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Binding to Galactoseα1→4Galactoseβ-Containing Receptors as Potential Diagnostic Tool in Urinary Tract Infection

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The diagnosis of urinary tract infection is based largely on quantitative urine cultures. The usefulness of qualitative information about the virulence of the infecting bacteria remains undefined. Ability to attach to human uroepithelial cells is one characteristic of the pyelonephritogenic clones, as well as a virulence factor per se. The identification of host cell receptors for attaching bacteria has permitted the construction of agglutination tests for simple detection of bacterial binding properties. In the present study, the reactivity with Galα1→4Galβ-latex [galactoseα(1→4)galactoseβ–latex] and globotetraosylceramide-latex was analyzed for strains from patients with acute pyelonephritis (n = 135), acute cystitis (n = 121), and asymptomatic bacteriuria (n = 119) and from the fecal flora of healthy children (n = 120) and compared with agglutination of human blood group P_1 and p, as well as guinea pig, erythrocytes. The reactivity by bioassay and the receptor-specific assays were significantly correlated. The frequency of positive reactions among the pyelonephritis isolates was 78.5% with the globotetraosylceramide-latex reagent, compared with 41% for the cystitis isolates, 25% for the asymptomatic bacteriuria isolates, and 13% for the fecal isolates. The combination of bioassays and receptor-specific assays increased the resolution of adhesins. Thus, adhesins reacting with human p erythrocytes frequently were coexpressed with Galα1→4Galβ-specific adhesins. The receptor-specific assays provide a refined reagent to resolve bacterial binding specificities, as well as a potential tool for clinical diagnosis.

Common infections, e.g., of the ear and the respiratory, digestive, and urinary tracts, are caused by bacterial species which also are part of the indigenous flora. The disease isolates may possess special characteristics, by which they differ from the indigenous variants of the same species; these characteristics may be used diagnostically. For species with a clonal structure, which coexpress virulence factors with other characteristics, diagnosis may be based on any marker which is sufficiently frequent among the virulent clones. An alternative is detection of single virulence factors.

The diagnosis of urinary tract infection (UTI) is based on quantitative urine cultures (13). The characteristics of *Escherichia coli*, which causes most UTI, have been shown to vary with the severity of infection and susceptibility of the host (24). Acute pyelonephritis in uncompromised hosts is caused by a restricted number of clones, identified by O:K:H serotype or multilocus enzyme electrophoresis (3, 4). These clones rarely occur in the normal intestinal flora or in patients with asymptomatic bacteriuria (ABU) (10, 17, 18, 20, 22–24). Adhesive capacity is both the single property most frequently expressed by the pyelonephritogenic clones (15, 22–25, 27) and a determinant of virulence in the urinary tract (9). The identification of the globoseries glycolipids with the minimal receptor Galα1→4Galβ [galactose-α(1→4)galactoseβ] as receptors on the uroepithelial cells (12, 14, 16) has allowed the construction of synthetic kits for the identification of bacteria with this receptor specificity (7, 16, 26).

In the present study, the frequencies of positive reactions with receptor-specific latex kits and bioassays were compared among isolates from patients with various forms of UTI. The results demonstrated a significant correlation between the severity of infection and reactivity with the latex kits.

MATERIALS AND METHODS

Patients and bacteria. The bacterial isolates were obtained from the urine of girls experiencing their first known episode of acute pyelonephritis (n = 135) or acute cystitis (n = 121). The origin of those strains has been described extensively (11, 17, 22). The urinary isolates from girls with ABU were obtained during a screening program among schoolgirls (n = 116). In addition, fecal *E. coli* isolates (n = 120) were obtained from nonbacteriuric children in the same population that was screened for ABU (17).

All patients had significant bacteriuria. Acute pyelonephritis was diagnosed by a temperature >38.5°C, loin pain, and two or three of the following pathological signs: elevated microsedimentation rate, C-reactive protein, and decreased renal concentrating capacity. Acute cystitis was characterized by dysuria, frequency, temperature <38°C, and determined normal laboratory tests. ABU was diagnosed when the initial screening culture and subsequent control culture showed the same bacterial strain (11).

A total of 492 isolates were included. They were identified as *E. coli* and saved in deep agar cultures. For analysis, the bacteria were initially grown on lactose-bromthymol blue agar plates to control for contaminants. Since the aim of the study was to investigate the frequency of adhesins binding to the globoseries glycolipid receptors, culture conditions promoting expression of these properties were selected. *E. coli* cells were cultured on tryptic soy agar plates (BBL Micro-

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biology Systems, Cockeysville, Md.). Plate-grown bacteria were harvested into phosphate-buffered saline (pH 7.2, 300 mosmol/liter). The bacterial concentration was adjusted to 10^9 per ml by optical density (optical density of 0.53 at 597 nm; Vitation, Hugo Tillquist, Gothenburg, Sweden).

Modification of latex beads for agglutination studies. Globotetraosylceramide was purified from human blood group P_1 erythrocytes by alkaline degradation, dialysis, and ion-exchange and silicic acid chromatography of the corresponding derivatives (2). The known glycolipid structures were confirmed by mass spectrometry, nuclear magnetic resonance spectroscopy, and degradative methods. Galα1→4Galβ-O-glycosides and lactose were synthesized as previously described (5, 6).

Polystyrene latex beads (0.81-μm diameter) were purchased from Seragen Diagnostics, Indianapolis, Ind. The beads were covalently linked to protein, bovine serum albumin (BSA; Kabi-Vitrum, Stockholm, Sweden), followed by coating with globotetraosylceramide or covalent coupling to Galα1→4Galβ-O or lactose via a spacer arm. The Galα1→4Galβ content of the beads was estimated by using a monoclonal antibody specific for this disaccharide. The particles were washed several times in buffer and water and suspended in water to a particle density of 5×10^9 per ml (for methods, see reference 7).

Latex bead agglutination. A drop of the latex bead suspension (20 μl) was mixed on a slide with 20 μl of the bacterial suspension to be tested. Each strain was tested in parallel with latex-BSA, latex–BSA–Gal1-4Gal, latex-BSA-globotetraosylceramide, and latex-BSA-lactose. After being mixed for 3 min at room temperature, agglutination was read with the naked eye and graded 0, +, ++, or +++.

Hemagglutination. Human erythrocytes of blood groups AP_1 and Ap, as well as guinea pig erythrocytes, were obtained from heparinized blood and used as 3% suspensions in phosphate-buffered saline or phosphate-buffered saline–0.1 mM α-methyl mannoside. The bacterial and erythrocyte suspensions (20 μl of each) were mixed on a microscope slide. After tilting, agglutination was read with the naked eye and registered as −, negative; +, weakly positive; or as ++ or +++, strongly positive. Agglutination reversed in the presence of α-methyl mannoside was designated mannose sensitive (MS); agglutination unaffected by α-methyl mannoside was designated mannose resistant (MR) (18).

TABLE 1. Frequency of agglutination with bioassays and latex beads

Assay/erythrocyte or glycolipid	% Positive *E. coli* strains[a]				
	Total	Pyelonephritis	Cystitis	ABU	Fecal
Hemagglutination					
Human					
MR P_1	40.4	77.0	41.3	22.7	15.8
MR p	5.4	4.4	9.0	1.7	0.0
No agglutination	52.1	23.0	36.4	72.3	80.8
Guinea pig					
MR	2.2	4.5	2.5	1.7	0.8
MS	29.5	17.9	52.9	18.3	30.0
No agglutination	68.1	77.6	44.6	80.0	69.2
Latex bead agglutination					
Globotetraosylceramide	40.4	78.5	37.2	27.7	12.7
Galα1→4Gal	35.4	65.9	27.3	21.2	14.3
Both	33.3	65.9	24.8	19.5	11.9

[a] Total number of strains, 492; pyelonephritis strains, 135; cystitis strains, 121; ABU strains, 116; fecal strains, 120.

TABLE 2. Correlation of bioassays with the latex bead agglutination

Human erythrocyte group(s) agglutinated	No. of strains positive in hemagglutination	% Strains positive in latex bead agglutination[a]	
		Globotetraosylceramide	Galα1→4Gal
MR P_1	174	95.4	88.9
MR P_1 + p	27	81.5	44.4
MS	36	5.7	2.9
No agglutination	261	3.5	3.1

[a] Percentage of strains reactive with human erythrocytes.

RESULTS

The overall frequency of strains with positive reactions by bioassay or latex bead agglutination is shown in Table 1. About 40% induced MR agglutination of human erythrocytes; 35% induced agglutination of P_1, and 5% induced agglutination of both P_1 and p erythrocytes. MR reactions of guinea pig erythrocytes were rare (2%). The globotetraosylceramide-latex beads were agglutinated by 40% of the strains; 35% agglutinated Galαl→4Gal-latex. The overall correlations between the assays are the topic of reference 7. Since no attempts were made to select for MS adhesins, e.g., by using special culture conditions, the frequencies of MS reactions are not discussed.

The isolates were subsequently analyzed in relation to clinical origin (Table 1). In the acute pyelonephritis group, the highest frequency of positive reactions occurred; 77% of the strains induced MR agglutination of human P_1 erythrocytes, 78.5% induced agglutination of the globotetraosylceramide-latex, and 66% induced agglutination of the Galαl→4Gal-latex beads. Thus, sensitivity of the human erythrocytes and globotetraosylceramide-latex tests were comparable in the pyelonephritis strains, suggesting that binding to globotetraosylceramide explained the hemagglutination in most of the strains in this group. In the cystitis isolates, there was a significant discrepancy between hemagglutination (41%), globotetraosylceramide-latex reactivity (37%), and Galα1→4Gal-latex reactivity (27%), suggesting a higher frequency of other binding specificities in this group. The proportion of isolates agglutinating p erythrocytes was greatest (about 10%) in the cystitis group.

Correlation with bioassay. The reactivity with globotetraosylceramide-latex and Galα1→4Gal-latex beads was correlated with hemagglutination (Table 2). Among the 174 strains inducing MR agglutination of human P_1 erythrocytes only, 95% reacted with globotetraosylceramide-latex and 89% reacted with the Galα1→4Galβ-latex beads. For the 27 strains reacting with both P_1 and p erythrocytes, a larger discrepancy between the two latex tests was found; 82% agglutinated globotetraosylceramide-latex, compared with 44% agglutinating Galα1→4Galβ-latex. Of the seven strains inducing MR agglutination of guinea pig erythrocytes also, five reacted with both latex kits. About 3% of strains negative for hemagglutination gave positive reactions with both latex kits.

The intensity of MR agglutination of human erythrocytes of each isolate was graded −, +, ++, or +++ and compared with the intensity of latex bead agglutination. Human

TABLE 3. Intensity of agglutination: Galα1→4Gal-latex assays versus globotetraosylceramide-latex assays

Globotetraosylceramide-latex agglutination	No. of strains showing Galα1→4Gal-latex intensity of:			
	−	+	++	+++
−	290	1	2	1
+	14	4	1	0
++	12	14	22	7
+++	4	5	32	83

P_1 erythrocytes reacted more strongly than the p erythrocytes (73% versus 52% with a +++ reaction). The Galα1→4Gal-latex gave significantly weaker agglutination than the globotetraosylceramide-latex beads (53% compared with 61% with a +++ reaction). The comparison between globotetraosylceramide-latex and Galα1→4Gal-latex was extended in Table 3, in which the intensity of agglutination was plotted pairwise for each strain. Of strains which were negative with Galα1→4Galβ-latex, 4 showed +++ reactions in globotetraosylceramide-latex, 12 showed ++, and 14 showed + ($P < 0.01$).

Receptor specificities. The use of both bioassays and receptor-specific agglutination assays provided increased resolution of receptor specificities compared with the bioassays. The expected pattern for strains recognizing the globoseries of glycolipid receptors was designated as follows: group 1, MR agglutination of human P_1, but not p, erythrocytes, and positive reactions with both globotetraosylceramide- and Galα1→4Gal-latex; group 2, MR agglutination of both P_1 and p erythrocytes; group 3, MR agglutination of human P_1 and p erythrocytes and guinea pig erythrocytes. The discernable specificities are shown in Table 4 and are outlined in footnote *a*. Most strains in group 1, designated group 1a strains, reacted in the expected way. The 20 strains of group 1b were identical to those of group 1a, except that the reactivity was restricted to globotetraosylceramide-latex (negative for Galα1→4Gal-latex). The strains reacting with both P_1 and p erythrocytes were divided into groups also (2a to 2c). Group 2a strains had no reactivity with globotetraosylceramide-latex or Galα1→4Gal-latex and thus fulfilled the criteria of the X adhesin group. Strains in groups 2b and 2c reacted with globotetraosylceramide and Galα1→4Gal-latex in addition to the p erythrocytes. The isolates inducing MR agglutination of guinea pig erythrocytes (group 3) were divided groups 3a to 3c. A single isolate agglutinated human P_1 and p erythrocytes and guinea pig erythrocytes but was unreactive with the latex reagents. The remaining isolates in this category reacted with globotetraosylceramide or Galα1→4Gal-latex or both.

The distribution of the adhesin specificity groups among isolates of different clinical origin is shown in Table 4. Group 1a, with specificity for the globoseries of glycolipid receptors, dominated in all clinical categories and differentiated well between acute pyelonephritis (79%), acute cystitis (34%), ABU (19%), and the normal fecal isolates (11%). The cystitis group contained the largest proportion of strains (4%) inducing isolated MR agglutination of p erythrocytes; the strains reactive with p erythrocytes in the pyelonephritis group also agglutinated Galα1→4Gal or globotetraosylceramide-latex or both. Few of the ABU or fecal isolates showed either of these reaction patterns.

DISCUSSION

The initial evidence for adherence as a virulence factor in UTI was obtained by comparing the ability of isolates causing acute pyelonephritis and ABU to attach to uroepithelial cells (25). In the present study, the bioassay was replaced with a synthetic latex assay detecting bacterial adhesins specific for the globoseries of glycolipid receptors. It was demonstrated that the frequency of positive reactions in the different groups of isolates paralleled those frequencies obtained by bioassay. The latex reagent provides a basis on which to evaluate the diagnostic potential of qualitative information about *E. coli* causing UTI.

Adherence satisfies two criteria for a potentially useful diagnostic marker: it contributes to virulence and is frequently expressed by the pyelonephritogenic clones. The pyelonephritogenic clones are identified by their combination of antigenic (O:K:H) or isozyme markers (3, 4, 29). The combination is necessary; a single antigen or isozyme has low discriminatory capacity (14). For diagnostic purposes, determination of multiple determinants is often too laborious. Instead, one may use the fact that the pyelonephritogenic clones to different serotypes coexpress the same set of virulence traits, e.g., resistance to serum-induced killing, adherence, and hemolytic capacity. Of these markers, hemolytic capacity occurred at too low a frequency to be useful as a detector of pyelonephritogenic clones (17, 18, 20). Serum antibody resistance did not discriminate sufficiently between pyelonephritis and ABU strains (17, 18, 22–24). Thus, adherence was the most suitable single marker so far examined for potential diagnostic use (26, 29).

The latex reagents detecting specific binding to the globoseries of glycolipid receptors are several steps removed

TABLE 4. Resolution of receptor specificities by the combination of bioassay and latex kits[a]

Group	Hemagglutination		Latex agglutination		% with this pattern			
	Human P_1/p	Guinea pig	Globotetraosylceramide	Galα1→4Gal	Pyelonephritis	Cystitis	ABU	Fecal
1a	MR/−	+/− MS	+++	+++	79	34	19	11
1b	MR/−	+/− MS	+++	−	12	1	4	1
2a	MR/MR	+/− MS	−	−	0	4	1	0
2b	MR/MR	+/− MS	+++	+++	4	2	1	0
2c	MR/MR	+/− MS	+++	−	2	5	0	0
3a	MR/MR	MR	−	−	0	1	0	0
3b	MR/MR	MR	+++	+++	2	2	0	1
3c	MR/MR	MR	+++	−	0	0	2	0

[a] Proposed receptor specificities: 1a, globoseries of glycolipids; 1b, additional determinant on globotetraosylceramide; 2a, X; 2b, 2a + 1a, ? = 2a + 1b; 3a, unknown + 2a; 3b, unknown + 3b + 1a; 3c, unknown + 2a + 1b.

from the functional parameter one would like to test, adherence to the mucosal lining in the urinary tract of the patient. Adherence to uroepithelial cells is a measure of the sum of affinities between the organism and the cell (16). Hemagglutination can be used to dissect the different specificities involved if the receptor composition of erythrocytes is known (8, 14–16). Latex reagents, on the other hand, give specific information about binding to the receptor analog coupled to the latex beads, but not about other specificities. This is illustrated by the results of the present study. The receptor-specific latex reagents gave good resolution of ability to bind globotetraosylceramide or Galα1→4Gal among the clinical isolates. The correlation with hemagglutination in the different diagnostic groups depended on the fraction of strains with this specificity. Thus, the best correlation occurred in the acute pyelonephritis isolates.

The combination of receptor-specific latex tests and bioassays provided increased resolution of receptor specificities of adhesins on uropathogenic *E. coli*. Previous classes, determined by bioassays, are as follows: P-fimbriated or Galα1→4Gal-recognizing adhesins, i.e., those agglutinating human P_1 but not p erythrocytes; X-fimbriated or non-Galα1→4Gal-specific adhesins, i.e., strains agglutinating both human P_1 and p erythrocytes, among which are adhesins specifically recognizing terminal *N*-acetyl-D-glucosamine, neuraminyl 2-3galactose, M antigen, etc. (21, 28, 30); and MR gp adhesins, i.e., adhesins inducing MR agglutination of both human P_1 and p erythrocytes and guinea pig erythrocytes (16). The latex reagents revealed coexpression of the specificities described above. Strains reactive with both globotetraosylceramide and Galα1→4Gal-latex were found among the strains defined as having X and MR gp adhesins by bioassays.

The reaction with both p erythrocytes and Galα→4Gal-latex may also be explained by binding to residual amounts of Galα1→4Gal-containing glycolipids in the p erythrocytes. Although serologic techniques do not demonstrate P_1, P, and p antigens in p erythrocytes, thin-layer chromatograms of the nonacid glycolipid extract from p erythrocytes revealed a band in the position of globotetraosylceramide (1, 19). Bacteria bound to this band (1). Possibly, isolates with a strong adhesin expression might agglutinate the p erythrocytes despite their low receptor density.

The occurrence of strains reactive with globotetraosylceramide-latex but not with Galα1→4Gal-latex is a novel observation in this study. Several mechanisms may be proposed; they need further analysis. First, globotetraosylceramide is a more efficient inhibitor of bacterial adherence than is Galα1→4Gal and might be a more efficient receptor on the solid phase (16). This was not supported by analyses of various concentrations of globotetraosylceramide and Galα1→4Gal on the latex beads (7). Second, the tetrasaccharide globotetraose exposes determinants in addition to Galα1→4Gal which may be recognized by the bacteria. Recognition of the terminal GalNAcβ1→3Gal is consistent with the finding that about 50% of strains recognizing the globoseries of glycolipids agglutinated sheep erythrocytes (15). For diagnostic purposes, it should be noted that strains reacting only with globotetraosylceramide-latex and those reactive with both globotetraosylceramide-latex and Galα1→4Gal-latex were overrepresented in the pyelonephritis group.

The latex reagents met the expectations for a diagnostic tool in that they had high reactivity and sensitivity for strains recognizing the globoseries of glycolipid receptors and a high frequency of positive reactions in the pyelonephritis group. However, the requirement for baterial virulence to cause acute pyelonephritis depends on the resistance of the host. The same clinical condition may be caused by an attaching *E. coli* strain in the resistance host or by a strain with characteristics of the normal fecal isolates in a child with, e.g., vesicoureteric reflux (18). This complexity must be considered, and the hope for diagnosis of bacterial virulence traits must be modified thereafter. Further studies are required to evaluate how this diagnostic procedure can be integrated into clinical praxis.

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