

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

D. Senior, N. Baker, B. Cedergren*, P. Falk⁺, G. Larson⁺, R. Lindstedt and C. Svanborg Edén

Departments of Clinical Immunology and ⁺Clinical Chemistry, University of Göteborg, Göteborg and *The Blood Bank, University Hospital, University of Umeå, Umeå, Sweden

Received 27 July 1988

Uropathogenic *Escherichia coli* strains designated as ONAP, based on their O negative A positive agglutination of human P₁ 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 AP₁, but not A $\bar{p}$ or OP₁ erythrocytes and by binding to the globo-A glycolipid on TLC plates. Neither Gal α 1 $\rightarrow$ 4Gal β nor the A trisaccharide GalNAc α 1 $\rightarrow$ 3(Fuc α 1 $\rightarrow$ 2)Gal β 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 Gal α 1 $\rightarrow$ 4Gal β disaccharide moiety of the globoseries of glycolipids [4,5]. The Gal α 1 $\rightarrow$ 4Gal β reactive strains agglutinate latex beads coupled with synthetic Gal α 1 $\rightarrow$ 4Gal β [16] and bind to glycolipids containing this sequence separated on thin-layer chromatograms (TLC) [7]. They also agglutinate human P₁ and P₂ 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 P₁, but not $\bar{p}$ erythrocytes or Gal α 1 $\rightarrow$ 4Gal β -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 Gal α 1 $\rightarrow$ 4Gal β alone, i.e. the globo-A structure.

Correspondence address: C. Svanborg Edén, Department of Clinical Immunology, University of Göteborg, Guldhedsgatan 10, S-413 46 Göteborg, Sweden

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 AP₁, OP₁, BP₁, 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 A₁ $\bar{p}$ 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-

Table 1

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

Hemagglutination blood group	Hemagglutination	
	Gal α 1 $\rightarrow$ 4Gal β -latex agglutination – (n = 16)	+ (n = 9)
A ₁ P 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	Gal α 1 $\rightarrow$ 4Gal β ^b
GalNAc α 1 $\rightarrow$ 3(Fuc α 1 $\rightarrow$ 2)Gal β	–	–
Gal α 1 $\rightarrow$ 4Gal β	–	++
Gal α 1 $\rightarrow$ 4Gal β 1 $\rightarrow$ 4Glc β	–	++
Gal α 1 $\rightarrow$ 4Gal β 1 $\rightarrow$ 4GlcNAc β	–	++
GalNAc β	–	–
GalNAc β 1 $\rightarrow$ 3Gal α	–	–
GalNAc β 1 $\rightarrow$ 3Gal α 1 $\rightarrow$ 4Gal β 1 $\rightarrow$ 4Glc β	+	++

^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 chloroform/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 CaCl₂ and 50 μ Ci [³⁵S]methionine (500 μ l) at 37°C for 15–18 h suspended to approx. 10⁸ 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 P₁ erythrocytes and the Gal α 1 $\rightarrow$ 4Gal β -latex beads but not $\bar{p}$ erythrocytes, and 16 strains agglutinated P₁ but not the $\bar{p}$ erythrocytes or the Gal α 1 $\rightarrow$ 4Gal β -latex beads. The strains with Gal α 1 $\rightarrow$ 4Gal β -specific adhesins agglutinated all P₁ erythrocytes regardless of ABH blood group. The second category reacted with the A₁P₁ 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 Gal α 1 $\rightarrow$ 4Gal β disac-

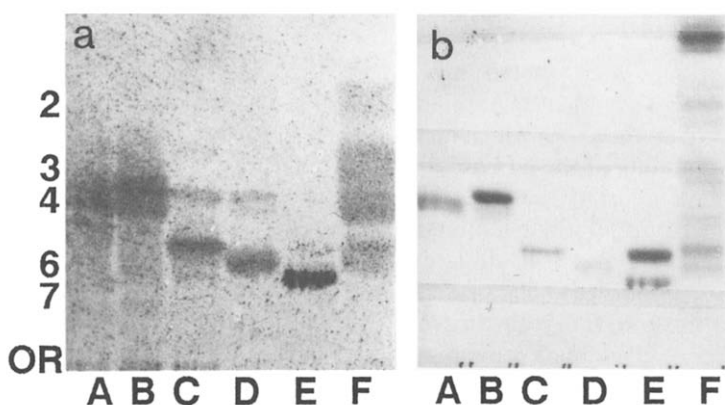


Fig.1. TLC with 3 μ g, respectively, of globotetraosylceramide with hydroxylated (lane A) and non-hydroxylated (lane B) ceramides; 1 μ g, β -Forssman glycolipid (lane C); 1 μ g, globo-H (lane D); 2 μ g respectively of blood group A hexa- and heptaglycosylceramides (lane E); and 20 μ g total non-acid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

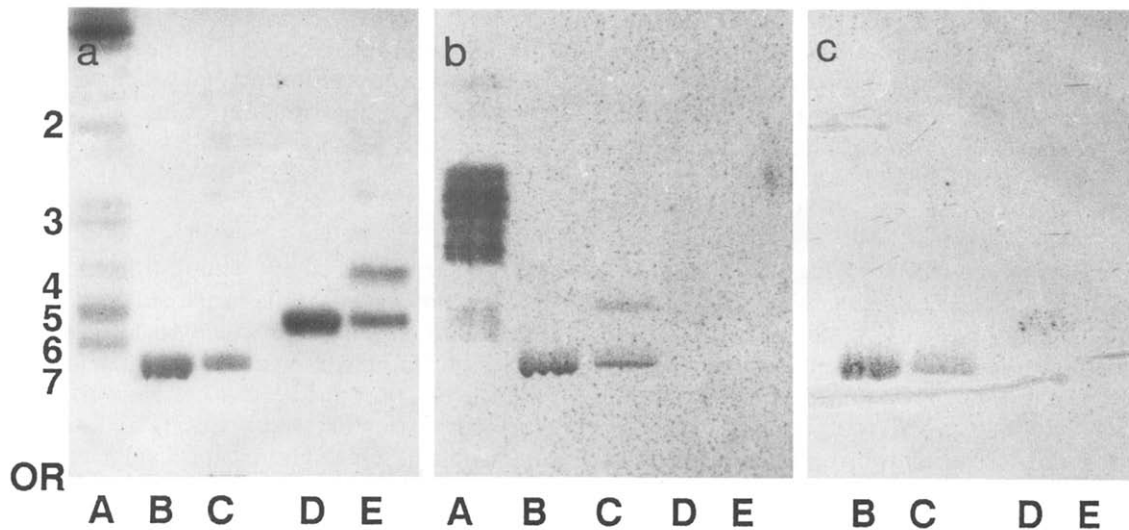


Fig.2. TLC of non-acid glycolipids of an O Lea-b+ secretor meconium sample (lane A), of blood group A hepta- and hexaglycosylceramide before (lanes B,D) and after 72 h of incubation with bacterial glycosidases (lanes C,E). (a) Chemical detection with copper acetate. (b) Autoradiogram after overlay with ^{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 $\text{Gal}\alpha 1 \rightarrow 4\text{Gal}\beta$ -specific strains was exclusive for $\text{Gal}\alpha 1 \rightarrow 4\text{Gal}\beta$ -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.

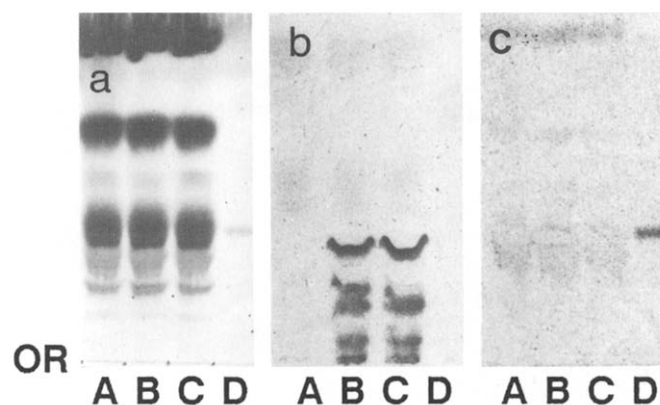


Fig.3. Thin-layer chromatograms of non-acid glycolipids from donors of blood groups Op (lane A), Ap (lane B), Ap (lane C), globotetraosylceramide (lane D). (a) Chemical detection with copper acetate. (b) Autoradiogram after overlay with anti-A antibody [20]. (c) Autoradiogram after overlay with *E. coli* 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 Gal α 1 $\rightarrow$ 4Gal β on latex beads and the P blood group related reactivity was limited to the A blood group. The strains with specificity for the Gal α 1 $\rightarrow$ 4Gal β disaccharide bound with comparable intensity to all the natural

Table 3
Structure and receptor activities of glycolipids

Structure		Receptor activities	
		<i>E. coli</i> ONAP	<i>E. coli</i> 506MR
Lactosylceramide	Gal β 1 $\rightarrow$ 4GlcCer	—	—
Galabiosylceramide	Gal α 1 $\rightarrow$ 4GalCer	(+)	+
Globotriaosylceramide	Gal α 1 $\rightarrow$ 4Gal β 1 $\rightarrow$ 4GlcCer	(+)	+
Globotetraosylceramide (globoside)	GalNAc β 1 $\rightarrow$ 3Gal α 1 $\rightarrow$ 4Gal β 1 $\rightarrow$ 4GlcCer	(+)	+
Globopentaosylceramide	Gal β 1 $\rightarrow$ 3GalNAc β 1 $\rightarrow$ 3Gal α 1 $\rightarrow$ 4Gal β 1 $\rightarrow$ 4GlcCer	n.d.	+
β -Forssman	GalNAc β 1 $\rightarrow$ 3GalNAc β 1 $\rightarrow$ 3Gal α 1 $\rightarrow$ 4Gal β 1 $\rightarrow$ 4GlcCer	(+)	+
H type 4 chain (globo-H)	Gal β 1 $\rightarrow$ 3GalNAc β 1 $\rightarrow$ 3Gal α 1 $\rightarrow$ 4Gal β 1 $\rightarrow$ 4GlcCer	(+)	+
	↑		
A type 2 chain	Fuc α 1 GalNAc α 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4GlcNAc β 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4GlcCer	—	—
	↑		
A type 1 chain	Fuc α 1 GalNAc α 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 3GlcNAc β 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4GlcCer	—	—
	↑		
ALe ^b (type 1 chain)	Fuc α 1 GalNAc α 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 3GlcNAc β 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4GlcCer	—	—
	↑ ↑		
A type 4 chain (globo-A)	Fuc α 1 Fuc α 1 GalNAc α 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 3GalNAc β 1 $\rightarrow$ 3Gal α 1 $\rightarrow$ 4Gal β 1 $\rightarrow$ 4GlcCer	+	+
	↑		
	Fuc α 1		

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 Gal α 1 $\rightarrow$ 4Gal β 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 GalNAc α 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 Gal α 1 $\rightarrow$ 4Gal β reactivity [18]. The functional importance of this specificity for adherence and infection remains to be established.

Acknowledgements: The synthetic oligosaccharides from Carbohydrates International, were synthesized and kindly provided by A.-C. Larsson, M. Back and L.E. Franzén. The A type 2 chain glycolipids were kindly provided by Dr Nicklas Strömberg and the bacterial enzymes by Dr L. Hoskins. The skilful typing of A.-C. Malmefeldt is gratefully acknowledged. This study was supported by grants from the Swedish Medical Research Council (nos 7934 and 8266), the Swedish Board for Technical Development, the Lundberg Foundation and the Medical Faculty, University of Göteborg.

REFERENCES

- [1] Hansson, G.C., Karlsson, K.-A., Larson, G., Strömberg, N., Thurin, J., Örvell, C. and Norrby, R. (1984) *FEBS Lett.* 170, 15-18.
- [2] Eidels, L., Proia, R.L. and Hart, D.A. (1983) *Microbiol. Rev.* 47, 596.
- [3] Mirelman, D. (1986) *Microbial Lectins and Agglutinins, Properties and Biological Activity*, pp. 84-110, Wiley, New York.
- [4] Leffler, H. and Svanborg Edén, C. (1980) *FEMS Microbiol. Lett.* 8, 127-134.
- [5] Källénus, G., Möllby, R. and Svensson, S.B. et al. (1980) *FEMS Microbiol. Lett.* 7, 297-302.
- [6] De Man, P., Cedergren, B., Enerbäck, S., Larsson, A.-C., Leffler, H., Lundell, A.-L., Nilsson, B. and Svanborg Edén, C. (1987) *J. Clin. Microbiol.* 25, 2, 401-406.
- [7] Bock, K., Breimer, M.E., Brignole, A., Hanson, G.C., Karlsson, K.-A., Larsson, G., Leffler, H., Samuelsson, B.E., Strömberg, N., Svanborg Edén, C. and Thurnin, J. (1985) *J. Biol. Chem.* 260, 8545-8551.
- [8] Marcus, D.M., Kundu, S.K. and Suzuki, A. (1981) *Semin. Hematol.* 18, 63-71.
- [9] Hull, R.A., Gill, R.E. and Hsu, P. et al. (1981) *Infect. Immun.* 33, 933-938.
- [10] Karlsson, K.-A. (1987) *Methods Enzymol.* 138, 212-220.
- [11] Larson, G. (1986) *Arch. Biochem. Biophys.* 246, 531-545.
- [12] Larson, G., Falk, P. and Hoskins, L.C. (1988) *J. Biol. Chem.*, in press.
- [13] Hansson, G.C., Karlsson, K.-A., Larson, G., McKibbin, J.M., Strömberg, N. and Thurin, J. (1983) *Biochim. Biophys. Acta* 750, 214-216.
- [14] Ångström, J., Karlsson, H., Karlsson, K.-A., Larson, G., Nilsson, K. (1986) *Arch. Biochem. Biophys.* 251, 440-449.
- [15] Hoskins, L.C., Agustines, M., McKee, W.B., Boulding, E.T., Krioris, M. and Niedermeyer, G. (1985) *J. Clin. Invest.* 75, 944-953.
- [16] Breimer, M.E. and Jovall, P.-Å. (1985) *FEBS Lett.* 179, 165-172.
- [17] Lund, B., Marklund, B.-I., Strömberg, N., Lindberg, F., Karlsson, K.-A. and Normark, S. (1988) *Mol. Microbiol.*, in press.
- [18] Enerbäck, S., Larsson, A.C. and Jodal, U. et al. (1987) *J. Clin. Microbiol.* 25, 407-411.