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Fecal excretion of intestinal glycosphingolipids by newborns and young children

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Glycosphingolipids were shown to persist in human fecal excretions from birth up 2 years of age. The pattern of glycosphingolipids was dependent on blood group and secretor status of the child and changed dramatically during the first months of life. Perinatally cerebroside, hematoside and blood group active fucolipids were dominating among fecal glycolipids. From the time of weaning lactosylceramide abruptly became and then persisted as a dominating glycolipid although cerebroside, complex gangliosides and blood group active fucolipids could still be detected in feces even at 2 years of age.

Blood group; Glycosphingolipid; Bacterial receptor; Weaning; (Human meconium, Feces)

1. INTRODUCTION

Blood group ABH-activity found in human meconium [1] was shown to rapidly diminish during the first few days of life [2]. The initial activity found in the water soluble mucin fraction was thought to be degraded by bacterial glycosidases as later illustrated on defined mucins in a series of papers by Hoskins et al. ([3,4] and references therein). More recently, however, blood group A-active oligosaccharides have been isolated in large quantities from feces of a breast-fed blood group A secretor infant [5].

Human meconium has been shown to be rich in blood group active glycosphingolipids reflecting the patterns of fetal intestinal cells [6]. After discovering glycosphingolipids. to be potential receptors for bacteria [7], an ecological and protective role for these substances in the meconium at the time of colonization was suggested [8]. Thus, by a specific binding of bacteria to luminal glycosphingolipids the uncontrolled infiltration and colonization of the intestinal epithelium could possibly be reduced and delayed until the protective factors of human colostrum and milk, i.e. bifidus factors, sectory IgA, lymphocytes and phagocytic cells reached their destinations in the gut [9].

Recently, we have found that glycosphingolipids excreted in the feces of conventional rats were enriched in lactosylceramide, depleted in hematoside (GM3) but contained all the blood group A-, B-, and H-activities of the intestinal cells of these rats and also of the feces of germ-free rats of the same strain [10]. The accumulation of lactosylceramide was of particular interest since this specific glycolipid has been shown in vitro to bind several anaerobic bacteria of the indigenous intestinal flora and also several pathogens [11,12].

The present paper is, to our knowledge, the first report of a persistent glycosphingolipid excretion

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in feces of newborns and young children and serves to illustrate the relevance of studies on luminal glycosphingolipids.

2. MATERIALS AND METHODS

Feces was collected from one child, M.L., at 1, 2, 3, 4 and 5 months of age and from another child, J.H., at 23 months of age. M.L. was completely breast-fed during the first 2 months, then fed on a mixed diet with decreasing amounts of breast milk for 3 months and with less than 5% being breast milk at 5 months of age. J.H. was taking only solid food. Feces samples were frozen (−20°C) immediately after defecation. Reference intestinal samples were taken at autopsy from a full term child who died at 2 weeks of age from a mechanical respiratory insufficiency. Epithelial cells and stromal residue of small intestine were obtained through a stepwise washing procedure [13]. Corresponding epithelial and stromal fractions from the large intestine were obtained by scraping the mucosa after a careful rinsing of the colon. Glycosphingolipids were isolated from lyophilized samples and wet cells as described [13,14].

The preparations and antigenic specificities of the mouse monoclonal antibodies CO 19-9 and CO 514 have been described [15,16] as has the overlay technique for immunostaining of glycosphingolipids [17,18]. Thin-layer chromatography was performed on HPTLC plates (Si60, Merck, Darmstadt) using glass support for chemical detection (anisaldehyde [19] or resorcinol [20]) and aluminia support for immunostaining with solvents chloroform/methanol/water, 60:35:8 (v/v), for non-acid glycolipids and chloroform/methanol/0.2% $CaCl_2$, 60:40:9 (v/v), and chloroform/methanol/2.5 M NH_3, 60:40:9 (v/v), for acid glycolipids. Blood group typing of M.L. was done on meconium glycosphingolipids [21].

3. RESULTS

The dry weights of the six collected feces samples varied from maximum 10.0 g (1 month) to minimum 0.8 g (5 months) and the yields of total non-acid glycolipids obtained from the feces during the 2 year period varied between 6.8 and 15.0 mg/g dry tissue. The child M.L. was typed as a blood group B Lewis$^{(a+b-)}$ non-secretor individual. The pattern of non-acid glycosphingolipids in the feces during her first breast-fed months was similar to that found in the meconium [21] of a blood group A Lewis$^{(a+b-)}$ non-secretor individual (fig.1, lanes A, 1 and 2). Thus, cerebroside and a pentaglycosylceramide (fig.1) were the dominating neutral glycosphingolipids in these excretions. Qualitative differences between the meconium and feces samples were seen mainly in the region of di- to tetra-glycosylceramides. This general pattern of two major neutral glycolipid species was also seen in the epithelial cell fractions from the autopsy material (fig.1, lanes ES and EL) suggesting the same non-secretor character of this individual. Minor differences between the glycolipid patterns of the small and large intestines (lanes ES and EL) were observed and also reflected in fecal fractions (lanes 1 and 2). For the small intestine no qualitative chemical differences were found between glycosphingolipids from villus tip and crypt cells (not shown) but as for the large intestine marked differences were found between epithelial cells and residual mesenchymal stroma (fig.1). At the time of weaning, when solid food was introduced, the more complex glycosphingo-

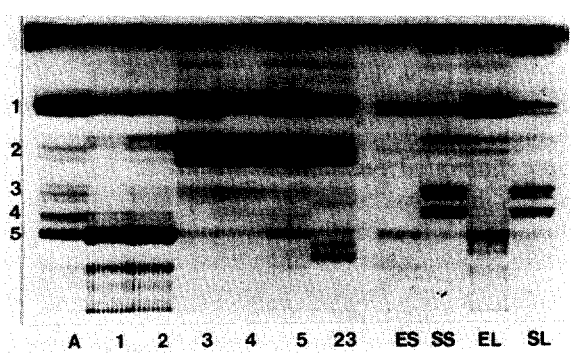

Fig.1. Thin-layer chromatogram of total non-acid glycolipids of the meconium of a blood group A Le$^{(a+b-)}$ non-secretor (lane A), of feces of the child M.L. at 1 (lane 1), 2 (lane 2), 3 (lane 3), 4 (lane 4), and 5 (lane 5) months and the child J.H. at 23 (lane 23) months of age, and of epithelial cells (lane ES) and residual stroma (lane SS) of small intestine and of large intestine (lanes EL and SL) from a perinatally diseased child. For the feces samples the amount spotted on each lane corresponds to 4 mg of dry feces. Figures to the left indicate the number of monosaccharide residues in the glycolipid carbohydrate chains.

lipids were generally diminished (fig.1) and simultaneously three strong bands of lactosylceramide appeared (fig.1). No increase in cerebrosides was observed. During the following months lactosylceramide remained a dominating glycolipid in the feces although quantitative changes of more slow moving glycolipids were seen. Also in the feces from J.H. (2 years old) lactosylceramide was a major glycolipid but the pattern of blood group active glycolipids was different here (fig.1, lane 23) and similar to that of the meconium of a blood group B Lewis$^{(a-b+)}$ secretor [22].

The general patterns of gangliosides of the various feces and tissue samples are illustrated in fig.2. Hematoside, GM3, and a slower moving ganglioside were dominating in feces during the first months of life. From the time of weaning GM3 was gradually lost while the other major ganglioside was secreted unchanged for an additional few months before its concentration in the feces also diminished. The fast-moving resorcinol-negative band seen in all the fecal fractions (fig.2) was identified using the meconium references, various solvent systems and the anisaldehyde reagent [23] as a mixture of cholesterol sulphate (CS) and sulphatide (S). Gangliosides were generally scarce in the small and large intestinal epithelial cells but enriched in the stromal fractions. These showed similar patterns for both acid and non-acid glycolipids along the intestinal tract.

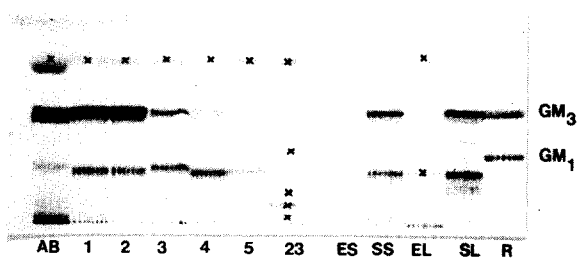

Fig.2. Thin-layer chromatogram of acid glycolipids of the feces and intestinal tissue samples of fig.1. Lane AB corresponds to the acid meconium fraction of a blood group AB child and lane R contains GM3 and GM1 of human brain. The amount spotted on each lane of the feces samples corresponds to 4 mg of dry feces. The plate was sprayed with the resorcinol reagent [20]. The bands marked with an x did not show the characteristic blue colour of gangliosides.

Using the blood group Lewis^a-specific monoclonal antibody (CO 514) [16] and the sensitive antibody overlay technique [16,17], the neutral pentaglycosylceramide of feces samples of child M.L. was shown to contain the Lewis^a-determinant. This determinant was also detected on glycolipids with 7 and 9 or more monosaccharide residues per ceramide and the quantitative changes with time were in agreement with those found using chemical detection. Lewis^a-active pentaglycosylceramides were also detected in the meconium references of the blood group A Lewis$^{(a+b-)}$ non-secretor (lane A) and the blood group B Lewis$^{(a-b+)}$ secretor (lane B) as well as in feces of child J.H. at 2 years of age (lane 23). The fecal excretion of the sialylated Lewis^a glycolipid (the tumor-associated 19-9 antigen) is shown in fig.3B. In meconium a very high activity was seen but after birth there was a gradual loss of this ganglioside in the feces. At 2 years only minute amounts were excreted from the intestines of the Lewis positive secretor positive individual.

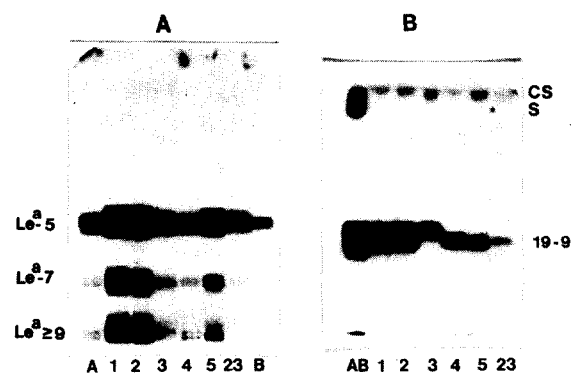

Fig.3. Autoradiograms of non-acid (panel A) and acid (panel B) glycolipids of feces of child M.L. at 1 (lanes 1), 2 (lanes 2), 3 (lanes 3), 4 (lanes 4) and 5 (lanes 5) months and J.H. at 23 (lanes 23) months of age after detection with Le^a specific antibody (CO 514) and with ganglioside 19-9 specific antibody (CO 19-9). References used were meconium samples of a blood group A Le$^{(a+b-)}$ non-secretor (lane A), a blood group B Le$^{(a-b+)}$ secretor (lane B) and a blood group AB child (lane AB). Feces samples correspond to 2 mg dry tissue for non-acid glycolipids and 4 mg of dry tissue for acid glycolipids. Abbreviations at the sides indicate specifically stained glycolipids. The strong staining of a front band with CO 19-9 was due to cholesterol sulphate (CS) and sulphatide (S).

4. DISCUSSION

We have shown for the first time that both acid and non-acid glycosphingolipids are excreted in the feces of humans from birth up to at least 2 years of age. The feces contains the final products of a very long series of events taking place within the gastrointestinal tract and the dynamics of fecal glycosphingolipids are now being studied. The problems of blood group individuality, stage-specific synthesis, luminal extrusion and bacterial degradation and contamination must be considered. The illustrated induction of lactosylceramide in the feces at the time of weaning was probably an effect of the microbial flora (unpublished) and is interesting with respect to the possible role for this glycolipid as a receptor for luminal bacteria [8,11,12]. The excretion of non-degraded glycosphingolipids in feces might be of value for the early post-natal diagnosis of sphingolipidoses and if excretion persists in adulthood for the diagnosis of both inflammatory and neoplastic intestinal diseases.

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