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Susceptibility of infant mice to F5 (K99) *E. coli* infection: differences in glycosyltransferase activities in intestinal mucosa of inbred CBA and DBA/2 strains

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Enterotoxigenic *Escherichia coli* (ETEC) strains expressing F5 (K99) fimbriae cause diarrhoea in the young animal through adhesion to specific sialoglycolipids of the small intestine surface. We studied here an infant mouse diarrhoea model, as CBA infant mice are susceptible to F5-positive ETEC infection, whereas DBA/2 ones are resistant. In an attempt to determine an enzymatic basis for susceptibility and resistance, we investigated the intestine ganglioside pattern in relation to the activity of glycosyltransferases responsible for the globo- and ganglio-series. We observed that the intestine of susceptible CBA infant mice displayed a characteristic sialoglycolipid pattern containing mainly the F5 receptors. The two murine strains differed in the relative activities of galactosyltransferases ($GbOse_3Cer$ and GM1 synthases), *N*-acetylgalactosylaminyltransferases (GA2 and GM2 synthases) and sialyltransferases (GM3 and GD3 synthases). An elevated GM3-synthase activity was observed in the intestine of susceptible CBA infant mice, at the age of high susceptibility. Hence, we conclude that the marked specificity of mouse type correlated with susceptibility and resistance to F5-positive ETEC infection which could be controlled through the regulation of glycosyltransferase activities.

Keywords: Enterotoxigenic *E. coli*, F5 positive-ETEC, inbred mice, glycosyltransferases, glycolipids, intestine

Abbreviations: NeuAc, *N*-acetylneuraminic acid; NeuGc, *N*-glycolylneuraminic acid; Glc, glucose; GalNAc, *N*-acetylgalactosamine; Gal, galactose; Cer, ceramide; LacCer, lactosylceramide (Galβ1-4Glcβ1-1Cer); GA2, asialo-GM2 ($GgOse_3Cer$); GA1, asialo-GM1 ($GgOse_4Cer$); NeuAc/NeuGc-GM1a, II^3 NeuAc/NeuGc-$GgOse_4Cer$; NeuAc/NeuGc-GM1b, IV^3 NeuAc/NeuGc-$GgOse_4Cer$; NeuAc/NeuGc-GM2, II^3 NeuAc/NeuGc-$GgOse_3Cer$; NeuAc/NeuGc-GM3, II^3 NeuAc/NeuGc-LacCer; NeuAc/NeuGc-GD1a, IV^3 NeuAc/NeuGc, II^3 NeuAc/NeuGc-$GgOse_4Cer$; NeuAc/NeuGc-GD1b, II^3 $(NeuAc/NeuGc)_2$-$GgOse_4Cer$; NeuAc/NeuGc-GD1c, IV^3 $(NeuAc/NeuGc)_2$-$GgOse_4Cer$; NeuAc/NeuGc-GD2, II^3 $(NeuAc/NeuGc)_2$-$GgOse_3Cer$; NeuAc/NeuGc-GD3, II^3 $(NeuAc/NeuGc)_2$-Lac Cer; NeuAc/NeuGc-GT1a, IV^3 $(NeuAc/NeuGc)_2$, II^3 NeuAc/NeuGc-$GgOse_4Cer$; NeuAc/NeuGc-GT1b, IV^3 NeuAc/NeuGc, II^3 $(NeuAc/NeuGc)_2$-$GgOse_4Cer$; NeuAc/NeuGc-GT1c, II^3 $(NeuAc/NeuGc)_3$-$GgOse_4Cer$; NeuAc/NeuGc-GT2, II^3 $(NeuAc/NeuGc)_3$-$GgOse_3Cer$; NeuAc/NeuGc-GT3, II^3 $(NeuAc/NeuGc)_3$-Lac Cer; NeuAc/NeuGc-GQ1b, IV^3 $(NeuAc/NeuGc)_2$, II^3 $(NeuAc/NeuGc)_2$-$GgOse_4Cer$; NeuAc/NeuGc-GQ1c, IV^3 NeuAc/NeuGc, II^3 $(NeuAc/NeuGc)_3$-$GgOse_4Cer$; NeuAc/NeuGc-GP1c, IV^3 $(NeuAc/NeuGc)_2$, II^3 $(NeuAc/NeuGc)_3$-$GgOse_4Cer$.

GD, GT and GQ: di-, tri- and tetra-sialoglangliosides. NeuGc-SPG, IV^3 NeuGc-$nLcOse_4Cer$. Glycosyltransferases assayed in this work are *N*-acetylgalactosaminyltransferases, UDP-GalNAc:lactosylceramide β1-4 *N*-acetylgalactosaminyltransferase or GA2 synthase (EC 2.4.1-) and UDP-GalNAc:(*N*-acetylneuraminyl)-lactosylceramide β1-4 *N*-acetylgalactosaminyltransferase or GM2 synthase (EC 2.4.1.92); sialyltransferases, CMP-*N*-acetylneuraminate: lactosylceramide α2-3 sialyltransferase (sialyltransferases I and IV) or GM3 synthase (EC 2.4.99.-) and CMP-*N*-acetylneuraminate:(*N*-acetylneuraminyl) lactosylceramide α2-8 sialyltransferase (sialyltransferase II) or GD3 synthase (EC 24.99.8); galactosyltransferases, UDP-galactose: *N*-acetylgalactosaminyl-(*N*-acetylneuraminyl) lactosylceramide β1-3 galactosyltransferase (galactosyltransferase II) or GM1a synthase (EC 2.4.1.62) and UDP-galactose:lactosylceramide α1-4 galactosyltransferase or $GbOse_3Cer$ synthase (EC 2.4.1-).

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Introduction

Enterotoxigenic *Escherichia coli* (ETEC) strains expressing F5 (K99) fimbrial adhesins cause diarrhoea in newborn piglets, calves and lambs. The adherence to epithelial cells is an important event, and sialic acids residues occupying terminal positions in sialoglycolipids and sialoglycoproteins are essential for the binding of F5 (K99) fimbriae to the intestinal mucosa [1–6]. Recent data has shown that the small intestine of neonatal calves contain mainly sialoparagloboside (SPG) and gangliosides NeuAc-GM3, NeuGc-GM3, GM2, GM1 and GD1 [7]. Previous studies from our laboratory have established the existence of two phenotypes of piglets based on the susceptibility of their enterocytes to adhesion of F5-positive ETEC and the distribution of sialoglycolipids [4]. The small intestine surface of susceptible piglets contains a higher content of the receptive monosialoglycolipids (NeuGc-GM3 and NeuGc-SPG) and oligosialo-gangliosides such as GD1b and GT1b when compared with the gangliosides NeuAc-GM3 and GM2 of non-receptive piglets.

Nevertheless, it remains to establish the molecular basis of the susceptibility to colibacillosis, and to determine if the control of expression, or the activity of the enzymes involved in their biosynthesis could be a possible basis for the elaboration of the F5-receptive animal phenotype. Hence, we used an infant mouse ETEC diarrhoea model [8–10]. The CBA and DBA/2 murine strains are attractive to use as a diarrhoea model with ETEC strains of bovine and porcine origin, because CBA infant mice are highly-susceptible to positive-K99 ETEC and DBA/2 exhibit complete resistance to the same ETEC strains [11]. Thus, we have studied the intestinal glycosphingolipids of these well defined experimental models to investigate the gangliosides in relation to glycosyltransferase activities. It has already been shown that the ganglioside biosynthesis is processed in Golgi apparatus where glucosylceramide is glycosylated by the sequential addition of galactose, *N*-acetylgalactosamine and sialic acid [12, 13]. The synthesis of gangliosides starts with lactoceramide, and gangliosides can be divided into several groups [13, 14], according to their biosynthetic pathways (Fig. 1).

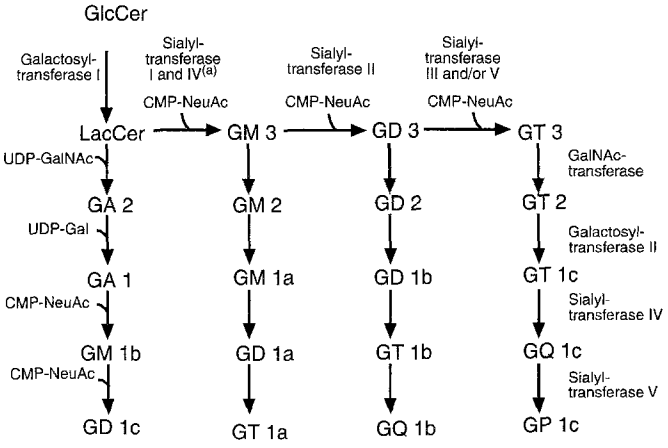

Figure 1. Scheme for ganglioside biosynthesis according to [13, 21, 22] obtained in rat liver Golgi. [(a)]Transfer of the same sugar to different glycolipid acceptors may be catalysed by the same enzyme. Sialyltransferase IV catalyses the transfer of sialic acid to galactose in α2-3 linkage; it converts both LacCer to GM3 and GA1 to GM1b, GM1a to GD1a and GD1b to GT1b [22].

Commonly used assay systems involve the determination of transfer of a radioactive sugar nucleotide to an acceptor. In the present study, we used crude supernatants of homogenized tissues as enzyme preparations [15–17], and glycolipids as acceptors, after separation by high-performance thin-layer chromatography [18].

Materials and methods

Animals

Inbred CBA and DBA/2 strains were obtained from IFA Credo (France). Animals were raised in an air-conditioned building with monitored lighting and were supplied with food and water *ad libitum*. Female mice were mated with males of the same strains at 6–8 weeks of age. Infant mice CBA (1.60 ± 0.14 g) and DBA/2 (1.10 ± 0.07 g) of 36-h-old, 6-w-old (15.6 ± 1.4 g and 14.5 ± 1.5 g, respectively) and 11-w-old mice of the two strains (16.5 ± 0.75 g and 15.3 ± 1.0 g, respectively), were fasted for 24 h before killing by decapitation after anaesthesia. The jejunum was sectioned (4 ± 0.2 cm and 3.5 ± 0.3 cm lengths in CBA and DBA/2 infant mice respectively; 12.4 ± 2 cm and 15.3 ± 2.6 cm in CBA and DBA/2 6-w-old mice respectively; 15 ± 0.6 cm and 16.6 ± 1.3 cm in CBA and DBA/2 11-w-old mice respectively). Jejunal fragments were rinsed with cold phosphate buffered saline, pH 7.4 (PBS). Tissues were longitudinally opened and immersed in Evans buffer pH 7.4 (5.6 mM KH_2PO_4, 1.5 mM KCl, 86 mM NaCl) containing 10 mM EDTA, for 15 min. Mucosa was gently scraped off and homogenized in Krebs Ringer bicarbonate buffer (KRB: 2.4 mM K_2HPO_4, 0.4 mM KH_2PO_4, 25 mM $NaHCO_3$, pH 7.4, 115 mM NaCl, 1.2 mM $MgCl_2$, 1.2 mM $CaCl_2$) with a Potter homogenizer (RW 20 DZM, Ikawerk). Homogenates were centrifuged at 1000 × **g** for 10 min, and supernatants were analysed for glycosyltransferase activities [16–18]. An aliquot was taken for protein determination and sugar asssays, and the remainder was kept at –80 °C. Protein determination was performed by the Lowry method. Sialylated and neutral sugars were assayed according to Svennerholm [19] and Rao [20], respectively.

Analytical methods

Scrapings were processed separately, glycolipids were

extracted with methanol and methanol:chloroform (2:1 then 1:2, v/v). Neutral and acidic glycolipids were separated by anion-exchange chromatography on DEAE-Sephadex A25 (Pharmacia) as previously described [4]. Two dimensional high-performance thin-layer chromatography was performed on Silica gel 60 (Merck). Gangliosides were separated using chloroform:methanol:water (60:35:8, by vol), 0.2% $CaCl_2$ (solvent A) in the first dimension and propan-1-ol:28% ammonia:water (75:5:25, by vol) in the second dimension. Glycolipids were visualized with orcinol-H_2SO_4 spray reagent and gangliosides by resorcinol-HCl. Spots were identified by comparing their migration to that of authentic reference markers isolated by us [4] or purchased from Sigma. Monosaccharide composition of glycolipids was analysed by gas chromatography.

F5-positive ETEC binding to intestinal glycolipids from various sources and pure sialoglycolipids were performed as previously described by us [4]. Briefly, gangliosides (from 1 to 10 µg of sialic acids) were chromatographed on thin-layer plates on solvent A. Plates were treated with 0.5% polyisobutylmethacrylate in diethyl ether followed by blocking of non-specific sites with 2% BSA in PBS. Then the plates were overlaid with ^{14}C-labelled *E. coli* B80 (1.6 kBq ml^{-1}), and the binding was assayed by autoradiography.

Assays of enzymes

Acceptors (GA3, NeuAc-GM3, NeuAc-GM2 from bovine brain; NeuGc-GM3 from horse erythrocytes) were chromatographed on thin-layer plates in solvent A. Plates were treated with 0.5% (w/v) polyisobutylmethacrylate (Plexigum P28, Rhöm) in diethyl ether, dried and stored at –20 °C until use. Glycosyltransferase assays were done on miniplates of 1 cm^2 at 37 °C for 30 min in a total volume of 100 µl containing enzyme solution (100 µg of protein) in KRB, 0.1% Triton X-100 [14, 17] and the activated sugar (20950 dpm in test). Uridine diphosphate galactose (UDP-Gal), uridine diphosphate-*N*-acetylgalactosamine (UDP-GalNAc) and cytosine monophosphate-sialic acid (CMP-sialic acid) (Sigma) were used to dilute uridine diphosphate-[U-^{14}C] galactose (12 GBq mol^{-1}), uridine diphosphate-[1-^{14}C] *N*-acetylgalactosamine (2.22 GBq mol^{-1}) and cytidine 5′-monophosphate *N*-acetyl-[4,5,6,7,8,9-^{14}C] neuraminic acid (11.5 GBq mol^{-1}) (Amersham). Incubations were initiated by the addition of enzyme to the isolated glycolipids. Optimal pH values were 6 for the sialyltransferase assay and 7.4 for the galactosyl- and *N*-acetylgalactosyl-transferases. The reaction was terminated followed by careful washing with PBS containing 0.2% Tween-80. Radioactivity incorporation was determined using a scintillation counter (LS 7000, Beckman). An endogenous acceptor control incubation, lacking exogenous glycolipid acceptor, was used for each set of assays. Endogenous and zero-time blanks were included for all assays and subtracted from the incorporated radioactivity. Activities were linear up to an incubation time of 60 min, and in the range of protein concentrations of 15–150 µg ml^{-1}. In the studies described below, relative glycosyltransferase activities were expressed as nmol of sugar transferred to the acceptor under the assay conditions (30 min).

Results

Relative affinity of binding of ETEC F5 to various sialoglycolipids

We estimated the binding of F5 positive ETEC to gangliosides obtained from different sources; the amounts

Table 1. Binding of ETEC F5 (K99) to purified glycolipids from the piglet and calf small intestine and from other sources

Glycolipid	*Binding*	*Source*
NeuGc-GM3	++++	Horse erythrocytes
	++++	Pig small intestine[a]
NeuGc-GD1b, NeuGc-GT1b	+++	Pig small intestine[a]
NeuGc-SPG	+++	Pig small intestine[a]
NeuGc-GM2	++	Pig small intestine[a]
NeuGc-GD1a	+	Calf small intestine
NeuAc-GM2	+	Bovine brain
NeuAc-GM1	+	Bovine brain
NeuAc-GM3	±	Human erythrocytes
NeuGc-GD3	–	Calf small intestine
NeuAc-GD3; NeuAc-GD1a,	–	Bovine brain
NeuAc-GD1b, NeuAc-GT1b	–	Bovine brain
LacCer	–	Bovine brain

Results were scored as (++++ and +++) for high, (++) moderate and (+, ±) weak F5 fimbriae affinities while (–) indicates no binding.
[a]Results are reproduced from previous work [4].

of these compounds in murine intestine were too small to use. As shown in Table 1, the *E. coli* F5 bound preferentially to glycolylated gangliosides NeuGc-GM3, NeuGc-GD1b, NeuGc-GT1b and NeuGc-SPG. Gangliosides GM3, GM2, GM1, GD3, GD1a, GD1b, GT1b containing an *N*-acetylated sialic acid and NeuGc-GD3, NeuGc-GD1a were weakly or not recognized by F5-positive *E. coli* strains.

Glycosylation of the intestinal tissue in CBA and DBA/2 murine strains

In the sensitive CBA mice, the concentration of intestine neutral sugars increased three-fold from the neonatal period ($172 \pm 13\ \mu g\ g^{-1}$ tissue) to weaning ($565 \pm 15\ \mu g\ g^{-1}$) and then remained high (Table 2). In the resistant DBA/2 mice, neutral sugars increased gradually from birth to adult age (100–522 $\mu g\ g^{-1}$ tissue).

The sialoglycoconjugate content was 1.6 times higher in new born CBA mice than in newborn DBA/2 mice (132 ± 6 and $82 \pm 14\ \mu g\ g^{-1}$ tissue, respectively; Table 2). Specifically, the sialylation of glycolipids (data not shown) was higher in intestinal tissues of newborn CBA mice than newborn DBA/2 mice, i.e. 88 $\mu g\ g^{-1}$ for CBA (66% of total sialic acids) and 41 $\mu g\ g^{-1}$ for DBA/2 (50% of total sialic acids).

The sialic acid content of intestine mucosa was age-dependent. It decreased in CBA mice (Table 2), whereas in DBA/2 intestines it increased at weaning and in adult animals, it was approximately the value observed at birth.

Postnatal changes in intestine sialoglycolipid patterns in the two murine strains

Each mouse strain presented a characteristic and complex pattern (Fig. 2A and B). First, intestinal mucosa of the two murine strains CBA and DBA/2 contained the neutral glycolipids Glcβ1-1Cer, Galβ1-1Cer and, LacCer which act as precursors for the biosynthesis of gangliosides. These glycolipids were in higher amounts in the intestinal tissue of weaned and adult mice than in newborn mice. Furthermore, the neutral globoside Galα1-4Galβ1-4Glc1-1Cer ($GbOse_3Cer$) was only detected in CBA intestine at birth.

In the neonatal period, CBA infant mice mainly expressed monosialylated ganglioside GM3 and di- and tri-sialylated gangliosides NeuGc-GD3, NeuGc-GD2, NeuGc-GD1 and NeuGc-GT1 (Fig. 2A). DBA/2 intestines contained gangliosides NeuGc-GD3, NeuGc-GM2, NeuGc-GD1a, and the complex sialoglycolipid GQ (Fig. 2B). In DBA/2 mice, only NeuGc-GM2 could be a receptor for F5 but the amount was very low and did not permit adhesion (results not shown). The sialoglycolipid pattern of the jejunum appeared to be age-dependent with a marked decrease of polysialylated gangliosides with age.

Postnatal changes in the relative activities of glycosyltransferases

The most striking finding to emerge from the glycosyltransferase measurements was that their relative activities in the two mice strains varied for enzymes involved in first steps of a- and b- series ganglioside and globoside pathways (Fig. 1). Sialyltransferases acting as GM3 and GD3 synthases, *N*-acetylgalactosaminyltransferases acting as GA2 and GM2 synthases and galactosyltransferases acting as $GbOse_3Cer$ and GM1 synthases had higher activities in the intestine of 36-h-old CBA mice than in DBA/2 mice.

Relative activites of sialyltransferases

At birth, GM3 synthase activity was significantly higher in infant mice of the CBA strain (4.6 ± 0.5 nmol sialic acid per mg tissue per 30 min). It decreased considerably in weaned and adult mice. GM3 synthase activity was only detected in adult DBA/2 mice (Fig. 3B). GD3 synthase activity was three times more active in the intestine from newborn CBA mice than in DBA/2 mice. This difference was the same whether NeuAc-GM3 or NeuGc-GM3 was used as an acceptor. In weaned CBA mice, GD3 activity decreased 1.5-fold and then could not be detected in adults (Fig. 3A). GD3 synthase was not detected in weaned or adult DBA/2 mice (Fig. 3B).

Relative activities of N-acetylgalactosaminyltransferases

GM2 synthase activity reached a maximum (7.4 ± 0.7 nmol GalNAc per g tissue per 30 min) at 6 weeks in CBA mice (Fig. 4A). In DBA/2 mice this enzyme was high (3.5 ± 0.5 and 2.2 ± 0.3 nmol GalNAc per g tissue per 30 min) at birth and at 11 weeks respectively but undetectable at 6 weeks of age (Fig. 4B).

GA2 synthase was high in newborn CBA mice (Fig. 4A), and the activity decreased with age. In DBA/2 mice,

Table 2. Amounts of neutral and sialylated sugars in intestinal glycoproteins and glycolipids

Age of animal	*Carbohydrate content in the jejunum*	
	CBA	*DBA/2*
Neutral sugars[a] ($\mu g\ g^{-1}$ dry tissue)[b]		
36 h	172 ± 13	133 ± 33
6 weeks	565 ± 15	264 ± 34
11 weeks	426 ± 26	463 ± 59
Sialic acids ($\mu g\ g^{-1}$ dry tissue)[b]		
36 h	132 ± 6	82 ± 14
6 weeks	113 ± 4	149 ± 41
11 weeks	60 ± 3	82 ± 46

[a]Neutral sugars (glucose, galactose, mannose residues) and sialic acid were assayed as described in Materials and methods.
[b]Mean value ± SD was determined by analysis of intestine from infant mice ($n = 16$ CBA and 20 DBA/2), 6-w-old mice ($n = 4$ CBA and 4 DBA/2, three females and one male) and 11-w-old mice ($n = 8$ CBA and 8 DBA/

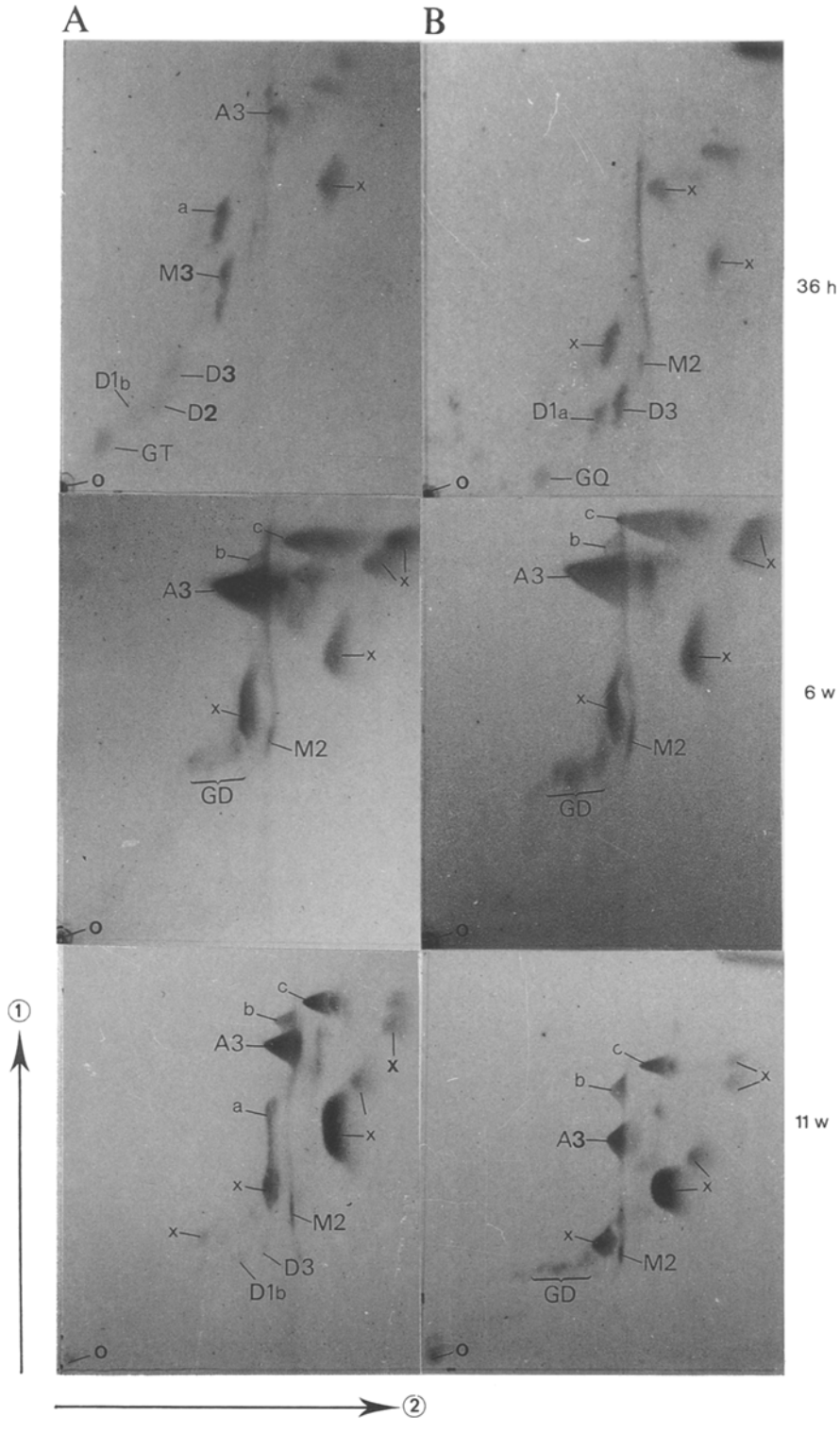

Figure 2. Two dimensional HPTLC of gangliosides extracted from the proximal jejunum of CBA (A) and DBA/2 mice (B), in neonates (36 h), at weaning (6 w) and in adults (11 w). Glycolipids (5 nmol of sialic acid) were spotted at origin (o) of the plate, which was developed twice with solvent A (chloroform:methanol:water, $CaCl_2$) in the first dimension and then solvent B (propan-1-ol:ammonia:water) in the second dimension. Each of the spots was estimated from their mobility relative to those of standard gangliosides and neutral glycolipids. A3, M3, M2, D3, D2, D1a, D1b, GT, GQ correspond to GA3, NeuGc-GM3, NeuGc-GM2, NeuGc-GD3, NeuGc-GD2, NeuGc-GD1a, NeuGc-GD1b, NeuGc-GT, NeuGc-GQ. Spots (a, b and c) correspond to neutral glycolipids $GbOse_3Cer$, Glcβ1-1Cer and Galβ1-1Cer respectively. (×) corresponds to an unidentified compound which is not coloured purple by orcinol spray reagent.

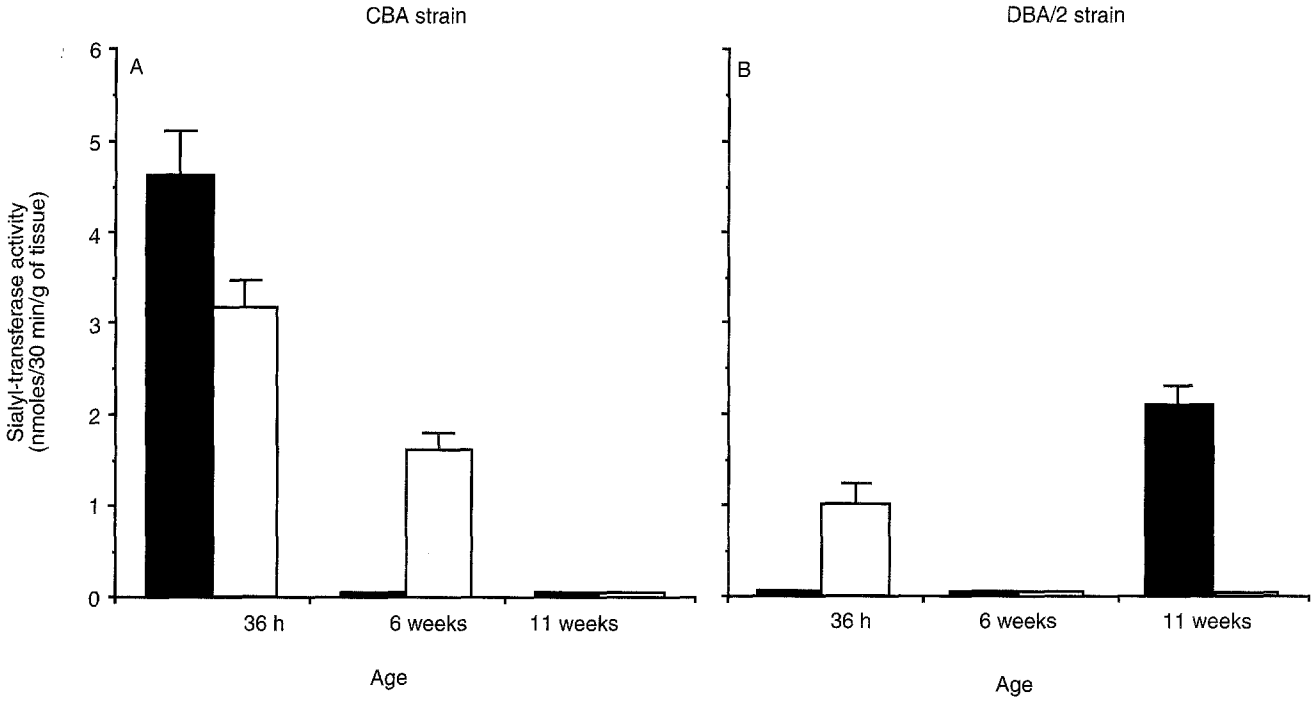

Figure 3. Activities of GM3 (■) and GD3 (□) synthases in small intestine of mice of 36-h-, 6-w- and 11-w-old CBA and DBA/2 mice (A and B). The data are given as mean values of five measurements.

GA2 synthase was undetectable in newborn mice but present in weaned and adult mice (Fig. 4B).

Relative activities of galactosyltransferases

Synthesis of GM1 from GM2 was important at the neonatal and weaning stages of CBA mice as shown by the high GM1 synthase activities (5.9 ± 0.4 and 2.3 ± 0.3 nmoles Gal per g tissue per 30 min respectively, Fig. 5A), but it was not detected in the DBA/2 mice at these times (Fig. 5B).

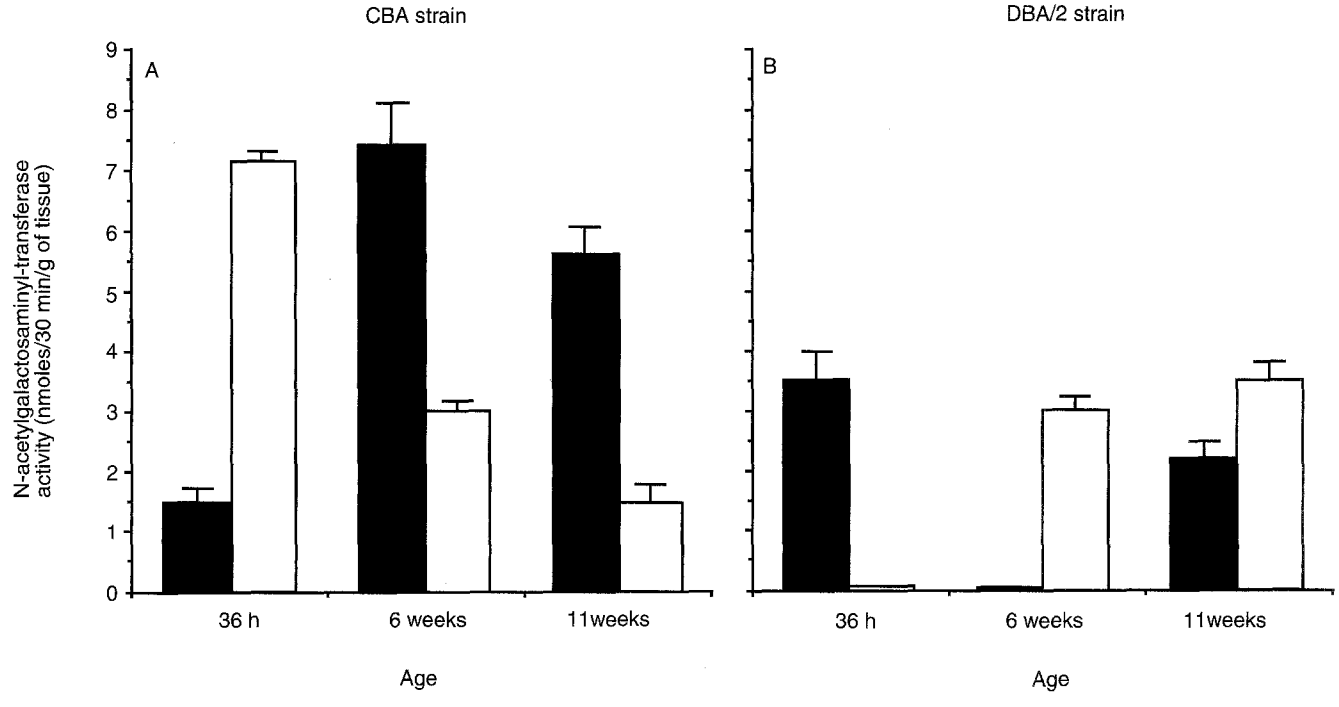

Figure 4. Activities of GM2 (■) and GA2 (□) synthases in small intestine of mice of 36-h-, 6-w- and 11-w-old CBA and DBA/2 mice (A and B). The data are given as mean values of five measurements.

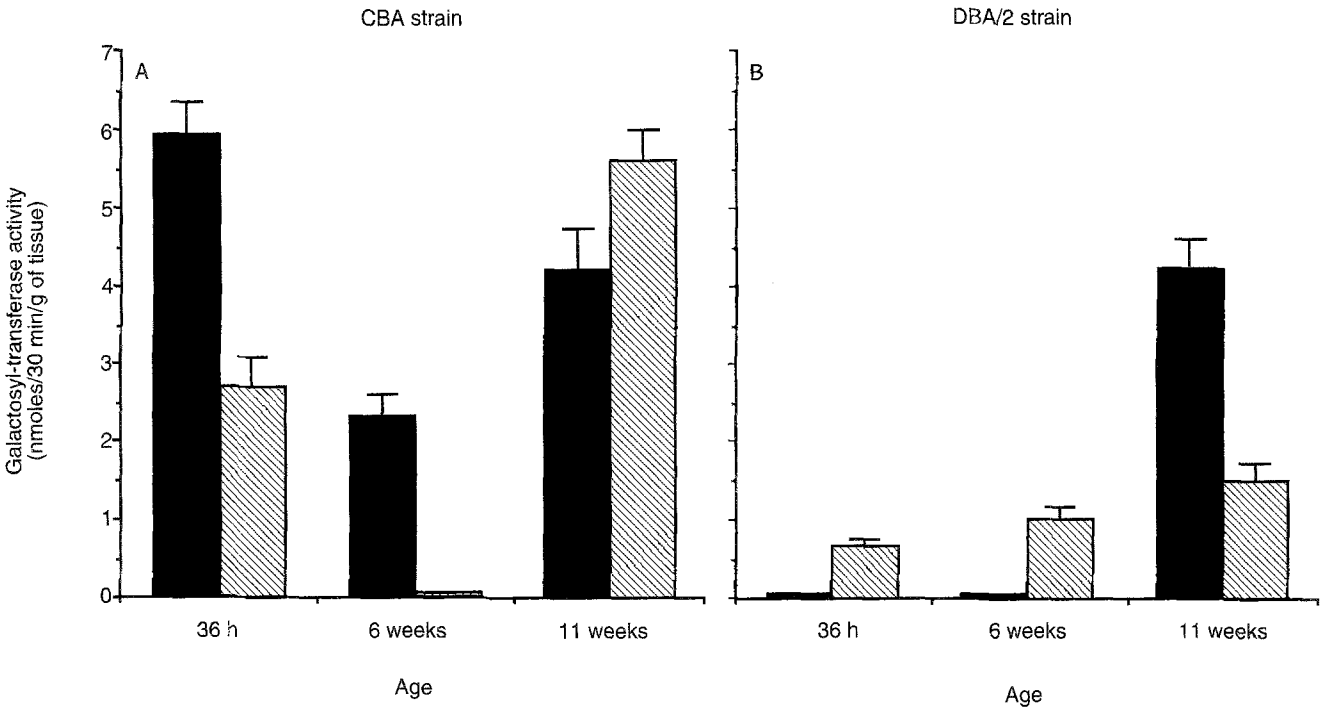

Figure 5. Activities of GM1 (■) and $GbOse_3Cer$ (▨) synthases in small intestine of mice of 36-h-, 6-w- and 11-w-old CBA and DBA/2 mice (A and B). The data are given as mean values of five measurements.

In CBA mice, 2.7 ± 0.4 and 5.6 ± 0.4 nmol of $GbOse_3Cer$ were generated from GA3 by $GbOSe_3Cer$ synthase, at the neonatal and adult ages. $GbOse_3Cer$ synthase activity was low in newborn DBA/2 mice and higher in older animals.

Discussion

First, we identified two ganglioside patterns in the intestine of CBA and DBA/2 murine strains, showing that these inbred strains belonged to two different phenotypes. As all inbred murine strains exclusively express NeuGc-containing gangliosides [23], the difference was not due to NeuAc/NeuGc polymorphism. We established that the ganglioside composition in the intestine of the resistant DBA/2 strain included NeuGc-GM2 and other various a-series gangliosides, whereas the sensitive CBA strain intestine contained NeuGc-GM3 and b-series gangliosides.

It has already been observed that the ganglioside expression varies with murine strains. GM2 is expressed as the sole major ganglioside in the liver of the DBA/2 strain (defined as negative GM1 type), whereas in some other strains such as SWR/J and SJL, GM1 and GD1a are expressed in addition to GM2 [23]. Moreover, it has been shown that asialo-GM1 is not expressed in intestinal epithelial cells of DBA/2 mice [24].

The ganglioside GM3 plays a remarkable role in ganglioside metabolism, it acts as glycolipid substrate for three enzymes, GM2 synthase, GD3 synthase and sialidase. Hence, variations in gangliosides may be due to either glycosyltransferases responsible for the glycosylation and/or degrading enzymes such as nucleotide pyrophosphatases, oxidases and proteases [25].

We have chosen to focus on the glycosyltransferases participating in ganglioside biosynthesis to understand the basis for variations in ganglioside pattern. We demonstrated significant modifications in the activities of enzymes responsible of intestine glycolipid glycosylation. Synthesis of the active-receptor, NeuGc-GM3, for the F5-positive *E. coli*, and high sialyltransferase activity were mainly detected in the CBA strain, just after the birth; a period of maximal susceptibility of the young animal to the colibacillosis. The elevated GM3-synthase activity in the CBA strain could be related to the sensitive phenotype, and might contribute to susceptibility to the F5-positive ETEC infection.

Nakamura *et al.* [26] have established polymorphic variations of ganglioside expression in erythrocytes and liver of several inbred strains of mouse in relation to genetically determined differences in the activity of certain glycosyltransferases. Duchet-suchaux *et al.*, using oral challenge and mating experiments, indicated that resistance of infant mice of DBA/2 strain is genetic [11], and dominant transmission of this characteristic may be

explained either by mixed control with a overdominant gene, or by a polygenic control with a large heterosis effect [27]. As GM3 synthase with either GD3 synthase or GM2 synthase is implicated in the synthesis of the b and a ganglioside series respectively, it is difficult to suggest which enzyme is defective in the recessive phenotype. Hence, further investigations are needed.

An age-dependent change in ganglioside expression occurred in the intestine as previously observed in murine liver [26] and rat intestine [28, 29]. CBA and DBA/2 variations in the glycosylation of intestine surface glycolipids could indicate a possible strain-type time-course of maturation, which could be related to the regulation of the expression of gangliosides. This hypothesis is supported by the evidence obtained on rat species, that cellular differentiation is mediated by the sialylation level of intestinal brush borders [29]. Hence, age-related modifications in sialylation of intestinal mucosa could be explained, in part, by the level of activity of sialyltransferases.

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