concentrations (23, 59, 60, 141, 142, 277, 435, 478). Monocytes and granulocytes are highly susceptible to hemolysin cytotoxicity, whereas lymphocytes are relatively resistant (143). Hemolysin production correlates closely with the toxicity of clinical *E. coli* isolates for hPMNLs (141, 142). Hemolysin stimulates superoxide anion and hydrogen peroxide release from and oxygen consumption by renal tubular cells (261) as well as histamine release from mast cells and basophils (168, 277, 478, 479). Hemolysin is also highly cytotoxic to chicken embryo cell cultures, but the hemolytic and cytotoxic factors may not be identical in this case (63).

Genetics and Secretion

Hemolysin production is encoded by a four-gene operon termed *hly* (Fig. 15) (152), which is located on the chromosome in human isolates of *E. coli* in contrast to the plasmid location common among animal strains (229, 605). Plasmid and chromosomal *hly* regions differ with respect to flanking and regulatory sequences and to the precise sequence of *hlyA*, the gene encoding the structural hemolysin protein (363, 581, 588, 604). The 110-kDa HlyA protein is unique among *E. coli* toxins in that it is secreted across both membranes without cellular lysis and without cleavage of a signal peptide (152, 591). To bind to erythrocytes (405) and have hemolytic activity (152, 591), HlyA must be activated by the 20-kDa intracellular protein HlyC (126, 369) prior to its secretion. Activation of HlyA by HlyC requires the presence of several repeat sequences present in the C-terminal end of HlyA (127).

Secretion. The first step in hemolysin secretion is an energy-dependent process (504) involving HlyB (174), whereas release from the outer membrane is passive (504)

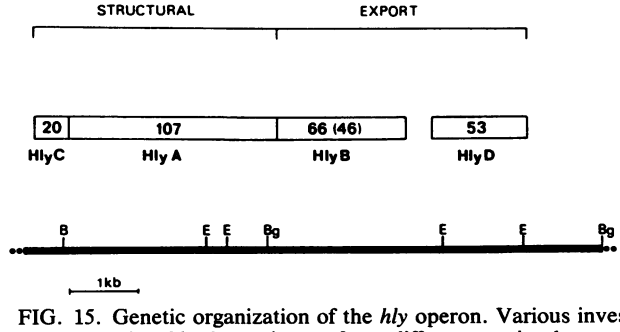

FIG. 15. Genetic organization of the *hly* operon. Various investigators studying *hly* determinants from different strains have reported minor differences in the sizes of the proteins HlyA, HlyB, HlyC, and HlyD. Symbols for endonucleases are B, *Bam*HI; Bg, *Bgl*II; and E, *Eco*RI. From reference 331, with permission from Springer-Verlag, Heidelberg, Germany.

and requires HlyD (591). In *hlyB* mutants, HlyA remains in the intracellular or periplasmic space, resulting in a nonhemolytic phenotype (152, 405, 592), whereas *hlyD* mutants accumulate hemolysin on the cell surface, giving small zones of clearing on blood agar plates but no free hemolysin in culture supernatants (174, 234, 592). The precise mechanism of hemolysin transport across both membranes and the periplasmic space remains conjectural (331).

Transcription. The *hly* operon is transcribed from a promoter upstream of *hlyC*, in the order *hlyCABD* (606). Different hemolytic strains have different regulatory sequences in this region and thus produce and secrete different amounts of hemolysin, with corresponding differences in strain virulence (286, 581, 604, 606). Production of excessive amounts of hemolysin is lethal to the *E. coli* cell (152). Transcription of the *hly* operon often terminates prematurely (284, 606), yielding transcripts of different lengths from the same promoter.

Regulation of Expression

In some strains, hemolysin production is suppressed in high-iron conditions and enhanced in low-iron conditions (296, 331, 589). Hemolytic activity is maximal in the supernatants of log-phase cultures of hemolytic strains (e.g., 2 to 2.5 at 37°C) and declines as cultures enter stationary phase (233), despite continued production of hemolysin protein (591). Hemolytic activity in culture supernatants is labile, decaying at a rate proportional to the temperature (233, 442, 497); this lability is not due to degradation of hemolysin (37), despite the sensitivity of hemolysin to proteases (442, 489). The amount of hemolytic activity in culture supernatants varies depending on the composition of the medium (16, 233, 442, 489, 497), probably not because of altered rates of hemolysin production (234) but because of substances in the medium (233, 442) that influence the rate of inactivation of hemolysin in the supernatant (331, 370, 489).

Molecular Epidemiology

All hemolytic UTI and urosepsis strains hybridize with the cloned hemolysin determinants from a pyelonephritis patient isolate (13, 15, 229, 605). Whereas *hlyB* and *hlyD* are highly conserved among different hemolytic *E. coli* strains, there are strain-to-strain differences within *hlyA* (21, 364). Multiple copies of the *hly* operon are present in a minority of strains (276).

The codon usage in *hly* is uncharacteristic of *E. coli*, and the G-C content (40%) is more like that of *Proteus* or *Pasteurella* spp. than of *E. coli* (126, 286, 602). These observations, and the presence of hemolysin determinants genetically related to the *E. coli hly* region in some *Proteus*, *Pasteurella*, and *Morganella* strains (285, 602), suggest that *E. coli hly* genes may have originated in one of these genera (286, 602). However, on the basis of codon usage and amino acid homology, it has also been proposed that HlyB may have a mammalian origin (148) in *mdr*, a 140-kDa membrane glycoprotein found in some human tumor cells that confers multidrug resistance by actively transporting compounds out of cells, a function similar to that of HlyB in hemolysin secretion (148, 164). *mdr* is essentially a tandem repeat of HlyB, including conserved transmembrane domains and an ATP-binding site near the C terminus (148, 164). Conversely, the mammalian gene could have come from bacteria via a transposable element of viral origin.

Association with Other VFs

Hemolytic uropathogenic strains almost always also express MRHA or P fimbriae (15, 77, 122, 162, 178, 198, 229, 393). *hly* sequences are sometimes genetically linked with determinants for other VFs, including P fimbriae (193, 322) and other fimbriae (193, 197), although the different genetic linkages in various strains indicate that they do not all carry the same block of VF genes. Hemolysin production is also associated with epithelial-cell adherence (198) and serum resistance (198, 199) but not with aerobactin production (415) or the AFA (291). Hemolysin production is especially common within certain O groups, e.g., O4, O6, O18, and possibly O75 (49, 77, 122, 178, 199, 393, 552) and in association with certain K antigens, e.g., K2, K5, K12, and K13 (122, 178); it is uncommon among O1, O2, O7, and O9 strains (199). Almost all strains of serotypes O4:K12:H5, O6:K2:H1, and O6:K13:H1 produce hemolysin; in contrast, hemolysin production is rarely or never encountered in strains of serotypes such as O1:K1:H$^-$, O2:K1:H4, and O2:K5:H4 (415). As a group, hemolytic strains are more closely related serologically, even in comparisons between fecal and urinary isolates, than are nonhemolytic strains (100), evidence which suggests that fecal hemolytic strains constitute a virulent subset of the fecal flora that can give rise to UTI under the appropriate circumstances.

Animal Studies

Hemolytic strains are more lethal than nonhemolytic strains for chicken embryos (116, 173, 206, 275, 353) and for mice or rats in models of peritonitis (116, 173, 175, 198, 275, 500, 603, 604), respiratory infection (115, 173), and hematogenous pyelonephritis (136, 137, 173, 310, 565, 566, 587, 589, 590), causing hemorrhagic lung lesions and hemoglobinuria in mice in addition to increased mortality (116, 500). In mouse and rat models of ascending UTI, hemolysin production is associated with increased bladder colonization and nephropathogenicity (97, 212, 265, 340).

Cell-free supernatants from hemolytic strains, but not from nonhemolytic strains, are lethally toxic when administered intravenously to mice, causing intravascular hemolysis and hemorrhagic pneumonitis in proportion to the level of hemolytic activity (497, 500). Coadministration of hemoglobin, iron, manganese, or hemolysin increases the lethality (but not the degree of organ pathology) associated with infection by nonhemolytic strains in mice to the same level seen with infection by hemolytic strains (310, 500, 589). These observations suggest that hemolysin promotes virulence both by providing iron for bacterial metabolism and by directly injuring host tissues. Active (310) or passive (115) immunity against hemolysin protects mice against infection with hemolytic strains.

Epidemiology

Expression during UTI. Several lines of evidence suggest that hemolysin is expressed in vivo, possibly by colonic bacteria and quite probably during acute UTI. The prevalence of antihemolysin serum antibodies among several mammalian species is proportional to the prevalence of fecal carriage of hemolytic *E. coli* in that species (497), and the prevalence of hemolysin seropositivity in humans increases from infancy to adulthood (200, 486). Anti-hemolysin antibody titers, which are higher in patients with UTI than in control subjects (486), are proportional to the severity of the UTI clinical syndrome and fall after therapy (115, 486).

TABLE 9. Association of hemolysin with clinical source of isolate

Reference	Proportion (%) of strains producing hemolysin[a]				
	PN	CY	ABU	Urine[b]	Fecal
13				7/20 (35)[c]	
15	20/49 (41)[d]	9/48 (19)		29/97 (30)[d]	12/40 (30)
48				40/112 (36)	5/40 (13)
49				45/105 (43)	
53					3/114 (3)
71				26/50 (54)	20/397 (5)
81				7/20 (35)	8/110 (7)
100				31/69 (45)	5/39 (13)
139	20/26 (77)			25/50 (50)[d]	
162				103/270 (38)	2/36 (6)
178				82/249 (33)	59/742 (8)
209				16/43 (37)	5/20 (25)
230				26/58 (45)[c,d]	
302			31/160 (19)	31/160 (19)	120/709 (17)
317	34/122 (29)[d]			34/122(29)[d]	
338	33/52 (63)		11/41 (27)	44/93 (47)	
343					17/61 (28)
354				29/59 (49)	1/20 (5)
393	9/12 (75)	15/26 (58)	2/6 (33)	26/44 (59)	10/73 (14)
415	76/139 (55)	57/119 (48)	20/112 (18)	153/370 (41)	19/97 (20)
470	49/120 (41)[d]	17/35 (49)		56/155 (36)[d]	
509	9/24 (37)[d]		9/37 (25)	18/62 (29)[d]	
557	40/67 (60)	16/60 (27)	10/60 (17)	66/187 (35)	5/50 (10)
566	6/12 (50)	25/64 (39)	3/13 (23)	34/89 (38)	6/30 (2)
607	39/57 (68)	19/43 (44)		58/100 (58)	
Total[e]	335/680 (49)	158/395 (40)	86/429 (20)	986/2,584 (38)	297/2,578 (12)

[a] PN, Pylonephritis or febrile UTI; CY, cystitis; ABU, asymptomatic bacteriuria.
[b] Preceding three columns plus any other urinary isolates of unspecificed clinical category.
[c] Urosepsis patient isolates.
[d] Includes patients with known compromising conditions.
[e] Because of differences in study design, methods, definitions, and populations in different studies, totaled results must be interpreted with caution.

Clinical studies. In human UTI, hemolysin production is most common among strains from patients with pyelonephritis (49%), followed by those from patients with cystitis (40%) and ABU (20%) (Table 9). Hemolysin production is more prevalent in UTI episodes localized to the upper (37 of 72 [51%]) than the lower (26 of 88 [30%]) urinary tract (48, 49). The prevalence of hemolysin production among bacteremia isolates (95 of 243 [39%]) and isolates from miscellaneous extraintestinal infections (68 of 160 [43%]) (13, 53, 95, 209, 230, 343, 354) is higher than among fecal strains (12%) (Table 9) but not as high as among pyelonephritis patient isolates. These data demonstrate an association of hemolysin production with invasive uropathogenic strains. From the limited available data it appears that hemolysin production among isolates from patients with pyelonephritis is less prevalent in strains from patients with compromising urological or medical conditions (19 of 59 [32%]) (15, 470), children with renal scarring (9 of 45 [21%]) (317), or pregnant women (19 of 48 [40%]) (470, 509) than in strains from comparable hosts without these compromising conditions (Table 9).

Summary

Hemolysin production is associated with human pathogenic strains of *E. coli*, especially those causing more clinically severe forms of UTI. Hemolysin is probably produced in vivo during UTI by uropathogenic strains. It is likely that the provirulence activity of hemolysin is multifactorial, including release of iron from erythrocytes, disruption of phagocyte function, and direct toxicity to host tissues. Antihemolysin immunity protects animals from infection with hemolytic strains and should be explored for human use.

CAPSULAR POLYSACCHARIDE (K ANTIGEN)

Structure

Capsular polysaccharides, of which *E. coli* has >80 types, are linear polymers of repeating carbohydrate subunits that sometimes also include a prominent amino acid or lipid component (Fig. 16) (233). They coat the cell, interfering with O-antigen detection (233) and protecting the cell from host defense mechanisms (see below) (233, 406). The capsules of most extraintestinal pathogenic *E. coli* strains are thin, patchy, acidic, thermostable, and highly anionic, characteristics that identify group II polysaccharides (233, 406, 408). Group II capsular polysaccharides, which aggregate spontaneously because of a phosphatidic acid group at the reducing end of the molecule (233), are allelic and include K1, K2, K5, K6, K12, K13, K14, K15, K20, K23, K51, K52, and K54 (233). The K1 polysaccharide is a homopolymer of NeuNAc (sialic acid) units linked 2-8 and randomly acetylated at C-7 and C-9 (Fig. 16) (243). It is structurally identical to the capsular polysaccharide of *Neisseria meningitidis* group B (451, 545) and related to human trisialogangliosides containing a NeuNAc-(2-8)-NeuNAc moiety (233, 503).

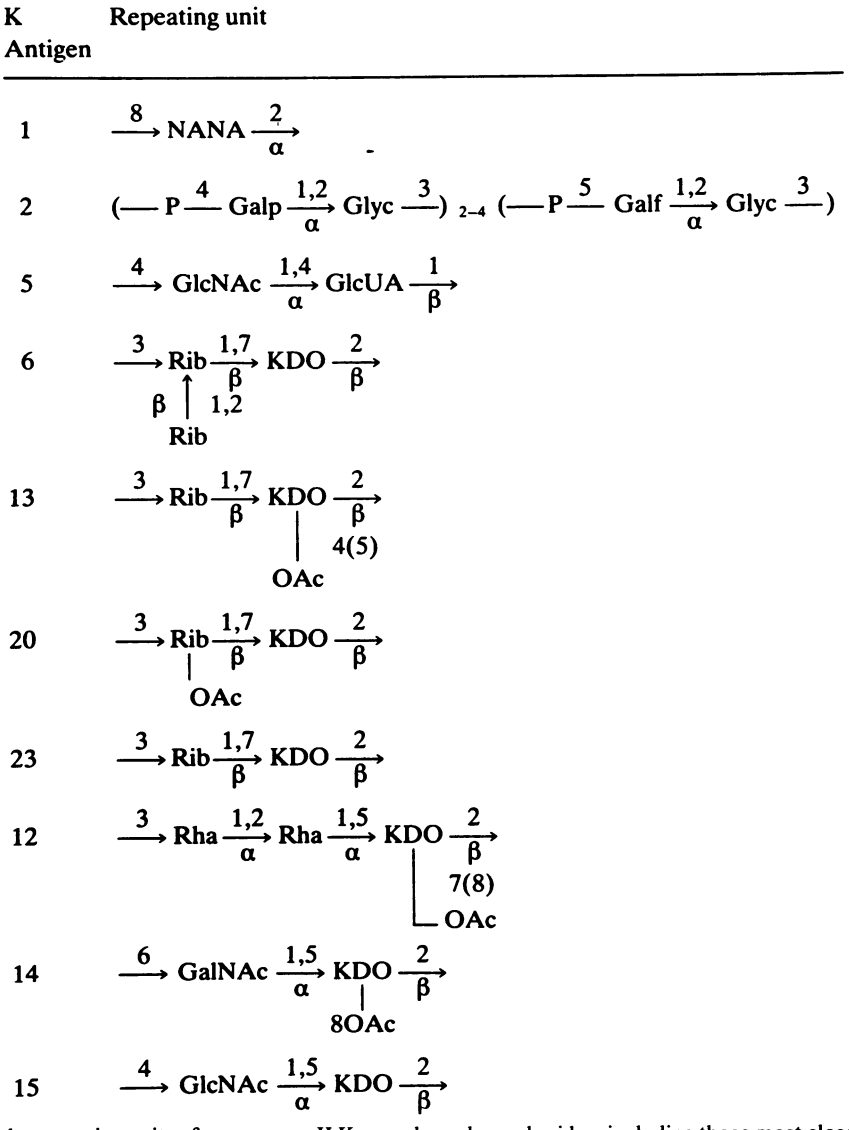

FIG. 16. Structure of the repeating units of some group II K capsular polysaccharides, including those most closely associated with urinary tract infection (e.g., K1, K5, K12, K13, and K20). NANA, *N*-acetylneuraminic acid; Gal, galactose; Glyc, glycerol; GlcNAc, *N*-acetylglucosamine; GlcUA, glucuronic acid; Rib, ribose; KDO, 2-keto-3-deoxyoctonic acid; OAc, acetyl; Rha, rhamnose; GalNAc, *N*-acetylgalactosamine. From reference 223, with permission from S. Karger AG, Basel.

Determination of K Capsular Types

Similar K antigens provide attachment sites for the same bacteriophages, which hydrolyze specific linkages in the polysaccharide and in effect drill through the capsule to attack the cell (233). This property is exploited in the identification of capsular types using K-specific phages (74, 167). Other tests used to identify the K1 capsule include agglutination with rabbit anti-K1 antiserum, immunodiffusion with equine group B meningococcal antiserum agar plates, and agglutination by murine monoclonal antibodies to the group B meningococcal capsule (74, 237).

Mechanisms of Virulence

Antiphagocytic and anticomplementary activities. With some exceptions (31, 539), encapsulated or $K1^+$ strains are phagocytosed less well by hPMNLs than nonencapsulated or $K1^-$ strains (9–11, 75, 111, 191, 196, 223, 411, 480, 507, 574, 575, 601). The degree of impairment of phagocytosis is proportional to the amount of polysaccharide (196). Phagocytosis of K1 strains increases after exposure to anti-K1 antibodies (9, 196) or disruption of capsular polysaccharide by heating (575). The negative charge and the hydrophilicity of the K1 polysaccharide are intrinsically antiphagocytic (111, 191, 223). In addition, capsular polysaccharide blocks opsonization by interfering with complement deposition in a dose-dependent fashion (196, 371). Erythrocytes coated with capsular polysaccharides are poorly lysed by complement even in the presence of anticapsular antibody (151, 196). K1 strains activate the alternative complement pathway poorly (433) unless rendered $K1^-$ (9, 575). The anticomplementary effect of capsular polysaccharides may occur in part because cell surface polysialic acids (such as the K1 polysaccharide) increase the binding of the inhibitor B1H to $C3_b$, thereby

preventing the formation of C3 convertase and blocking activation of the complement cascade (191, 300).

Serum resistance. The anticomplementary activity of capsular polysaccharide probably also contributes to the increased survival in serum of some encapsulated strains (411, 578). The degree of serum resistance of K^+ strains has been reported to be proportional to the amount of capsular material present (49) and to vary with the K type (398, 523). In studies of selected strains, the K1 polysaccharide is associated with increased serum resistance (147, 300, 434, 499). This effect can be overcome with the addition of anti-K1 antibody (9), presumably because of activation of the classical complement pathway. The threshold amount of K1 polysaccharide required for serum resistance is generally present in K1 strains during log-phase growth (577). Paradoxically, despite this laboratory evidence that the K1 capsule in particular and capsular polysaccharides in general contribute to serum resistance, most studies of collections of clinical isolates (including urinary strains) fail to identify an association between encapsulation or the amount of capsular polysaccharide present and serum resistance (31, 347, 396, 539, 574). Thus, encapsulation may be but one among other more important determinants of serum resistance (see below) (540).

Genetics

Group II capsular polysaccharides are encoded by a cluster of genes located near the *serA* locus on the *E. coli* chromosome (233, 406). One group of genes is responsible for synthesis and polymerization of subunits, another is responsible for postpolymerization modification of the polysaccharide (e.g., addition of phosphatidic acid) and transport across the cytoplasmic membrane, and a third is responsible for translocation across the outer membrane to the cell surface (39, 107, 287, 491, 492). As might be expected, the regions encoding subunit biosynthesis are specific for the different group II K types (459). In contrast, the postpolymerization and translocation regions of different K types are closely related (452, 453). Despite the similarity of the *E. coli* K1 and the *N. meningitidis* group B capsular polysaccharides, there is no homology between the capsular synthesis genes of these organisms (107).

Immunogenicity of Capsular Polysaccharides

Capsular polysaccharides from pathogenic *E. coli* strains are poor immunogens in animals (238, 246) and humans (187, 239, 467). The K1 polysaccharide is particularly nonimmunogenic, yielding a measurable antibody response in only one-third of rabbits immunized with killed K1 organisms (238) and 12% of humans with pyelonephritis due to a K1 strain (467). This may be because of molecular mimicry, with the host immune system possibly "blind" to the K1 polysaccharide because of this compound's similarity to host structures (see above) (490). Conjugation of capsular polysaccharides to bovine serum albumin or tetanus toxoid increases their immunogenicity (240, 243, 245), although the K1 conjugate is still less immunogenic than others (240). Similarly, protein conjugates of the structurally identical *N. meningitidis* group B polysaccharide are much less immunogenic than conjugates of the group A and C polysaccharides (225).

O-Serogroup Associations

The K1 capsule is associated with O1, O2, O7, O16, and O18 strains (77, 241, 294, 557), with one-half of K1 clinical isolates belonging to serogroups O1, O7, and O18 (294). In contrast, K1 capsule is uncommon among O4, O6, and O75 strains (557). The amount of K1 polysaccharide produced is similar among strains within a clonal group (294). The K5 capsule is associated with O2, O6, O18, and O75 strains (77), and the K12 capsule is associated with O4 strains (241).

Association with Other VFs

The K1 capsule is associated with MRHA (122), especially in strains of serogroups O1 and O2 but not of O18 (77). Among isolates from patients with pyelonephritis, this association is probably explained by the association of K1 and P fimbriae (553). Hemolysin production is associated with K5 strains (198) but not K1 strains (77, 198). K12 strains exhibited increased uroepithelial-cell adherence in one study (578), but this was attributed to other associated traits.

Animal Models

Among wild-type uroisolates, the presence and the amount of capsular polysaccharide in general, or of the K1 polysaccharide in particular, are associated with increased lethality, bladder and renal colonization, and renal pathology in experimental infections in mice (97, 257, 265, 360, 371, 578). The nephropathogenicity of K1 strains is more apparent with ascending than with hematogenous infection (360, 566, 578). Nonencapsulated mutant strains are less virulent in mice than their encapsulated parents (76, 499, 523, 565, 578), although exceptions occur (76). Acquisition of K1 capsule production by avirulent, nonencapsulated laboratory strains has little impact on their virulence (491, 499), evidence suggesting that additional factors must be present for virulence even in the presence of the K1 capsule.

Actively or passively acquired anticapsular immunity to the K1, K2, K6, or K13 capsular polysaccharide protects mice, rats, and rabbits from hematogenous or ascending pyelonephritis with the homologous strain (240, 243–246). The degree of protection per gram of antibody differs between different capsular types (240). Anti-group B meningococcal antiserum protects mice from infection with *E. coli* K1 before, but not after, removal of anti-K1 activity by adsorption with *E. coli* K1 (451).

Epidemiology

Capsular polysaccharides in general. Among human strains, a greater proportion of urinary than fecal *E. coli* isolates is encapsulated (552) and is typeable with standard anti-K sera (241). Certain K types, including K1, K2, K3, K5, K12, K13, K20, and K51, are overrepresented among isolates from patients with cystitis and especially pyelonephritis in comparison with fecal strains (241, 243, 411, 470, 490, 509, 516, 545, 607). Capsular types K1 and K5 include 63% of isolates from women with pyelonephritis (470), and capsular types K1, K2, K3, K12, and K13 account for 70% of isolates from girls with pyelonephritis (241). Encapsulated urinary strains produce greater amounts of capsular substance than do encapsulated fecal strains (48, 49, 150, 236, 302, 308). The association of certain capsular types with UTI is influenced by the O group; e.g., O18:K1 strains are rare in UTI, but O18:K5 and O1:K1 strains are common (290).

K1 capsule. K1 is the most commonly encountered capsular type among both urinary and fecal strains (406). The prevalence of fecal carriage of K1 strains increases with age, climbing from 22% of premature infants to 45% of adult

TABLE 10. Association of K1 antigen with clinical source of isolate

Reference	Proportion (%) of strains with K1 capsule[a]				
	PN	CY	ABU	Urine[b]	Fecal
42					22/99 (22)
48				37/112 (33)	14/40 (35)
75				21/193 (11)	9/50 (18)
77	14/155 (9)	10/170 (6)	4/59 (7)	28/384 (7)	17/233 (7)
139	9/26 (35)			12/50 (24)[c]	
198				13/93 (14)	
236	23/41 (56)	12/31 (39)	0/1	35/72 (49)	10/37 (27)
241	39/118 (33)	16/108 (15)	29/120 (24)	84/346 (24)	26/100 (26)
399				39/100 (39)	9/50 (18)
451					29/218 (13)
470	44/120 (37)[c]	6/35 (17)		50/155 (32)[c]	
472	70/174 (40)[c]		42/152 (28)[c]	112/326 (34)[c]	26/100 (26)
473				37/284 (13)	558/2,181 (26)
509	8/24 (33)	5/16 (31)	4/37 (11)	13/110 (11)	
553	11/32 (34)			11/32 (34)	
557	21/67 (31)	9/60 (15)	14/60 (23)	44/187 (24)	11/50 (22)
601				17/69 (25)	
607				7/55 (13)	
Total[d]	239/757 (32)	58/420 (14)	93/429 (22)	560/2,568 (22)	731/3,158 (23)

[a] PN, Pyelonephritis or febrile UTI; CY, cystitis; ABU, asymptomatic bacteriuria.
[b] Preceding three columns plus any other urinary isolates of unspecified clinical category.
[c] Includes patients with known compromising conditions.
[d] Because of differences in study design, methods, definitions, and populations in different studies, totaled results must be interpreted with caution.

women (473). The best known association of the K1 capsule with human disease is in meningitis, in which 79% of *E. coli* isolates are positive for the K1 antigen (77, 451, 473). Most bacteremia isolates from neonates express the K1 capsule (51 of 139 [37%]) (451, 473); in contrast, among other bacteremic strains, the K1 capsule is no more prevalent (269 of 1,158 [23%]) than among fecal strains (23%) (Table 10) (42, 75, 77, 359, 399, 451, 473, 601).

In the aggregate, K1 strains are distinctly more common in patients with pyelonephritis than in patients with other UTI syndromes or in the normal fecal flora (Table 10). In contrast, they are no more common in patients with ABU and may even be less common in patients with cystitis than in fecal strains (Table 10). Among K1 strains, isolates from patients with more severe forms of UTI produce greater amounts of capsular substance (48, 236). As with P fimbriae, the prevalence of K1 capsule is paradoxically similar among UTI isolates localized to the upper instead of the lower urinary tract (48). These findings suggest that the K1 capsular polysaccharide contributes to the development of meningitis, neonatal bacteremia, and pyelonephritis but is not particularly important in the pathogenesis of bacteremia after the neonatal period or in cystitis or ABU.

Summary

Acidic capsular polysaccharides and the K1 capsule in particular contribute to virulence by shielding bacteria from phagocytosis and possibly from serum killing, in part by blocking activation of the alternative complement pathway. Activation of complement via the classic pathway is impaired as well because many K antigens, particularly K1, are poor immunogens, resulting in low or absent anti-K-antibody levels in most individuals. Since a limited number of capsular types accounts for most cases of human pyelonephritis and since anticapsular immunity is protective in animal models of UTI, anticapsular immunity (possibly stimulated through the use of protein conjugate vaccines) would be of particular benefit in preventing human pyelonephritis.

SERUM RESISTANCE

Mechanisms and Measurement of Serum Killing

As reviewed recently by Taylor (540), bacteria are killed by normal human serum through the lytic activity of the complement system (396). The alternative pathway is activated by bacteria in the absence of specific antibody and plays a more important role in serum killing than does the classic pathway (540). Lipid A can activate the classic pathway in the absence of antibody, but its location deep within the outer membrane probably makes it inaccessible to complement components in intact bacteria (except perhaps in rough strains) (540). Both arms of the complement cascade converge in the formation of the C_{5-9} membrane attack complex (MAC), a short hollow cylinder with an inner diameter of 10 nm, an outer diameter of 22 nm, and an M_W of 2×10^6. The MAC inserts into the outer membrane, forming a pore through which lysozyme gains access to the peptidoglycan cell wall. Digestion of the cell wall allows the MAC to insert into the inner membrane, leading to cell lysis (540).

Bacterial susceptibility to serum killing is measured by assessing regrowth after incubation in serum (396) or growth rates in dilute serum (357). Many technical factors influence the results of such assays, including the growth phase of organisms at the time of exposure to serum and the dilution of serum used (dilutions greater than 1/16 have insufficient complement activity to cause killing) (540). Bacterial resistance to killing by serum results from the individual or combined effects of capsular polysaccharide, O-polysaccharide side chains, and surface proteins (359).

Capsular Polysaccharides in Serum Resistance

As discussed above, the evidence supporting a major role for capsular polysaccharides in serum resistance is contradictory, leading to conflicting opinions by authorities in the field (111, 223, 411, 540, 545, 577). Although the K1 capsule is important in certain strains, other mechanisms appear to be more significant determinants of serum resistance in populations of *E. coli* isolates.

O Polysaccharide in Serum Resistance

On the whole, smooth strains are more serum resistant than rough strains (49, 307, 396, 411, 540, 545), and the degree of serum resistance is proportional to the amount of lipopolysaccharide the strain contains (157, 396). The O polysaccharide is a component of smooth-type lipopolysaccharide. Acquisition of the O18 antigen by *E. coli* K-12 (rough) enhanced its serum survival (538), and loss of the O18 antigen increased the serum sensitivity of an O18 wild-type strain (434). Serum-resistant strains with abundant O polysaccharide appear to activate and consume complement to as great a degree as do less-well-shielded serum-sensitive strains. This evidence suggests that O-antigen polysaccharide side chains may protect against complement lysis not by blocking complement activation but by causing complement to be activated at a location distant from sensitive membrane target sites (157, 540). If membrane fluidity is important in MAC insertion, the decreased membrane fluidity associated with smooth-type lipopolysaccharide (540) might protect against effective MAC insertion. Whether the association of specific O serogroups with serum resistance (see below) is due to properties of the particular O polysaccharides per se or to other associated factors is unknown.

Plasmid-Associated Proteins in Serum Resistance

Certain plasmids confer slightly increased serum resistance on host strains, especially when the host strain is already partially serum resistant (540, 541). Incompatibility group F plasmids, e.g., ColV plasmids (FI) and the resistance plasmids R100 and R6-5 (FII), are the best studied of these (30, 259, 358, 540, 541, 545), although plasmids of other incompatibility groups (N, O, S, T, and U) may also confer enhanced serum resistance (128).

ColV and *iss*. Strains carrying ColV plasmids (which encode production of and resistance to colicin V) are overrepresented among human pathogenic strains of *E. coli* (78) and are associated with pyelonephritis (139, 393), although possibly not with UTI in general (78). ColV plasmids from wild-type strains also increase the virulence of *E. coli* K-12 in various animal models (419, 608). The provirulence effect of ColV plasmids is not mediated by ColV, however, as demonstrated by the lack of toxicity of colicin-containing supernatants (419) and the absence of a decrease in virulence after inactivation of colicin production (9, 436, 611).

Several phenotypic traits other than colicin production per se may contribute to the virulence associated with ColV plasmids, including autoagglutination and hypomotility at 37°C (which may impede phagocytosis) (464, 542, 543), enhanced survival in the mouse peritoneum (419), increased adherence to mouse intestinal cells and increased fimbriation (65), aerobactin production (see above), impaired phagocytosis (9), and increased serum resistance (9, 29, 30, 259, 373, 498, 540, 541). pColV, I-K94 (a well-studied ColV plasmid) also confers increased acid susceptibility (72). Of these properties, aerobactin production (see above) and serum resistance are the best characterized. In one study, 9 of 14 ColV plasmids conferred serum resistance on serum-sensitive strains (373, 498). Serum resistance mediated by ColV plasmids is due to impaired killing by complement (9, 498) and in some cases is attributable to the *iss* (increased serum survival) protein (29, 30, 540, 545). The cloned *iss* gene (which is closely linked with colicin V production in pColV, I-K94) (29) increases by 100-fold the serum resistance and virulence for chicks of an avirulent, serum-sensitive *E. coli* strain (29, 30) without quantitatively altering deposition or consumption of complement (30), possibly through inhibition of MAC activity at the outer membrane (540).

***traT*.** The *traT* protein is a 25-kDa outer membrane lipoprotein that mediates surface exclusion among strains carrying certain F-like plasmids such as R6-5, R100, and some ColV plasmids (30, 358, 359, 373, 540, 545). It is a major outer membrane component in these strains (373) and confers a moderate degree of serum resistance by interfering with complement-mediated killing (373, 540, 545) without affecting complement deposition or inactivating soluble complement (30, 359), possibly by inactivating the MAC (359). The cloned *traT* gene increases the serum resistance of unencapsulated strains when present in low copy number, whereas with encapsulated strains, higher copy numbers of *traT* must be present to affect serum resistance (8). *traT* is always carried on large IncF plasmids (259, 359). Although *traT* is more common among extraintestinal isolates (58%) than fecal strains (38%) (259), it is not clearly associated in the aggregate with serum resistance (259, 359), except possibly among UTI isolates (359), a finding which casts doubt on the importance of *traT* as a significant determinant of serum resistance in pathogenic strains.

Serogroup Associations

Serum resistance is common among O6, O7, O18, and O50 strains (199, 540, 586) and uncommon among O1, O2, O4, O9, O16, and O75 strains (540, 586). In the aggregate, strains of the most common O groups (O1, O2, O4, O6, O7, O18, and O75) (see Serotype below) are more serum resistant than are strains from uncommon O groups, which in turn are more serum resistant than spontaneously agglutinating (O-polysaccharide-deficient) strains (397). The clinical source of the strain influences the association of O group and serum resistance, with isolates from patients with pyelonephritis having the highest and isolates from patients with ABU the lowest levels of serum resistance, even within O-group categories (i.e., common, uncommon, and spontaneously agglutinating) (see also Clinical studies below) (307, 396).

Epidemiology

Animal models. Serum-resistant strains are usually more nephropathogenic than comparison serum-sensitive strains in a variety of models of UTI (97, 172, 212, 340, 351, 360), even though these resistant strains may not be associated with increased lethality (172, 360). Serum resistance is variably associated with virulence in models of other types of infection (173, 198, 496, 499).

Clinical studies. In the aggregate, isolates from patients with pyelonephritis and cystitis are more commonly serum resistant than are those from patients with ABU or fecal isolates (Table 11). Pyelonephritis and cystitis patient isolates are similar with respect to the prevalence of serum

TABLE 11. Association of serum resistance[a] with clinical source of isolate

Reference	Proportion (%) of strains resistant to serum killing[b]				
	PN	CY	ABU	Urine[c]	Fecal
49				94/149 (63)	
161				13/44 (30)	
302			14/115 (12)	38/170 (22)	340/709 (48)
307	82/119 (69)	78/109 (72)	14/113 (12)	174/341 (51)	
316	94/186 (50)[d]	23/59 (39)	72/277 (23)	189/552 (36)[d]	
317	85/122 (70)[d]			85/122 (70)[d]	
338	36/52 (69)		28/41 (68)	64/93 (69)	
343					26/61 (43)
397	94/149 (63)	71/101 (70)	22/116 (19)	187/366 (51)	64/102 (63)
470	57/120 (48)[d]	21/35 (35)		78/155 (50)[d]	
472	37/56 (66)[d]		16/32 (50)[d]	53/88 (60)[d]	
509	19/24 (83)		19/37 (51)	38/61 (62)	
586				66/97 (68)	94/141 (67)
Total[e]	504/828 (61)	193/304 (63)	185/731 (25)	1,079/2,238 (48)	524/1,013 (52)

[a] Serum resistance is variably defined. Table reflects designations used by different investigators.
[b] PN, Pyelonephritis or febrile UTI; CY, cystitis; ABU, asymptomatic bacteriuria.
[c] Preceding three columns plus any other urinary isolates of unspecified clinical category.
[d] Includes patients with known compromising conditions.
[e] Because of differences in study design, methods, definitions, and populations in different studies, totaled results must be interpreted with caution.

resistance (Table 11), as are upper and lower urinary tract isolates (49, 161). These observations suggest that serum resistance is important in the pathogenesis of symptomatic UTI, regardless of the severity.

ABU. In contrast to strains causing symptomatic UTI, the prevalence of serum resistance among ABU patient isolates is even lower than among fecal strains (Table 11; 32), suggesting that serum-sensitive strains may be selected from the fecal flora for asymptomatic colonization of the urinary tract or, alternatively, that fecal strains entering the bladder may adapt to the new environment by becoming more serum sensitive. The latter hypothesis is supported by the finding that successive isolates of the same organism showed progressively decreasing serum resistance among four of six patients with ABU whose urine organisms were initially serum resistant (307). Isolates from patients with ABU of unknown duration are more serum sensitive than isolates from patients with recently acquired ABU, data again supporting the concept that prolonged asymptomatic colonization is associated with a shift to lower serum resistance (396). This phenomenon may be due to a loss of O antigens during prolonged bladder colonization as an adaptive response to the presence of urinary anti-O antibodies (307).

Compromised hosts. Patients with pyelonephritis are less likely to have a serum-resistant strain when underlying medical illnesses or urological abnormalities are present (84 of 170 [49%]) than when such compromising conditions are absent (141 of 234 [60%]) or even when the patient is pregnant (40 of 60 [67%]) (316, 317, 470, 472, 509). The decreased prevalence of serum resistance among isolates from compromised hosts may be related to host defects in serum killing. Some patients with upper tract infection have a defect in serum killing that is specific for the patient's infecting strain (161) and that in some instances is due to an immunoglobulin G inhibitor that can be removed by adsorption with the urine organism (536). Other patients with pyelonephritis or chronic bacteriuria have a more global defect in serum killing (258).

Bacteremia. The prevalence of serum resistance among isolates from blood (120 of 151 [79%]) (343, 586) is higher than in any of the UTI-associated categories (Table 11), evidence suggesting that serum resistance is a major determinant of bloodstream invasiveness. Among bacteremic strains, serum resistance is associated with a greater incidence of shock and death (347).

Summary

Bacteria escape complement-mediated killing by blocking activation of the complement cascade (acidic polysaccharides), by denying complement components access to critical membrane target sites (O polysaccharides), or by preventing the C_{5-9} MAC from functioning normally even if it is able to reach the outer membrane (*traT* and *iss*). Serum resistance is often multifactorial, with no one bacterial property satisfactorily accounting for serum resistance in the majority of resistant strains. Isolates from patients with pyelonephritis, cystitis, and especially bacteremia are typically serum resistant, whereas ABU patient strains are characteristically even more serum sensitive than fecal strains. The development of interventions against serum resistance will require further delineation of the bacterial mechanisms that block serum killing.

SEROTYPE

Epidemiology of O Groups

As a group, strains of *E. coli* causing UTI can be differentiated from fecal strains by their expression of specific O-polysaccharide antigens (191). Urinary isolates are more commonly typeable with batteries of common O antisera than are fecal strains (552, 557). All colonies are more likely to be of the same O group in urine cultures than in fecal specimens (550, 552). In addition, UTI isolates are less serologically diverse than fecal strains, with a small number of O groups accounting for the majority of urinary strains (13, 552). The same O groups predominate among both urinary and fecal strains (550), but certain of the common O groups are significantly more prevalent among urinary than among fecal strains (48, 49, 71, 98, 169, 209, 302, 307, 316,

TABLE 12. Association of O group with clinical source of isolate

Reference	Proportion (%) of strains from eight common O serogroups[a]				
	PN	CY	ABU	Urine[b]	Fecal
209				21/43 (50)	12/41 (30)
302			52/160 (32)	60/170 (35)	226/709 (32)
307	95/120 (79)	64/108 (59)	36/113 (32)	195/341 (57)	
413	99/144 (69)	71/123 (58)		170/267 (64)	
472	35/56 (62)		10/32 (31)	45/88 (51)	
552				70/82 (85)	155/290 (53)
586	47/52 (91)	81/108 (75)	41/60 (68)	169/220 (77)	158/392 (40)
Total[c]	276/372 (74)	216/339 (64)	139/365 (38)	730/1,211 (60)	551/1,432 (38)

[a] Includes O1, O4, O6, and O75 in all studies plus O2 (209, 302, 307, 413, 472, 552), O7 (302, 307, 413, 472, 552, 586), O8 (209), O16 (302, 307, 413, 472), O16/72 (586), O18 (209, 302, 307, 413, 472, 552), O25 (209, 552, 586), and O50 (586). PN, Pyelonephritis or febrile UTI; CY, cystitis; ABU, asymptomatic bacteriuria.
[b] Preceding three columns plus any other urinary isolates of unspecified clinical category.
[c] Because of differences in study design, methods, definitions, and populations in different studies, totaled results must be interpreted with caution.

329, 338, 393, 397, 413, 437, 472, 509, 518, 552, 557, 585, 607).

The association of individual O groups with UTI is somewhat difficult to discern from the literature because investigators commonly report prevalence figures for groups of 3 to 10 UTI-associated O groups rather than for individual O groups. However, regardless of the number of O groups included in the UTI-associated category in a given study, the prevalence of the UTI-associated O groups is greatest among pyelonephritis patient isolates, lower among cystitis patient isolates, and lowest among ABU patient and fecal isolates. The most commonly used grouping is of eight UTI-associated O groups (Table 12), including combinations of O1, O2, O4, O6, O7, O8, O16, O16/72, O18, O25, O50, and O75 (Table 12). There is a clear association of the UTI-associated O groups with more severe forms of UTI and little apparent difference between fecal and ABU patient isolates (Table 12). The UTI-associated O groups are also more prevalent among episodes of UTI localized to the upper rather than the lower urinary tract (48, 49).

Supporting the hypothesis that strains of these UTI-associated O groups are particularly urovirulent is the observation that individuals colonized vaginally with O2, O4, O6, and O75 strains commonly go on to develop UTI with these strains, in contrast to those colonized with strains of other O groups (71). O16 strains are specifically associated with febrile UTI in infants (338) and ABU in girls (302), O18 strains are associated with ABU in infants (338), and O8, O14, O25, and O83 strains are associated with cystitis in girls (307). ABU patient isolates are more commonly spontaneously agglutinating (i.e., O-polysaccharide deficient) than are fecal or other urinary strains (307, 316, 472), which may be due to loss of O antigens during prolonged bladder colonization as an adaptive response to the presence of urinary anti-O antibodies (307) (see Serum Resistance above). Among pyelonephritis patient isolates, UTI-associated O groups are less prevalent in the setting of VUR (316, 411) but not during pregnancy (473, 509).

Animal Models

In hematogenous pyelonephritis models in mice, strains of the same O group exhibit similar levels of toxicity (496) and O-typeable strains (especially those of the UTI-associated groups O2, O6, and O18ac) show enhanced toxicity compared with strains of other O groups (496, 564, 566). In contrast, no association of virulence and O group was found when a model of ascending UTI in mice was used (212).

VF Associations, O:K:H Serotype, and the Clone Concept

Although O polysaccharides function directly to protect against complement-mediated bacterial killing (see above), it seems likely that in many cases the apparent virulence associated with certain O groups may be mediated through other VFs (e.g., P fimbriae, MRHA, hemolysin, and serum resistance), which are more commonly present in strains of the UTI-associated O groups than in other strains (as discussed above in the sections on individual VFs). However, the association of certain O antigens with other VFs is inconsistent, as reflected in contradictory findings regarding the associations of hemolysin with the O1 and O7 antigens (122, 557), of P fimbriae with the O2 and O75 antigens (393, 553, 557), and of serum resistance with the O1, O2, O4, and O75 antigens (307, 397, 537). These apparent inconsistencies may be attributable in part to the limited ability of the O antigen alone to define genetically homogeneous groups of strains. Some investigators consider that the O group designation reveals little about a strain, since for some isolates with the same O antigen, the divergence of electrophoretic type (by multilocus enzyme electrophoresis) approaches or equals that of randomly chosen isolates (58).

The O antigen alone may be inadequate for stratification of strains according to clinical category of UTI (329); however, combinations of the 164 O groups, the approximately 100 K capsular antigens, and the 56 H flagellar antigens of *E. coli* yield some 10,000 theoretically possible O:K:H serotypes, offering much finer resolution of genetic differences and similarities (407). The O:K:H serotype has traditionally been used to define genetically distinct clones of *E. coli*, a concept that is still useful despite evidence from recent studies that genetic relationships with *E. coli* are more complex than previously suspected (407). Illustrative of this complexity is the presence of several outer membrane protein groups (290) or electrophoretic types (58, 428, 432, 509) within individual O:K:H serotypes, with uniform expression of other phenotypic properties occurring within but not between these subgroups within a given O:K:H serotype. Similarly, electrophoretic types sometimes overlap more than one O:K:H serotype. Notwithstanding these limitations of the O:K:H system, certain associations emerge from the analysis of O:K:H serotype and clinical manifestations of UTI. Al-

though no O:K:H groups are uniquely associated with pyelonephritis and ABU patient isolates or with fecal isolates (415), there is no overlap in O:K:H serotype between pyelonephritis and ABU patient isolates (509). Cystitis patient isolates share O:K:H serotypes with both these categories (509); however, at least one serotype (O75:K100:H5) is uniquely associated with cystitis (413, 415). The following O:K:H serotypes are reported to be overrepresented among pyelonephritis patient isolates: O1:K1:H7, O1:K1:H$^-$, O2:K1:H4, O2:K1:H5, O2:K1:H$^-$, O2:H4, O4:[K5]:[H9], O4:K12:H1, O4:K12:H5, O6:K2:H1, O6:H1, O7:K1:H$^-$, O9:K34:H$^-$, O16:K1:H6, O16:K1:H$^-$, O18:K5:H7, O18:K5:H$^-$, O25:H1, O75:K5:H$^-$, and O75:H$^-$ (317, 329, 338, 407, 411, 413, 470, 509). For a number of these serotypes, association with pyelonephritis was noted in two or more sources. O6:K13:K1 strains are particularly associated with cystitis (411). Finally, O4:K$^-$ strains with a distinctive outer membrane protein pattern (OMP 2) constitute a clone unique to UTI isolates from boys (607).

Summary

Although the precise definition of genetically homogeneous clonal groups within *E. coli* is problematic, certain groups of strains sharing the same O antigen or complete O:K:H serotype are associated with UTI in general or, in some cases, with specific UTI syndromes. The presence of other uro-VFs in certain lineages of *E. coli* probably accounts for most of these associations. The direct contribution of specific O (and possibly K and H) antigens to virulence awaits clarification through studies of genetically manipulated strains.

VFs IN COMBINATION

Epidemiology

Although the association of VFs with UTI is usually approached from the perspective of each individual VF in isolation, it is clear that urinary strains commonly express multiple VFs simultaneously (48, 49, 139, 178, 229, 316). In some strains this multiple expression is due to the presence of a block of genetically linked determinants for different VFs (193, 275, 322). Expression of multiple VFs is more common among UTI isolates than among fecal or periurethral isolates (48, 49, 178) and is more common among pyelonephritis patient or upper tract isolates than among cystitis patient, ABU patient, or lower tract isolates (48, 49, 139, 316). Among patients with pyelonephritis or urosepsis, in which most strains express multiple VFs, the presence of compromising host conditions is associated with a decreased likelihood of multiple-VF expression (229, 316). These observations suggest that VFs function additively or synergistically in overcoming normal host defenses, that strains with a more extensive complement of virulence traits are more effective urinary pathogens, and that compromising host conditions decrease the need for multiple VFs in strains causing serious UTI.

Animal Studies

In models of hematogenous or ascending pyelonephritis in mice, the virulence of wild-type UTI isolates is not clearly associated with the number of VFs expressed (97, 566). In contrast, studies using genetically manipulated, multiple-VF strains suggest that each individual VF contributes to the net virulence of the organism (180, 265, 340, 499). These observations indicate that whereas the virulence of an organism cannot be accurately predicted on the basis of its measurable VF phenotype (probably because there are many as-yet-undefined properties contributing to virulence), the presence of multiple VFs does increase the organism's virulence. Further studies using appropriate animal models and genetically engineered strains are needed to clarify the interactions between the VFs most commonly present together in UTI isolates (e.g., P fimbriae, type 1 fimbriae, hemolysin, aerobactin, and serum resistance).

MISCELLANEOUS PUTATIVE VFs

Metabolic Enzymes

A variety of bacterial properties in addition to those already discussed have been proposed as possible VFs in UTI. No single biochemical test differentiates bacteremic isolates of *E. coli* (including many urosepsis strains) from fecal strains, although the overall biotype yields subclusters more closely associated with bacteremia (42). Salicin (but not dulcitol) fermentation is more prevalent among UTI strains than among periurethral strains and among upper urinary tract than among lower urinary tract isolates (48, 49, 257). However, dulcitol-fermenting uroisolates are more nephropathogenic (but not more lethal) in mice than non-dulcitol-fermenting strains (172, 212, 257, 360). No information is available on the virulence in animals of salicin-fermenting strains. The slower-electrophoretic-mobility variant of carboxylesterase B is more prevalent among *E. coli* strains causing extraintestinal infections (including UTI) in humans than among human or animal intestinal isolates (159, 160). The B_2 variant of carboxylesterase B (as determined by combining electrophoretic mobility analysis with isoelectric focusing) is associated with human pathogenic strains, hemolysin production, and MRHA (160). It seems likely that any association of these metabolic and isoenzyme allelic traits with virulence is due to their linkage with other properties that contribute more directly to virulence, although this remains to be determined.

Growth Characteristics and Motility

Growth in minimal medium is more common among lower urinary tract than upper urinary tract isolates (49). In view of this, it is somewhat surprising that this property is associated with greater nephropathogenicity in mice (172). Motility (or the presence of flagellar antigen) has been reported to be more (48) or no more (162) common among UTI isolates than among fecal strains and to be more (470) or no more (192) common among pyelonephritis patient isolates than among cystitis patient isolates, with pyelonephritis patient isolates possibly exhibiting even less motility than cystitis strains (192). Motile wild-type strains are no more uropathogenic or toxic in mice than nonmotile strains (172, 265, 360).

Cytotoxin and Protease Production

Some (but not all) hemolytic strains produce a cytotoxic necrotizing factor that causes dermonecrosis in rabbits, is lethal for guinea pigs, and stimulates the formation of multinucleate giant cells in Chinese hamster ovary, Vero, and HeLa cell monolayers (52). Rabbit dermal necrotoxic activity was much more prevalent among UTI isolates and among vaginal isolates from women who subsequently de-

veloped UTI than among vaginal isolates from women who did not later develop UTI (71), although whether this dermonecrotic factor was the same as cytotoxic necrotizing factor (52) is unknown. Production of cytotoxic necrotizing factor is much more prevalent among clinical isolates than among fecal strains (53) and is limited to strains that also produce alpha hemolysin (53, 54). This 110-kDa protein, which is distinct from *E. coli* stable toxin, labile toxin, and Vero toxin (52), promotes cell spreading and multinucleation through changes in cytoskeletal actin and tubulin (129) and is associated with O2, O4, O6, O22, O75, and O83 strains (54). The production of an immunoglobulin A protease by a small proportion of UTI isolates of *E. coli* (in contrast to none of the stocked strains tested) in one preliminary study suggested that this property also might be a uro-VF (350).

Other Properties

The ability of certain strains to cause ureteral paralysis in monkeys has been proposed as a VF (253, 454, 456) and has been attributed to P fimbriae (253, 456). However, *E. coli* culture supernatants (544) and even endotoxin alone (368) inhibit the spontaneous contractions of sheep ureters (544) and block the phenylephrine- or norepinephrine-stimulated alpha adrenergic contractions of feline bladder strips (368). Internalization of *E. coli* into human renal tubular cells has been proposed as a possible marker of invasiveness (595), but the observation that a similar proportion of fecal and pyelonephritis patient isolates in the pilot study exhibited this property (595) casts doubt on this hypothesis. An antiphagocytic factor that is expressed by several strains at 37°C but not at 17°C and that is removed by treatment with EDTA was proposed as a uro-VF (213). Finally, surface hydrophobicity was greater among strains from patients with recurrent UTI than among isolates from patients with occasional UTI (462). The epidemiological significance and contribution to urovirulence of all of these properties is unknown.

RENAL SCARRING

Infection-related renal scarring, a theoretically preventable cause of renal dysfunction that develops during childhood in some individuals, has proven difficult to attribute to any specific bacterial property (516). Although children with pyelonephritis are at greater risk of developing renal scarring if they have VUR (86), most renal scarring develops in children who do not have demonstrable VUR when studied between UTI episodes (613). These observations, plus the finding that ureteral infection with P-fimbriated organisms induces VUR and intrarenal reflux in monkeys (457), has led some investigators to propose that pyelonephritis due to P-fimbriated organisms may lead to renal scarring by inducing reversible VUR in children with otherwise normal urinary tracts (253, 533, 613). However, isolates from children with pyelonephritis and scarred kidneys are actually significantly less likely to express P fimbriae than isolates from children with pyelonephritis and no scarring (86, 191, 317, 516). This evidence suggests that P fimbriae may somehow protect against scarring, possibly by stimulating inflammation and thereby producing symptoms that lead to prompt treatment, a hypothesis supported by the observation that UTI episodes in unscarred infants are associated with greater elevations of body temperature than episodes occurring in infants with renal scarring (86). Alternatively, patients predisposed to scarring may also have some defect in host defenses that allows infection with VF-deficient strains, a scenario favored by the observation that hemolysin production and serum resistance (as well as P fimbriation) are no more common among isolates from scarred children with pyelonephritis than among those from unscarred children with pyelonephritis (317). It has been argued that the finding of VF-deficient strains in pyelonephritis episodes in children with preexisting renal scarring provides little information about the virulence of the organisms that caused the scarring initially (546). However, in a recent study, VF-deficient strains were isolated even during episodes of pyelonephritis associated with the appearance of new renal scarring in previously unscarred children, suggesting that the VFs usually considered important in the development of acute pyelonephritis truly do not contribute to renal scarring (317, 320).

As described above (Type 1 Fimbriae), the degree of renal scarring developing in rats after intrarenal injection of *E. coli* as well as the degree of chemiluminescence and neutral protease release stimulated by the incubation of bacteria with hPMNLs is greatest among type 1-fimbriated strains (547), evidence suggesting that type 1 fimbriae may contribute to renal scarring by stimulating phagocyte-induced renal injury (547). Other bacterial components that cross-react serologically with human renal tissue may elicit an injurious immune response in the infected host (194), possibly contributing to scarring. However, there is no evidence that persistent bacterial O antigens in renal tissue lead to immune-mediated scarring in humans (186). Thus, although certain bacterial factors have been implicated in renal scarring, consensus is lacking as to whether renal scarring is primarily due to special characteristics of the pathogen (547) or of the host (317).

CONCLUSION

Several bacterial properties (including P fimbriae, type 1 fimbriae, hemolysin, aerobactin, serum resistance, and the K1 capsule) are fairly well established as VFs in acute, symptomatic *E. coli* UTI. This conclusion is based on the epidemiological observation that these properties are overrepresented among isolates from patients with UTI in general or with more severe forms of UTI, confirmation from animal models of UTI that these (and not associated) properties contribute directly to virulence, and clarification in the laboratory of the mechanisms whereby these properties help overcome host defenses or injure host tissues. For several other properties (such as the UTI-associated O antigens), the evidence for a direct role in UTI pathogenesis is not so clear-cut. The currently recognized VFs account for only a fraction of the total virulence of wild-type strains, evidence suggesting that other as-yet-unidentified properties that are important determinants of virulence await discovery and characterization.

Along with the search for new VFs, certain aspects of the currently defined VFs require further study. Surprisingly little is known regarding the interactions of different VFs, in view of the fact that the most urovirulent strains (in animal models or human UTI) commonly express multiple VFs simultaneously. The same sophisticated techniques used to study individual VFs in isolation should now be applied to the study of multiple VFs acting in concert. The expression of VFs in situ is another important unknown area, since most of the evidence regarding VF expression comes from the study of urinary bacteria or cultured organisms rather than of bacteria in the bladder or kidney. Renal scarring remains enigmatic; whether a specific bacterial property is responsi-

ble for the development of renal scars in some patients or whether this phenomenon reflects a unique host susceptibility to injury from infection with ordinary bacteria has yet to be determined. More-detailed attention to particular patient populations is needed to clarify the role of VFs in RUTI, UTI in men, and UTI in patients with specific compromising conditions.

The prevailing trend in *E. coli* uro-VF research appears to be toward further refinement of understandings of VF structure and mechanisms at the molecular level. However, to date none of the tremendous accumulated body of knowledge regarding the genetics, structure, and function of uro-VFs has been translated into clinical interventions for human use. Anti-VF interventions effectively block the function of several of the defined VFs in the laboratory and protect animals from UTI due to VF-expressing strains. The next decade could possibly see the development of practical anti-VF interventions and their introduction into the human clinical arena. Fulfillment of this promise will require that committed clinician-scientists supplement the pursuit of basic knowledge as an end unto itself by endeavoring to apply the fruits of basic investigation to the prevention and treatment of human disease. The decreased importance of certain VFs (e.g., P fimbriae, hemolysin, and serum resistance) in UTI in compromised hosts will handicap efforts to protect such individuals from UTI through the use of anti-VF interventions. However, even compromised hosts may benefit from interventions directed against other VFs (e.g., aerobactin) that are not influenced by the presence of compromising host conditions. Thus, although a great deal has been learned regarding *E. coli* virulence mechanisms in UTI, much remains to be learned, and the practical application of our growing understanding of *E. coli* VFs to the prevention and treatment of UTI has only just begun.

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