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DEVELOPMENTAL CONTROL OF BACTERIAL RECEPTORS IN THE GASTROINTESTINAL TRACT

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INTRODUCTION

Recent advances on glycobiology research have rapidly expanded our knowledge of the importance of oligosaccharide structure in bacterial interaction with host cells. Cellsurface carbohydrates with a structural diversity are potential candidates as recognition sites for bacteria and their toxins. The recognition sites or "receptors" may exist on different glycoproteins and/or glycolipids. Several lines of evidence indicate that glycolipids are much more common than glycoproteins as receptors for bacterial adherence and toxin binding (*Karlsson*, 1989). Furthermore, cell-surface carbohydrates are expressed in a species-specific, tissue-specific and developmentally regulated manner (*Hakomori*, 1981; *Feize*, 1985, *Rademacher* et al., 1988). Therefore, the control mechanisms by which the expression of cell-surface carbohydrates are regulated may have a direct impact on the host, age and tissue specificities of different bacterial infections. In this article, we will summarise available information regarding intestinal receptor expression and its relevance to the host response to bacterial toxins.

MEMBRANE RECEPTORS FOR BACTERIAL TOXINS

Cholera and *Escherichia coli* enterotoxins

Table 1 summarises membrane receptors for several bacterial toxins that cause infectious diarrhoea. The best-known glycolipid receptor is the ganglioside GM1 for cholera toxin (CT) and *E. coli* heat-labile enterotoxin (LT) (*Eidels* et al., 1983). Both the terminal galactose and sialic acid of GM1 are required for the binding. These two toxins also bind to receptor variants, N-glycolyl-GM1 and GD1b, in human intestine, but with a much lower affinity (*Holmgren* et al., 1985). In addition, LT binds to a galactoprotein receptor in human (*Holmgren* et al., 1985), rabbit (*Holmgren* et al., 1982; *Griffiths* et al., 1986) and rat (*Zelmelman* et al., 1989) intestine. The receptor for the *E. coli* heat-stable toxin (STa) has been identified as a glycoprotein of 200 kilodaltons in rat intestine (*Kuno* et al., 1986). This STa receptor contains three peptides (80, 68 and 60 kilodaltons) with binding epitopes for the toxin, but the specific role of the sugar sequence in receptor binding bas not been established.

Clostridium difficile toxin

Toxin A from *Clostridium difficile* binds to hamster intestinal (*Krivan* et al., 1986) and rabbit erythrocyte (*Clark* et al., 1987) glycolipids with a terminal Galα1-3Galβ1-4GlcNAc sequence. The

Table 1: Membrane Receptors for Bacterial Toxins

Microbe	*Vibrio cholera*	
Toxin	Cholera toxin (84 KD, AB_5)	
Receptor	GM1	Galb1-3GalNAcb1-4 (NeuAca2-3)Galb1-4Glcb1-1Cer
Microbe	*Escherichia coli* (ETEC)	
Toxin	Heat-labile toxin (91 KD, AB_5)	
Receptor	GM1	Galb1-3GalNAcb1-4 (NeuAca2-3)Galb1-4Glcb1-1Cer
Gacactoprotein	(Determinant probably like GM1)	
Microbe	*Escherichia coli* (ETEC)	
Toxin	Heat-stable toxin STa (2 KD)	
Receptor	Glycoprotein (Determinant unknown)	
Microbe	*Clostridium difficile*	
Toxin	Toxin A (250 KD)	
Receptor	nLC_5Cer	Gala1-3Galb1-4 GacNAclb1-3Glcb1-4Glcb1-1Cer
Microbe	*Shigella dysenteriae*	
Toxin	Shiga toxin (70 KD, AB_5)	
Receptor	Gb3	Gala1-4Galb1-4Glcb1-1Cer
Microbe	*Escherichia coli* (EPEC and EHEC)	
Toxin	Shiga-like toxin (70 KD, AB_5)	
Receptor	Gb3	Gala1-4Galb1-4Glcb1-1Cer

proposed receptor sequence primarily exists as neolactopentaosylceramide (nLc_5Cer,Galα1-3Galβ1-4GlcNAcβ1-4Galβ1-4Glcβ1-1Cer) on the cell surface. However, the presence of this glycolipid structure in the human large intestine, the target site for toxin action, has yet to be detected (*Karlsson*, 1989).

Shiga and Shiga-like toxins

Shiga toxin is a protein cytotoxin produced by *Shigella dysenteriae* type 1. The glycolipid receptor for Shiga toxin has been identified as globotriaosylceramide (Gb3, Galα1-4Galβ1-4Glcβ1-1Cer) in rabbit intestine and HeLa cells (*Jacewicz* et al., 1986; *Lindberg* et al., 1987; *Mobassaleh* et al., 1988). *Escherichia coli* can produce cytotoxins that are similar in both structure and function to Shiga toxin. It has been shown that the Shiga-like toxin (or Verotoxin) also binds to the glycolipid Gb3 as a functional receptor (*Samuel* et al., 1990).

MEMBRANE RECEPTORS FOR BACTERIAL CELLS

Attachment to the host is required for the colonisation or infection with bacteria. This step is considered an important virulence factor in microbes. Cell-cell interaction between the host and bacteria is mediated by specific molecular recognition. The substances involved are bacterial proteins called adhesins and receptor proteins or carbohydrates on the epithelial surface. Adhesin molecules are expressed as bacterial cell surface components known as fimbriae or pili. The "receptor" sites are cell-surface glycoconjugates of the host. A bacterial cell may have a primary and secondary receptor

Table 2: Membrane attachment sites for bacterial cells

Sugar specificity or receptor epitope	Microbe	Adhesin
Mannose	*E. coli*	Type 1 fimbriae
Sialic acid		
NeuAcα2-8	*E. coli*	CFA/I
NeuAc, NeuGc	*E. coli*	CS2 or CFA/II
NeuGcα2-3Galβ1-4Glcβ1-1Cer	*E. coli*	K99 fimbriae
Galabiose		
Galα1-4Gal	*E. coli* (Uropathogenic)	Type P fimbriae
Lactosylceramide		
Galβ1-4Glcβ1-1Cer	*Bacteroides* *Lactobacillus* *Fusobacterium* *Clostridium* *Shigella* *Vibrio cholera*	

and the receptor epitope can be either a terminal or internal sugar sequence. Table 2 lists several species of bacteria that recognise specific sugar sequences on cell-surface glycoconjugates of the host.

Mannose receptor

Type 1 fimbriae are common on most *E. coli* bacteria and show mannose-sensitive haemagglutination (*Sharon*, 1987). Since glycolipids do not contain mannose, type 1 fimbriated *E. coli* binds to mannose receptors on glycoproteins instead.

Sialic acid receptor

Colonising factor antigens (CFA/I and CFA/II) of enterotoxigenic *E. coli* (ETEC) strains are known to be associated with infantile diarrhoea and travellers' diarrhoea in humans. CFA/I binds to sialic acid receptors with NeuAcα2-8 specificity (*Lindahl* et al., 1982). Recently, CS2 subtype of *E. coli* CFA/II has been shown to bind to a sialic acid (NeuAc or NeuGc)-containing receptor (*Sjoberg* et al., 1988). K99 is a colonising factor antigen of ETEC that causes enteric disease in piglets, calves and lambs. The glycolipid receptor for *E. coli* K99 has been identified as N-glycolyl-GM3 (NeuGcα2-3Galβ1-4Glcβ1-1Cer) (*One* et al., 1989; *Kyogashima* et al., 1989).

Galabiose receptor

Type P fimbriae of uropathogenic *E. coli* recognise the glycolipid receptor containing Galα1-4Gal (galabiose) sequence (*Bock* et al., 1985). This is the same receptor sequence recognised by Shiga toxin.

Lactosylceramide receptor

Many bacteria carry the lactosylceramide (Galβ1-4Glcβ1-1Cer) specificity with a relatively low binding affinity (*Karlsson*, 1989). Both Gram-negative and Gram-positive bacteria with various colonisation tissues are binders including both normal flora and pathogens. The dominate anaerobes of normal flora in the human

Table 3: developmental changes in the levels of toxin receptors in the small intestine

Toxin	Receptor	Species	Change with age
Cholera toxin GM1		rat	↔
E. coli LT	GM1	rat	↔
	Galactoprotein	rat	↑
E coli STa	Glycoprotein	rat	↓
		human	↓
Shiga toxin	Gb3	rabbit	↑
		rat	↑
C. difficile toxin A	nLc_5Cer	rabbit	↑

large intestine, including *Bacteroides*, *Clostridium*, *Fusobacterium* and *Lactobacillus*, may all use this specificity. *Vibrio cholerae* and *Shigella dysenteriae* are also shown to express lactosylceramide binding. These bacteria may compete for common receptor binding sites for adherence and colonisation. This competition may explain why overgrowth of *C. difficile* organism is induced after antibiotic treatment, which eliminates antibiotic sensitive strains such as *Bacteroides* (*van der Waaij*, 1989). Thus, competition for common attachment sites could be one of the important mechanisms by which normal flora of the gastrointestinal tract contribute to the protective barrier to intestinal colonisation by pathogens.

DEVELOPMENTAL CHANGES IN MEMBRANE RECEPTORS AND HOST RESPONSE

Increased toxigenic diarrhoea in the immature intestine due to receptor-dependent and -independent mechanisms

Age-related differences in the structure and function of membrane receptors for bacterial toxins (Table 3) have been studied using binding assays or glycolipid analysis. Table 4 summarises developmental variations in the host susceptibility to various bacteria-induced diarrhoeal diseases. In the case of glycolipid GM1, there is no change in the level of this glycolipid receptor in rat intestine during postnatal development (*Chu* et al., 1989). So the increased host responsiveness of the immature enterocyte to cholera toxin (*Chu* et al., 1989) and *E. coli* heatlabile toxin (LT, unpublished data) seems to be independent of the initial receptor occupancy (*Chu* et al., 1989). In contrast, the increased enterocyte response to *E. coli* heatstable toxin (STa) can be explained by an increased receptor number in the immature gut of rats (*Cohen* et al., 1986) and young children (*Cohen* et al., 1988). These two examples suggest that both receptor and/or postreceptor events may contribute to the developmental variations in host responsiveness to toxigenic diarrhoea. One of the postreceptor events appears to be due to the

Table 4: Age-related changes in the host susceptibility to infectious diarrhea in children and animal models

Pathogen	Host	Target tissue	Change with age
Vibro cholera	children	small intestine	↓
Enterotoxigenic *E. coli* (ETEC)	children	small intestine	↓
Clostridium difficile	children rabbit	large intestine ileum	↑ ↑
Shigella dysenteriae type 1	children rabbit	large intestine ileum	↑ ↑

underdeveloped activity of Na^+,K^+-ATPase (*Chu* et al., 1989), the sodium pump, that is the driving force for the intestinal absorption of ions and water. In addition, another functional galactoprotein receptor for LT is expressed in the adult, but not in the neonatal rat intestine (*Zelmelman* et al., 1989). Because of this glycoprotein's low binding affinity for LT, naturally occurring quantities of toxin most likely affect the epithelium primarily via the GM1 high affinity receptor in the rat intestine. However, the possibility that the LT glycoprotein receptor may play a role in travellers' diarrhoea in adult humans cannot be excluded.

Protection from toxigenic diarrhoea in the immature intestine due to a receptor-dependent decrease in host responsiveness

In the case of Shiga toxin and *C. difficile* toxin A, a positive correlation between the receptor expression and toxin effects has been demonstrated. Age-dependent increases in the expression of the glycolipid receptor Gb3 for Shiga toxin have been noted in the rabbit intestine (*Mobassaleh* et al, 1988; *Mobassaleh* et al., 1989). In rats, Gb3 is the major glycolipid of crypt cells of adult intestine and is detectable only after weaning (*Bonhours* and *Bonhours*, 1981). Similarly, the binding of *C. difficile* toxin A to membrane receptors in the rabbit intestine is shown to increase after weaning (*Eglow* et al., 1989). The underdeveloped expression of toxin receptors in the immature intestine could explain the relative resistance of human neonates to clinical shigellosis (*Keusch*, 1982). It could also explain the finding that infancy is the only population in which *C. difficile* toxin A is frequently detected in the absence of any clinical symptoms (*van der Waaij*, 1989).

MECHANISM FOR DEVELOPMENTAL CONTROL OF RECEPTOR EXPRESSION

Developmental regulation of glycosyltransferase and surface-carbohydrate expression

As mentioned, a defined oligosaccharide structure is needed for the binding of bacterial toxins and cells to membrane receptors. Therefore, the regulation of specific sugar sequence expression can control the expression of bacterial receptors during development. Although mechanisms for developmental regulated carbohydrate expression on the intestinal surface are not completely understood, one of the

Table 5: Developmental changes in the intestinal glycosyltransferase activities

Glycosyl-transferase	Donor substrate	Sequence formed	Change with age
Sialyltransferase (ST)			↓
Galα2,6-ST	CMP-NeuAc	**NeuAcα2-6**Galβ1-4GlcNAc-R	
Galα2,3-ST		**NeuAcα2-3**Galβ1-4GlcNAc-R	
Fucosyltransferase (FucT)			↑
GlcNAcα1,3-FucT	GDP-Fuc	Galβ1-4 (**Fucα1-3**)GlcNAc-R	
Galα1,2-FucT		**Fucα1-2**Galβ1-4GlcNAc-R	
		Fucα1-2 Galβ1-3GlcNAc-R	
Galactosyltransferase (GalT)			
GalNAcβ1,4-GalT	UDP-Gal	**Galβ1-4**GlcNAc-R	↑
Galα1,3-GalT		**Galβ1-3**Galβ1-4GlcNAc-R	↑
N-Acetylgalactosaminyl-transferase (GalNAcT)			
Galα1,3-GalNAcT	UDP-GalNAc	**GalNAcα1-3**(Fucα1-2)Gal-R	↑

major regulatory mechanisms appears to be operated via control of glycosyltransferase expres-sion. Table 5 illustrates examples of several glycosyltransferase activities of rat intestine that are under developmental control. A reciprocal relationship between a decrease in sialyltransferase activity and an increase in fucosyltransferase activity (*Chu* and *Walker*, 1986) appears to be well correlated with a shift from sialylation to fucosylation of microvillus membrane glycoproteins and glycolipids in the rat small intestine after weaning (*Torres-Pinedo* and *Mohmood*, 1984; *Pang* et al., 1987). Specifically, the increased sialylation in the neonatal intestine is reflected by an increased level of gangliosides (i.e. sialic acid containing glycolipids), mainly as the form of GM3 (*Bouhours* and *Bouhours*, 1983). Furthermore, Western blots of microvillus membrane glycoproteins probed with a sialic acid (α2-3)-specific lectin, MAA (*Maackia amurensis* agglutinin) and a sialic acid (α2-6)-specific lectin, SNA (*Sambucus nigra* agglutinin) also showed an increase in both α(2-3)- and α(2-6)-linked sialoglycoproteins in the immature intestine (unpublished data). It is our hypothesis that the presence of the sialic acid-rich glycoconjugates may possibly cause a barrier dysfucntion vis-à-vis receptor availability and binding capacity in the neonatal mucosa, and therefore may favour the colonisation and penetration of sialic acid-binding bacteria (e.g. ETEC) leading to neonatal bacterial infection. Two other glycosyltransferases, galactosyl- (*Pang* et al., 1987) and N-acetylgalactosylaminyltransferase activities (*Biol* et al., 1987), are noted to increase with age. The increased activities of these two enzymes may enhance the expression of glycolipids and glycoproteins containing galactose and N-acetylgalactosamine in the mature intestine, but their role in affecting the expression of sugar sequences involved in toxins receptors remains to be determined.

Regulation of glycolipid biosynthesis and toxin receptor expression

Most of the biosynthetic pathways for both acidic (i.e. gangliosides) and neutral glycolipids are well established

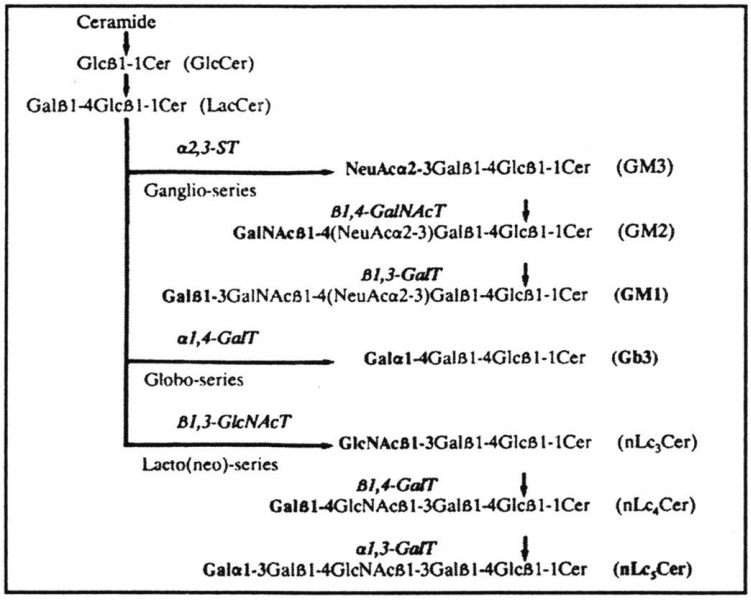

Figure 1: Biosynthetic pathways of glycolipid receptors for bacterial toxins.
Glycosyltransferases involved in these pathways are α2,3-sialyltransferase (α2,3-ST), β1,4-N-acetylgalactosaminyltransferase (β1,4-GalNAcT), β1,3-galactosyltransferase (β1,3-GalT) for cholera toxin receptor GM1; α1,4-galactosyltransferase (α1,4-GalT) for Shiga toxin receptor Gb3; and β1,3-N-acetylglycosyltransferase (β1,3-GlcNAcT), β1,4-galactosyltransferase (β1,4-GalT) and α1,3-galactosyltransferase (α1,3-GalT) for *C. difficile* toxin A receptor nLc_5Cer.

(*Wiegant*, 1985). Figure 1 depicts the biosynthetic pathways leading to the expression of glycolipid molecules, which are known receptors for bacterial toxins. Lactosylceramide (LacCer, Galβ1-4Glcβ1-1Cer) is the common precursor for the expression of the glycolipid receptors for cholera toxin, Shiga toxin, and *C. difficile* toxin A. Ganglioside GM1, the receptor for cholera toxin, is formed by the sequential addition of sialic acid, N-acetylgalactosamine and galactose to LacCer, catalysed by three enzymes, α2,3-sialyl-, β1,4-N-acetylgalactosaminyl-, and β1,3-galactosyltransferases, respectively. Shiga toxin receptor, Gb3, is formed by the addition of a galactose to LacCer, catalysed by α1,4-galactosyltransferase. *Clostridium difficile* toxin A receptor, neolactopentaosylceramide (nLc_5Cer) is formed by the sequential addition of N-acetylglucosamine, β-linked galactose and α-linked galactose, catalysed by β1,3-N-acetylglucosyl-, β1,4-galactosyl-, and α1,3-galactosyltransferases, respectively. Conceivably, developmental regulation of these lipid glycosyltransferases, especially the ones involved in the rate-limiting step of the glycosylation pathway, can markedly influence the expression and prevalence of toxin receptors on the intestinal surface during development. It may be assumed that the absence of receptor expression for both Shiga toxin and *C. difficile* toxin A is due to the underdevelopment of glycosyltransferases involved in the synthesis of Gb3 and of nLcCer as proposed in Figure 2. However, additional studies on developmental changes of these glycolipid glycosyltransferases in the gastrointestinal tract are needed to prove this hypothesis.

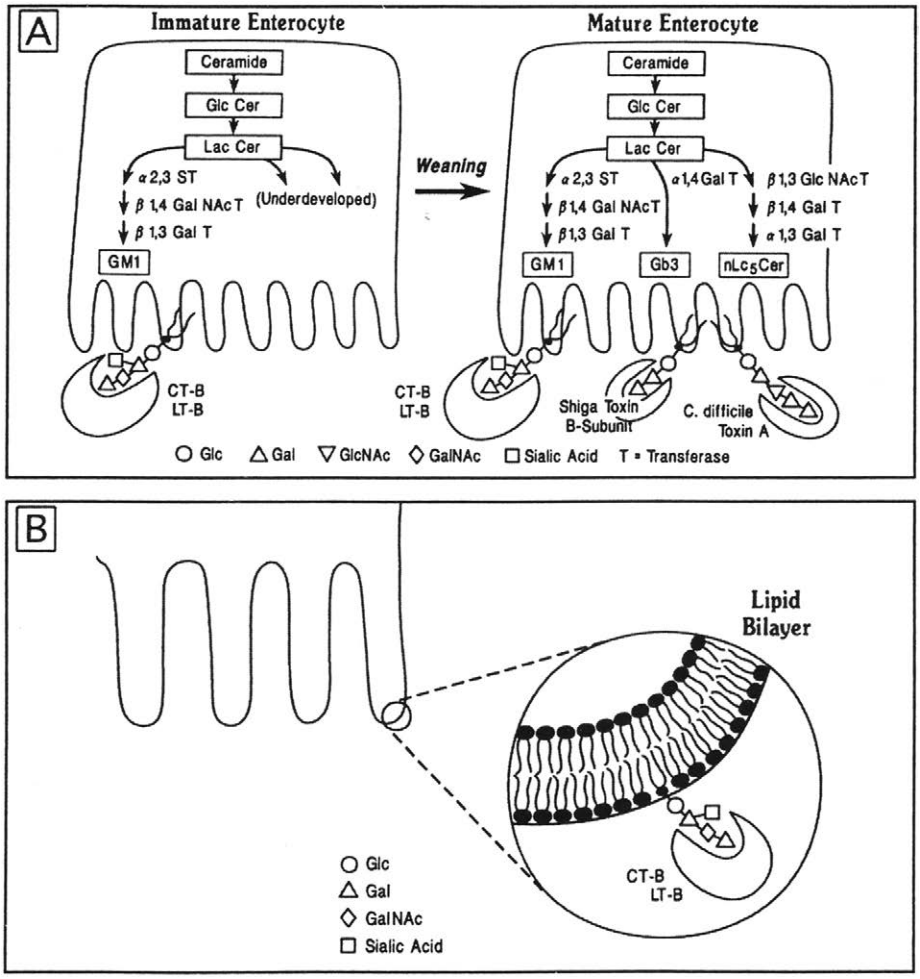

Figure 2: A proposed model for the control of glycolipid biosynthesis and toxin receptor expression during enterocyte development.
A. Immature vs. mature enterocytes
B. Detail of microvillus membrane showing expression of toxin receptor on lipid bilayer.

External factors that may influence developmental regulation

Alterations in the carbohydrate structures of glycolipids can be attributed to the variable level of glycosyltransferase activity during development. However, the glycoconjugate patterns formed through activity of glycosyltransferases in tissues may vary extensively depending on a number of external factors. These include the presence of chemical stimuli such as phorbol ester (*Ozaki* et al., 1989) and sodium butyrate (*Simmons* et al., 1975), or nutritional factors such as a vitamin A deficiency (*DeLuca* et al., 1970), dietary composition changes (*Biol* et al., 1984), or hormone stimuli such as cortisone (*Ozaki* et al., 1989; *Sato* et al., 1984) and thyroxin (*Sato* et al., 1984) administration, as well as the stimuli derived from bacterial colonisation itself (*Ozaki* et al., 1988; *Umesaki* et al., 1982; *Lucas* et al., 1989). These external factors may affect the developmental signals which modulate the

control of gene expression of glycosyltransferases probably at the transcriptional level (*Chu* et al., 1990; *Paulson* and *Colley*, 1989). On the other hand, other regulatory mechanisms may operate through the regulation of substrate availability as well as through the phosphorylation-dephosphorylation regulation (*Burczak* et al., 1984) of enzyme activities responsible for specific sugar sequences. Thus, intestinal microflora and nutritional conditions may modulate regulatory mediators that regulate the neonatal expression of bacterial receptors during development. However, these observations need more definitive studies to establish the specific mechanism of developmental regulation.

CONCLUSIONS AND SPECULATION

The molecular nature of membrane receptors for bacterial cells and toxins on intestinal epithelial cells points to the importance of the intestinal surface-carbohydrate expression in host-pathogen interactions. With the improved techniques for characterising receptor binding and receptor biochemical structure, the availability of several human intestinal epithelial cell lines (e.g. T84, HT-29, Caco-2) and of carbohydrate-specific monoclonal antibodies, we may identify additional membrane receptors and the receptor sugar sequences in the near future. Subsequent studies on the intestinal expression and developmental regulation of individual glycosyltransferases responsible for the addition of receptor sugar sequences should be pursued. By understanding the molecular nature of bacterial receptors in the intestine, developmental programming and environmental influence on receptor expression, and biological significance in neonatal host defences, new approaches may soon be available in the prevention and treatment of young infants with bacterial diseases.

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