MOLECULAR CHARACTERIZATION OF CELL-SURFACE ANTIGENS OF HUMAN FETAL TISSUE

Meconium, a rich source of epithelial blood-group glycolipids

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1. Introduction

Molecules of many different classes, proteins [1], glycoproteins [2], oligosaccharides [3] and glycolipids [4], display blood-group activity. The sparse occurrence of blood-group-active glycolipids in the erythrocyte membrane [5], the classical object of study, has hampered more precise chemical studies. The discovery [6] of much larger amounts of complex glycolipids in the gastro-intestinal tract has intensified research on their chemical structure [7,8] and on the relation of these glycolipids to cell differentiation [9].

During fetal development, epithelial cells lining the intestine are extruded into the intestinal lumen [10]. This process starts in gestation week 7, at the time of the first formation of villi, and continues until birth. This material constitutes a major part of the meconium, the first feces of the new-born child. Meconium has been shown to be immunologically active, including ABH, Lewis and MN activities, but few chemical investigations have been done [11–13] and so far none on glycolipids, although a lipid extract was found blood-group active [14].

The purpose of the present investigation was to find out the level of blood-group glycolipids in human meconium. Provided there is no degradation in the intestinal lumen before birth, a careful analysis should provide information on glycolipids from most of the fetal period.

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2. Materials and methods

Meconia were collected individually from newborns delivered by elective cesarian section at the Obstetric Clinic, Östra Sjukhuset, Göteborg. The blood groups were determined routinely on cord blood. The meconium material was stored at +4°C up to a week, and then lyophilized and kept at −20°C. The preparation of total glycosphingolipids followed conventional steps [15]. The detailed procedure, adapted to a large and a small scale, will be described separately (K.-A.K. to be published). The dried tissue was extracted with chloroform–methanol in a Soxhlet apparatus, and the non-acid glycolipids were isolated by mild alkaline degradation, DEAE-cellulose chromatography, and silicic acid chromatography of both native and acetylated [16] glycolipids. Thin-layer chromatography was done on small (10 × 20 cm) microanalytical plates (HPTLC-precoated silica gel 60 plates, Merck, Darmstadt). The reference glycolipids used were prepared in this laboratory.

The major tetraglycosylceramide was isolated from the intestinal content of a week 38 human fetus by silicic acid column chromatography of acetylated and native glycolipids, using increasing amounts of methanol in chloroform as eluant. The purified glycolipid was analysed by mass spectrometry on an MS 902 instrument (AEI Ltd, Manchester) as in [17].

Table 1

The weight (mg) of fractions in the isolation of glycosphingolipids of meconium of single individuals

Blood-group	A	B	O	O
Wet wt meconium	32×10^3	61×10^3	57×10^3	47×10^3
Dry wt meconium	7.0×10^3	13.3×10^3	12.5×10^3	10.3×10^3
Total extract	2.0×10^3	3.8×10^3	3.3×10^3	2.1×10^3
Cholesterol fraction	0.6×10^3	2.4×10^3	1.1×10^3	0.6×10^3
Sphingomyelin fraction	20	10	18	16
Total acid glycosphingolipids	178	224	144	163
Total non-acid glycosphingolipids	181	173	196	157

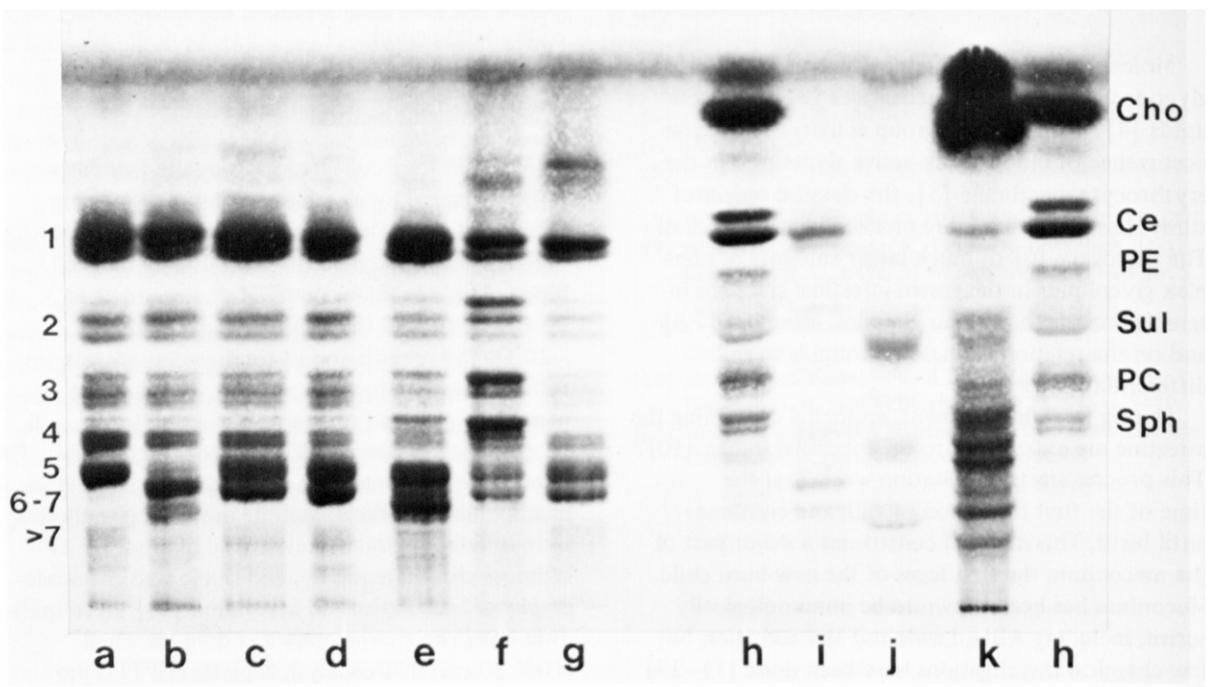

Fig.1. Thin-layer chromatogram of lipid samples of meconium or intestine of separate individuals. Samples (a–g) are 70 μg each of total non-acid glycosphingolipids of the following origins. (a–d): Samples are from meconia of blood-groups A (a), blood-group B (b) and blood-group O (c,d) individuals, respectively. (e): Sample is from whole intestine of a week 17 old fetus. (f,g) Samples are from intestinal wall and intestinal contents, respectively, of a week 38 fetus. The figures to the left indicate the number of sugars in the glycolipid molecules. The band inbetween location 3 and 4 and of higher intensity in fetal intestinal wall (f) represents globoside. All numbered bands were coloured green by the anisaldehyde reagent. Sample (k) is the total lipid extract of meconium of the blood-group B individual, and (i) and (j) are the non-acid and the acid glycolipids, respectively, obtained from double the amount of total lipids as shown in (k). The designations to the right of the reference sample (h) of human brain lipids stand for cholesterol (Cho), cerebrosides or monoglycosylceramides (Ce), ethanolamine phosphoglycerides (PE), sulphatides (Sul), choline phosphoglycerides (PC), and sphingomyelins (Sph). Chloroform/methanol/water, 60:35:8 (by vol.) was used as solvent and the anisaldehyde reagent [15] for detection.

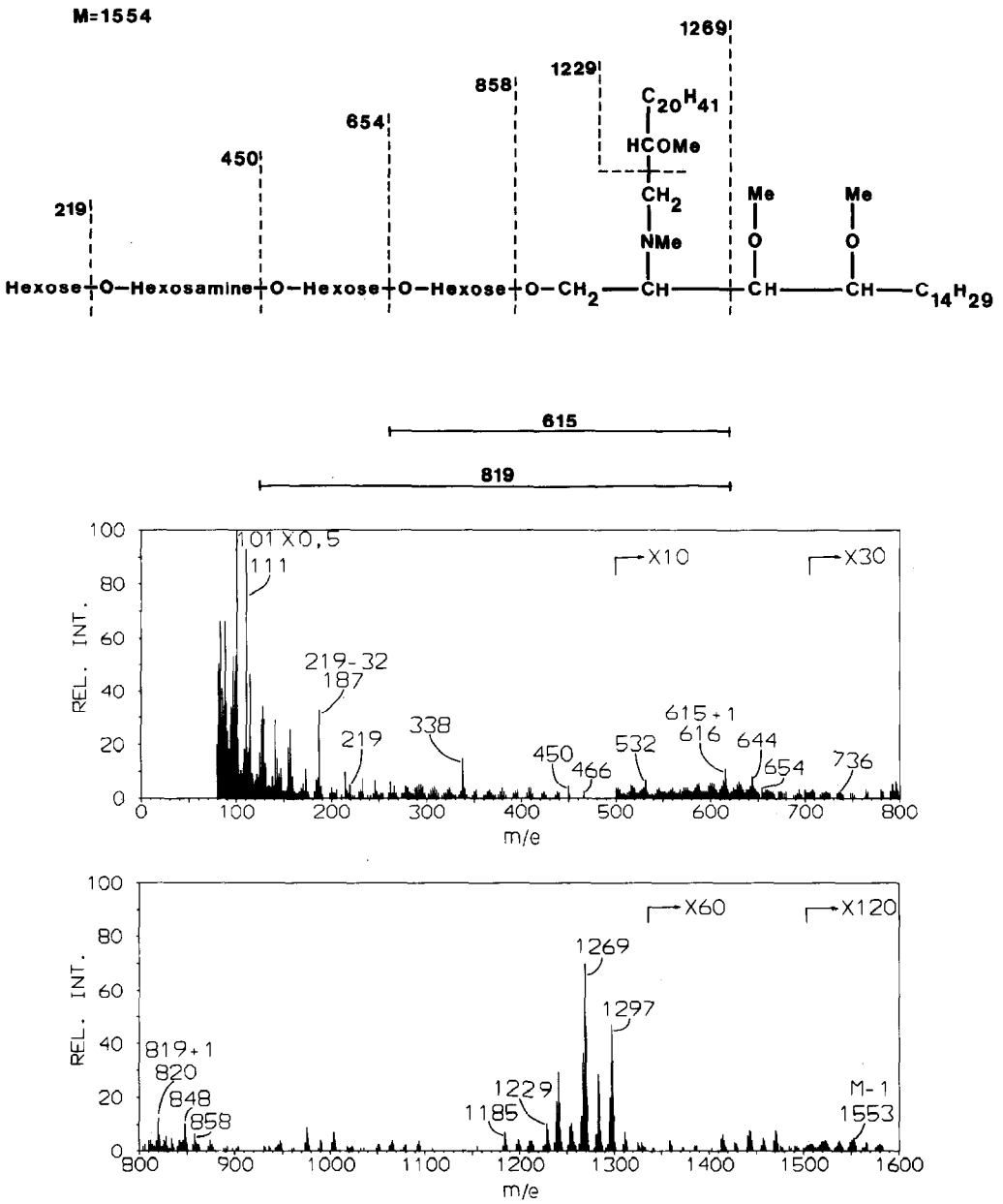

Fig.2. Mass spectrum of the methylated and reduced derivative [17] of a novel tetraglycosylceramide isolated from fetal intestine and corresponding to location 4 of fig.1. The glycolipid in the top formula was concluded from this spectrum and the spectrum of the methylated (not reduced) derivative (not shown), to be the major molecular species present. This was on the basis of known fragmentation patterns of these derivatives [17]. The relatively strong peaks at *m/e* 1185–1297 are diagnostic for the sugar and fatty acid compositions (three hexoses and one hexosamine, and 2-hydroxy fatty acids with 16–24 carbon atoms). The sugar sequence is evident from *m/e* 187, 219, 450, 466 (450+16), 654 and 858, but corresponding peaks are stronger for the methylated (not reduced) derivative. Evidence for phytosphingosine (trihydroxy base) as the major base are the ions at *m/e* 1229 and 1553, and several ions from the methylated (not reduced) derivative. The absence of *m/e* 182 in the spectrum of the methylated (not reduced) derivative is strong evidence for a type-1 saccharide chain [17], i.e., a hexose-1→3-hexosamine linkage. The conditions of analysis were: electron energy 46 eV, filament current 0.5 mA, acceleration voltage 4 kV, ion source temperature 300°C and probe temperature 200°C.

3. Results

Some preparative data on meconium of individuals of separate blood groups are collected in table 1, and a thin-layer chromatogram of selected fractions is shown in fig.1. The most abundant individual glycolipid was found in the acid fraction with a mobility and colour after detection similar to those of brain sulphatides. The high level of this lipid may be related to the demand on Na^+-dependent transport of intestinal epithelial cells [18]. The total non-acid glycolipid fraction varied between 157 mg and 196 mg, which is in the range of the corresponding fraction isolated from whole small intestine of adult humans ([18] and unpublished). The latter, however, which originated in both epithelial cells and intestinal wall tissue, was dominated by glycolipids with 3 and 4 (globoside) sugars [18]. Instead meconium contained relatively large amounts of slow-moving glycolipids with 5 or more sugars and a glycolipid moving just below globoside. The two major slow-moving spots of sample (g) in fig.1 were shown by mass spectrometry of a mixture [17] to be a monofucosyl-pentaglycosylceramide and a difucosyl-hexaglycosylceramide, respectively [17]. The glycolipid below globoside was isolated and shown by mass spectrometry (fig.2) to be a tetraglycosylceramide, preliminarily identified as lacto-N-tetraosylceramide, a glycolipid not detected before in human tissues and a probable precursor of blood-group fucolipids [2].

4. Discussion

Meconium is usually regarded as a mixture of desquamated intestinal and skin cells, red and white blood cells, lanugo hair, fatty material from vernix caseosa, amniotic fluid and various intestinal secretions [10]. The non-acid glycolipids, which show a rather small quantitative variation between individuals (table 1), are probably mostly derived from epithelial cells of the intestine (to be published), while cholesterol, with a larger variation (table 1), is probably mostly a skin product. The great heterogeneity of the meconium fraction is, however, of minor importance for the study of fetal glycolipid antigens. Noteworthy is the high level of fucolipids, which is about 2 times higher than for adult whole small intestine and about 50 times higher than for 1 unit (400 ml) fresh adult blood (unpublished data).

Although the novel tetraglycosylceramide could be a result of enzyme hydrolysis of more complex glycolipids, the absence of the corresponding triglycosylceramide with a terminal hexosamine (shown by mass spectrometry and gas chromatography) and the large amount of fucolipids present speak against this (see also [19]). As meconium is being produced during most of the fetal period, the glycolipids therefore should be an important source of transiently-expressed fetal antigens [20], if these exist among glycolipids. These may be of oncofetal type, cross-reacting with tumor-associated antigens and of probable relevance for immunological tumor therapy [20].

The blood-group activity of meconium was early shown to correspond to that of red cells of the same individual [11]. The findings reported here of very high levels of fucolipids in meconium are of importance for future chemical studies of blood-group polymorphism [2].

The detailed chemical structure of several of the glycolipids detected in meconium will be the subject of separate communications.

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