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Globo-A – a new receptor specificity for attaching *Escherichia coli*

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Uropathogenic *Escherichia coli* strains designated as ONAP, based on their O negative A positive agglutination of human P_1 erythrocytes, were shown to prefer the globo-A glycolipid as a receptor structure. The dependence on both the A terminal and the globoseries chain was confirmed by agglutination of human AP_1, but not $A\bar{p}$ or OP_1 erythrocytes and by binding to the globo-A glycolipid on TLC plates. Neither Galα1→4Galβ nor the A trisaccharide GalNAcα1→3(Fucα1→2)Galβ alone functioned as receptors. The bacteria thus appeared to recognize an epitope resulting from the combination of the terminal and internal structures.

Bacterial attachment, Glycolipid receptor; Globoseries; (*E. coli*)

1. INTRODUCTION

Glycoconjugates serve as receptors for microbial ligands such as viral binding proteins, bacterial toxins and adhesins [1-3]. One well-characterized receptor for attaching bacteria is the Galα1→4Galβ disaccharide moiety of the globoseries of glycolipids [4,5]. The Galα1→4Galβ reactive strains agglutinate latex beads coupled with synthetic Galα1→4Galβ [16] and bind to glycolipids containing this sequence separated on thin-layer chromatograms (TLC) [7]. They also agglutinate human P_1 and P_2 but not $\bar{p}$ erythrocytes which lack serologically detectable amounts of the globoseries of glycolipids [4,5,8]. We recently identified a group of *E. coli* isolates which agglutinated human P_1, but not $\bar{p}$ erythrocytes or Galα1→4Galβ-latex beads (Senior et al., to be published). The present study demonstrates that those strains recognize an epitope on the globoseries of glycolipids other than Galα1→4Galβ alone, i.e. the globo-A structure.

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2. MATERIALS AND METHODS

The 82 *E. coli* isolates from the urine of dogs were stored and kept in deep agar stab cultures.

Two type strains with known specificity for the globoseries of glycolipids were used: the wild-type isolate *E. coli* 36692 [4,7] and *E. coli* 506MR, derived from the faecal isolate 506P by transformation with the chromosomal DNA fragment *pap* [9] encoding pili and adhesins specific for the globoseries of glycolipids.

Erythrocytes from citrated human blood of groups AP_1, OP_1, BP_1 $A\bar{p}$ and $O\bar{p}$, were diluted to 3% suspensions (v/v) in phosphate-buffered saline (PBS) containing 2.5% α-methyl-mannoside. Synthetic oligosaccharides were coupled to bovine serum albumin (BSA), covalently linked to latex beads [6]. Bacteria were cultured and agglutinations of erythrocytes and latex beads performed as described [6].

Total non-acid glycosphingolipid fractions [10] and purified glycolipids [11-14] from mouse and dog intestines, human meconium and erythrocytes were tested for binding activities. The structures of these glycolipids have all been confirmed by mass spectrometry (VG ZAB-HF, Manchester, England) of the permethylated derivatives.

Crude glycolipid extracts were obtained from 1 ml of packed human erythrocytes of blood group $A_1\bar{p}$ and $O\bar{p}$ which were mixed with methanol (1:2, v/v), sonicated for 10 min, heated at 70°C for 60 min and centrifuged at 3500 rpm for 10 min. The sediment was re-extracted with 2 ml methanol and with 1.5 ml chloroform/methanol (2:1, v/v). The pooled supernatants were adjusted to a solvent composition of chloroform/methanol/water of 1:10:9 (by vol.), and applied three times to 1 ml pre-

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Table 1

Agglutination of human erythrocytes in relation to A, B, H and P blood groups

Hemagglutination blood group	Hemagglutination	
	Galα1→4Galβ-latex agglutination	
	−(n=16)	+(n=9)
A_1P_1	+ + +	+ + +
BP_1	(+)	+ + +
OP_1	(+)	+ + +
$A_1\bar{p}$	−	−
$O\bar{p}$	−	−

Hemagglutination reactions were performed in the presence of 2.5% α-methylmannoside.

Table 2

Agglutination of saccharide-latex bead conjugates by ONAP strains

Saccharides	Receptor specificity	
	ONAP[a]	Galα1→4Galβ[b]
GalNAcα1→3(Fucα1→2)Galβ	−	−
Galα1→4Galβ	−	+ +
Galα1→4Galβ1→4Glcβ	−	+ +
Galα1→4Galβ1→4GlcNAcβ	−	+ +
GalNAcβ	−	−
GalNAcβ1→3Galα	−	−
GalNAcβ1→3Galα1→ 4Galβ1→4Glcβ	+	+ +

[a] n=16

[b] *E. coli* 36692 and 506MR

packed Bond Elute C-18 columns (Analytichem, Harbor City, CA) pre-conditioned in this solvent. The glycolipids were eluted with 10 ml chlorofom/methanol (2:1, v/v), dried under nitrogen and resuspended in chloroform/methanol (2:1, v/v).

Glycolipids were degraded using a mixture of extracellular α-glycosidases from *Ruminococcus* AB strain VI-268 at 37°C for 72 h [12,15].

Antibody and bacterial overlay [7,12] was performed with slight modifications, using TLC plates treated with polyisobutylmethacrylate in diethyl ether/hexane (1:1, v/v) or in pure diethyl ether at concentrations ranging from 0.15 to 0.35% (w/v) and bacteria grown in Luria broth with 1 mM $CaCl_2$ and 50 μCi [^{35}S]methionine (500 μl) at 37°C for 15–18 h suspended to approx. 10^8 CFU/ml in PBS (spec. act. 100–200 cpm/cfu).

3. RESULTS

Two categories of mannose-resistant adhesins were observed among the 82 *E. coli* isolates. Nine strains agglutinated P_1 erythrocytes and the Galα1→4Galβ-latex beads but not $\bar{p}$ erythrocytes, and 16 strains agglutinated P_1 but not the $\bar{p}$ erythrocytes or the Galα1→4Galβ-latex beads. The strains with Galα1→4Galβ-specific adhesins agglutinated all P_1 erythrocytes regardless of ABH blood group. The second category reacted with the A_1P_1 erythrocytes but poorly with B or O erythrocytes, and were designated as ONAP (O negative, A positive, table 1).

The ONAP strains did not react with the A trisaccharide or terminal Galα1→4Galβ disac-

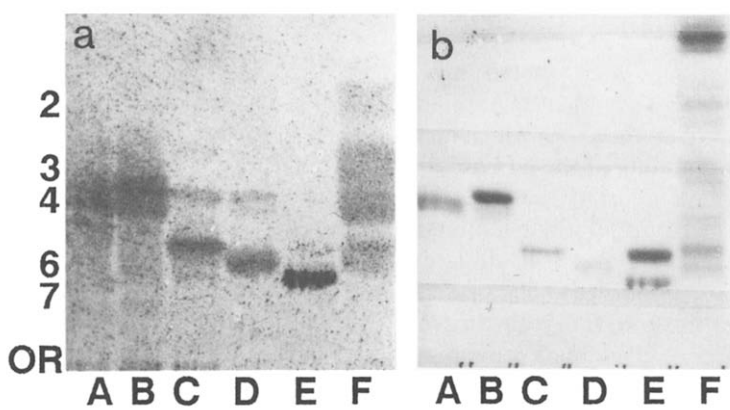

Fig.1. TLC with 3 μg, respectively, of globotetraosylceramide with hydroxylated (lane A) and non-hydroxylated (lane B) ceramides; 1 μg, β-Forssman glycolipid (lane C); 1 μg, globo-H (lane D); 2 μg respectively of blood group A hexa- and heptaglycosylceramides (lane E); and 20 μg total non-acid glycolipids of meconium of a blood group O Lea − b + secretor individual (lane F). (a) Autoradiogram after overlay with ^{35}S-labelled *E. coli* ONAP strain 1484. (b) Chemical detection of the glycolipid fractions as shown by copper acetate staining.

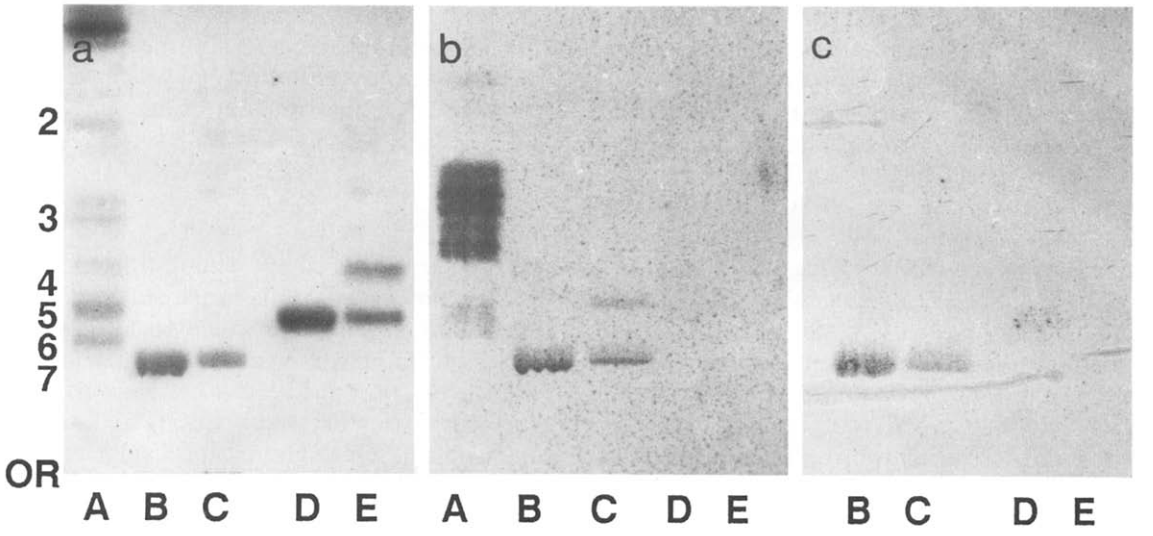

Fig.2. TLC of non-acid glycolipids of an O Lea−b+ secretor meconium sample (lane A), of blood group A hepta- and hexaglycosylceramide before (lanes B,D) and after 72 h of incubation with bacterial glycosidases (lanes C,E). (a) Chemical detection with copper acetate. (b) Autoradiogram after overlay with ^{35}S-labelled *E. coli* strain 506MR. (c) Autoradiogram after overlay with ONAP strain 1484. Lane A was omitted from c.

charide as tested by agglutination of oligosaccharide-latex bead conjugates, but reacted weakly with globotetraose-coupled beads (table 2). As expected the reactivity of the Galα1→4Galβ-specific strains was exclusive for Galα1→4Galβ-containing saccharides.

Fig.1 shows bacterial binding to a series of blood group A and globoseries-related glycolipids separated on TLC plates. The ONAP strain bound strongly to glycolipids in the seven-sugar region of the fractions in lane E containing mainly blood group A active mono- and difucosylated hexa- and heptaglycosylceramides, as confirmed by staining with anti-A antibodies, and weakly to globotetraosylceramides from meconium, to β-Forssman from erythrocytes and to globo-H from meconium.

The identity of the heptaglycosylceramide as globo-A was first indicated by mass spectrometry

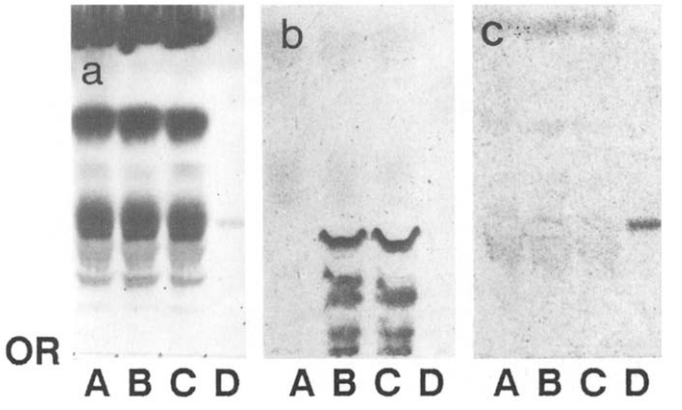

Fig.3. Thin-layer chromatograms of non-acid glycolipids from donors of blood groups O$\bar{p}$ (lane A), A$\bar{p}$ (lane B), A$\bar{p}$ (lane C), globotetraosylceramide (lane D). (a) Chemical detection with copper acetate. (b) Autoradiogram after overlay with anti-A antibody [20]. (c) Autoradiogram after overlay with *E. coli* strain 1484.

of the permethylated sample, with specific ions [16] at *m/z* 606 and 638 (terminal A trisaccharide determinant), *m/z* 851 and 883 (tetrasaccharide determinant), *m/z* 1087 (pentasaccharide), *m/z* 1291 and *m/z* 1495 (hexa- and heptasaccharide). Secondly, glycosidases of *Ruminococcus AB,* which degrade monofucosylated but not difucosylated blood group A active type 1 chain glycolipids to lactotetraosylceramide, partially degraded the monofucosylated A hexaglycosylceramide, but not the difucosylated heptaglycosylceramide (fig.2a). The degradation of type 4 chain glycolipids was shown by the reactivity of both substrate and product, probably globopentaosylceramide, with *E. coli* 506MR (fig.2b). The minor heptaglycosylceramide component was thus tentatively identified as globo-A. The ONAP strain bound to the substrate of the enzymatic degradation, i.e. globo-A, but not to the product globopentaosylceramide at the concentrations used (fig.2c).

The lack of reactivity with A type 1 and A type 2 chain glycolipids was confirmed using crude glycolipid extracts from $A\bar{p}$ and $O\bar{p}$ erythrocytes, a non-acid glycolipid fraction of human meconium and a purified A-type 2 chain glycolipid (fig.3).

4. DISCUSSION

The present study has demonstrated specificity for the globo-A oligosaccharide sequence (table 1) among uropathogenic *E. coli* designated as ONAP based on O negative A positive hemagglutination.

Like the previously known strains recognizing the globoseries of glycolipids, these isolates demonstrated P blood group dependent hemagglutination. In contrast, they did not bind Galα1→4Galβ on latex beads and the P blood group related reactivity was limited to the A blood group. The strains with specificity for the Galα1→4Galβ disaccharide bound with comparable intensity to all the natural

Table 3

Structure and receptor activities of glycolipids

Structure	Receptor activities	
	E. coli ONAP	*E. coli* 506MR
Lactosylceramide	–	–
Galabiosylceramide	(+)	+
Globotriaosylceramide	(+)	+
Globotetraosylceramide (globoside)	(+)	+
Globopentaosylceramide	n.d.	+
β-Forssman	(+)	+
H type 4 chain (globo-H)	(+)	+
A type 2 chain	–	–
A type 1 chain	–	–
ALe^b (type 1 chain)	–	–
A type 4 chain (globo-A)	+	+

glycolipids containing this disaccharide. The ONAP strains bound strongly to globo A but weakly to globotetraosylceramide, the β-Forssman glycolipid hapten and the diglycosylceramide region on TLC plates. An influence of the A determinant per se was contradicted by the lack of reactivity with $A\bar{p}$ erythrocytes, glycolipid extracts and with the synthetic A trisaccharide coupled to latex beads. The specificity for the globoseries showed that the Galα1→4Galβ moiety was required for the receptor conformation. These results suggest that the bacteria recognize a conformation afforded by the type 4 chains only, which is not satisfied by any of the tested terminal or internal di- or trisaccharide combinations. Recent evidence indicates stronger binding to α-Forssman, suggesting that the binding epitope may be a terminal GalNAcα on an extended globoseries oligosaccharide common to Forssman and globo-A. It remains to be shown if this specificity is similar to that of the recently reported *prs* clone from *E. coli* J96 [17].

The specificity for globo-A was found in 19% of the *E. coli* strains from dogs with urinary tract infection and in a subgroup of human strains with globotetraosylceramide, but not Galα1→4Galβ reactivity [18]. The functional importance of this specificity for adherence and infection remains to be established.

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