HUMAN BLOOD GROUP A-POSITIVE AND -NEGATIVE STRAINS OF RAT. CHEMICAL BASIS AS SHOWN BY FUCOLIPIDS OF SMALL INTESTINE

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1. Introduction

Experimental animals with cell surface antigens in common with man may be of importance for model studies in the field of tissue transplantation. With improved techniques of chromatography in combination with mass spectrometry it is now possible to reveal subtle differences in glycolipid antigens between single individuals [1–3]. In the present work this technique was applied on small intestine of two strains of rat. In one of these blood group A-type fucolipids and in the other H-type fucolipids were identified, corresponding to human blood group A and O activity, respectively. This is of interest since A-negative rats have not been reported before as far as known to us.

2. Materials and methods

Two inbred strains of rat were used, a white and black-white (hooded). The animals were sacrificed by decapitation after ether anesthesia and the small intestine was rapidly taken out. Lyophilized tissue was cut into small pieces and extracted in two steps in a Soxhlet apparatus, one day with chloroform–methanol 2:1 (by vol.) and one day with chloroform–methanol 1:9 (by vol.). The combined extracts were evaporated and subjected to mild alkaline degradation, dialysis, DEAE and silicic acid chromatography mainly as described [4]. To free the non-acid glycolipid fraction of alkali-stable phospholipids, acetylation and silicic acid chromatography were used [5].

The pure non-acid glycolipids were then fractionated as acetylated derivatives on a column of silicic acid using methanol in chloroform as eluting agent. From the black-white rat seven fractions and from the white five fractions were taken out. After deacetylation the fractions were subjected to thin-layer chromatography and immunological analysis by hemagglutination inhibition using commercial anti-A and anti-B human antisera. All fractions were analysed by mass spectrometry as permethylated and as $LiAlH_4$-reduced permethylated derivatives [1–3].

In a separate set of experiments the epithelial cells of small intestine were prepared according to a slightly modified technique of Weiser [6]. The total non-acid glycolipids were derivatized and characterized by selected ion monitoring [2,3].

3. Results

Fig.1 shows a thin-layer chromatogram of the fractions eluted from a silicic acid column as acetylated derivatives and deacetylated. The weight and immunological activity of these fractions are gathered in table 1. Although all fractions were analysed in form of both derivatives, mass spectra are reproduced only for permethylated-reduced derivatives of fraction B_6 (fig.2) and fraction W_3 (fig.3) to illustrate differences.

The spectrum of fig.2 has traits in common with a corresponding spectrum of a blood group A active hexaglycosylceramide of human erythrocyte [1], although the ceramide differed. The rather intense peaks at *m/e* 1560–1672 are due to all sugars and the non-hydroxy fatty acids with 16 (*m/e* 1560), 20 (*m/e* 1616), 22 (*m/e* 1644), and 24 (*m/e* 1672) carbon atoms (see top formula). Hydroxy fatty acids were also present as shown by peaks at *m/e* 1590 (16 carbon atoms) and *m/e* 1702 (24 carbon atoms). Saccharide sequence ions from an A-type saccharide are shown at *m/e* 157,189,246,262,855 and 871 but

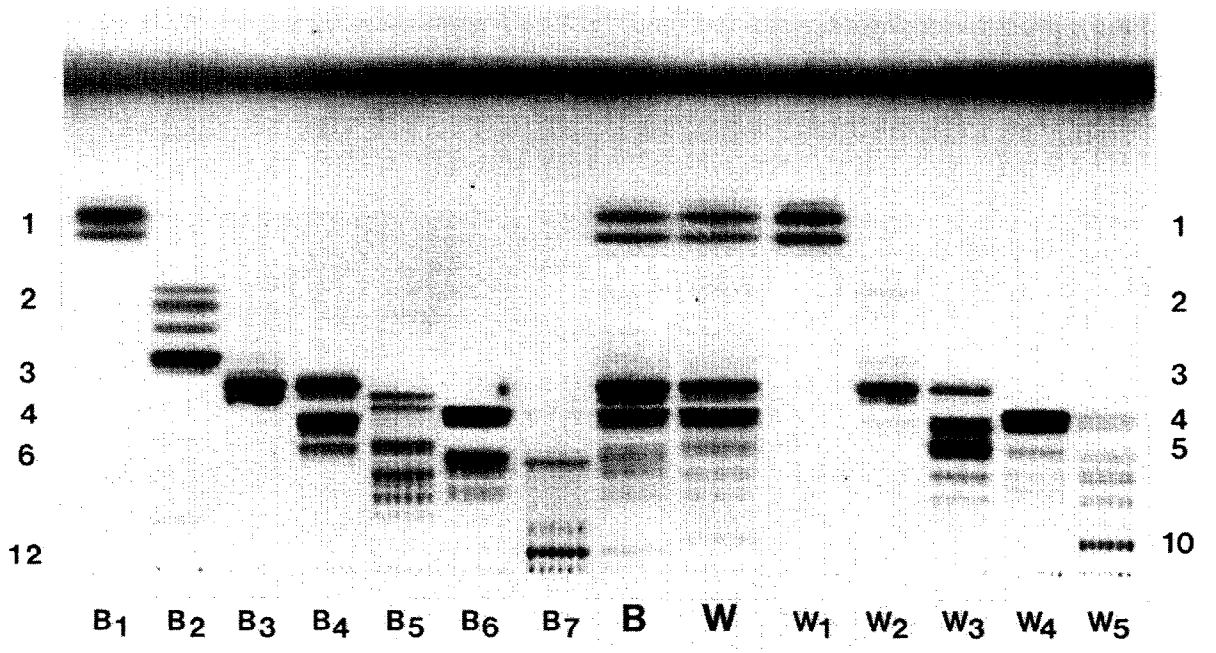

Fig.1. Thin-layer chromatogram of deacetylated fractions obtained from silicic acid chromatography of acetylated total non-acid glycosphingolipids of small intestine of a black-white strain of rat (B_1-B_7 and a total fraction B) and a white strain of rat (W_1-W_5 and a total fraction W). The numbers to the left and right indicate number of sugars found in the glycolipids of the separate regions. About 10 μg were applied of separate fractions and 40 μg of total samples. HPTLC plates (Merck) were developed with chloroform–methanol–water 60:35:8 (by vol.). Bands were detected with anisaldehyde reagent [4].

also by fragments explained below the formula and containing the fatty acid and an increasing part of the saccharide (for the 24 carbon non-hydroxy fatty acid at *m/e* 614, 818, 1049 and 1427). Although the fraction B_6 of fig.1 contained two groups of glycolipid bands, the spectrum reproduced was dominated by the A-type hexaglycosylceramide. This is because the faster-moving component (globotetraosylceramide) was distilled off in the ion source before the actual spectrum was recorded (compare the distillation process in fig.4). The existence in fraction B_6 of a saccharide sequence corresponding to a blood group A determinant was confirmed by immunological analysis (table 1).

In fig.3 the mass spectrum of fraction W_3 is shown. The spectrum was recorded late during the evaporation and therefore only the five-sugar component was detected (compare chromatogram of fig.1). In this

Table 1
The weight and immunological activity of the different glycolipid fractions from the two strains of rat

Fractions	Black and white rat								White rat					
	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B	W	W_1	W_2	W_3	W_4	W_5
Weight (mg/rat)	2.9	0.6	1.6	0.6	0.7	1.1	0.6	8.1	4.8	1.4	1.0	0.9	0.9	0.6
Weight (mg/g dry weight)	2.0	0.4	1.1	0.4	0.5	0.7	0.4	5.5	4.4	1.3	1.0	0.8	0.8	0.5
Percentage	37	7	20	7	9	13	7	100	100	30	22	18	18	12
Blood group A-activity[a]	ND	–	–	–	1+	4+	4+	4+	–	ND[b]	–	–	–	–
Blood group B-activity[a]	ND	–	–	–	2+	–	–	–	–	ND[b]	–	–	(+)	–

[a] Dilution of antisera 1:2
[b] ND, not determined

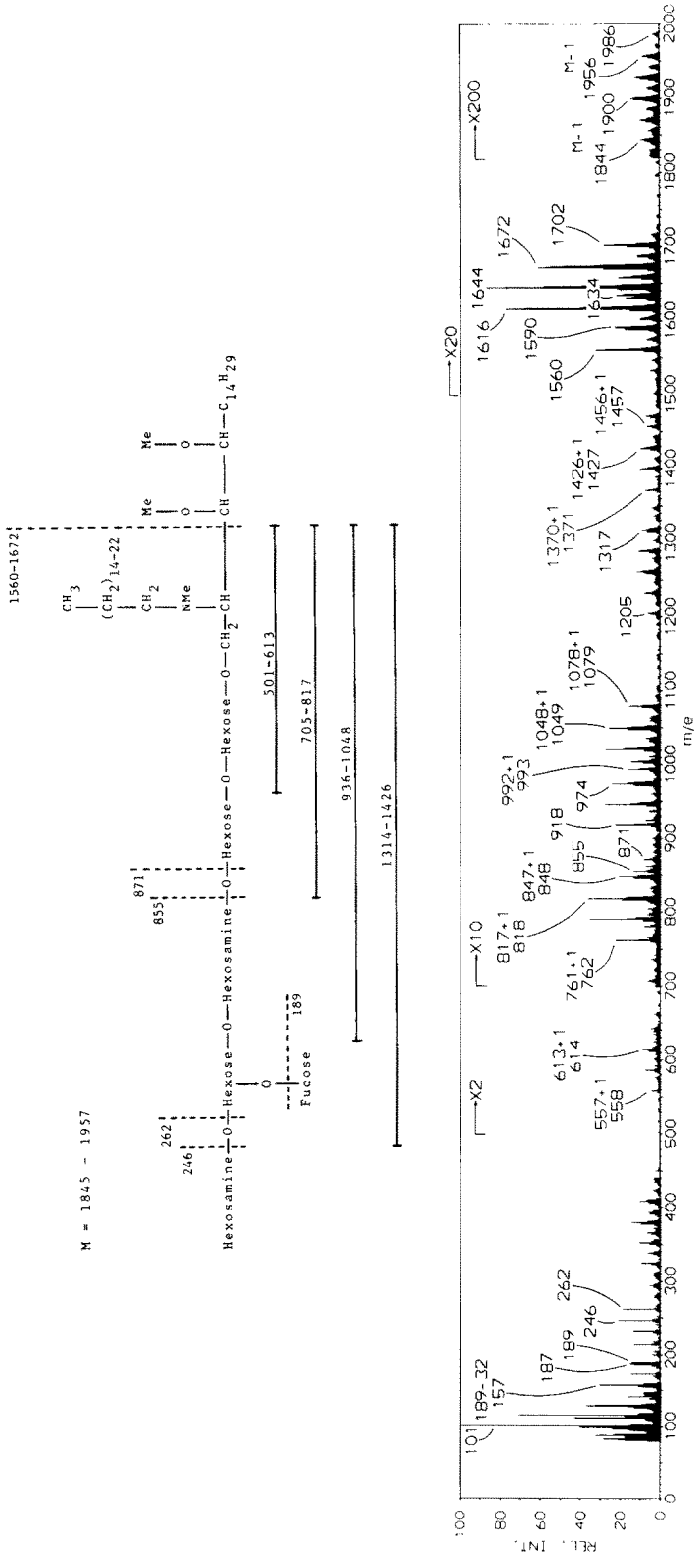

Fig.2. Mass spectrum of the permethylated-reduced derivative of fraction B_6 of fig.1 (black-white strain). Sample amount 140 μg, electron energy 38 eV, acceleration voltage 4 kV, trap current 500 μA, ion source temperature 300°C, and probe temperature 315°C. The instrument used was an AE1 MS 902 mass spectrometer.

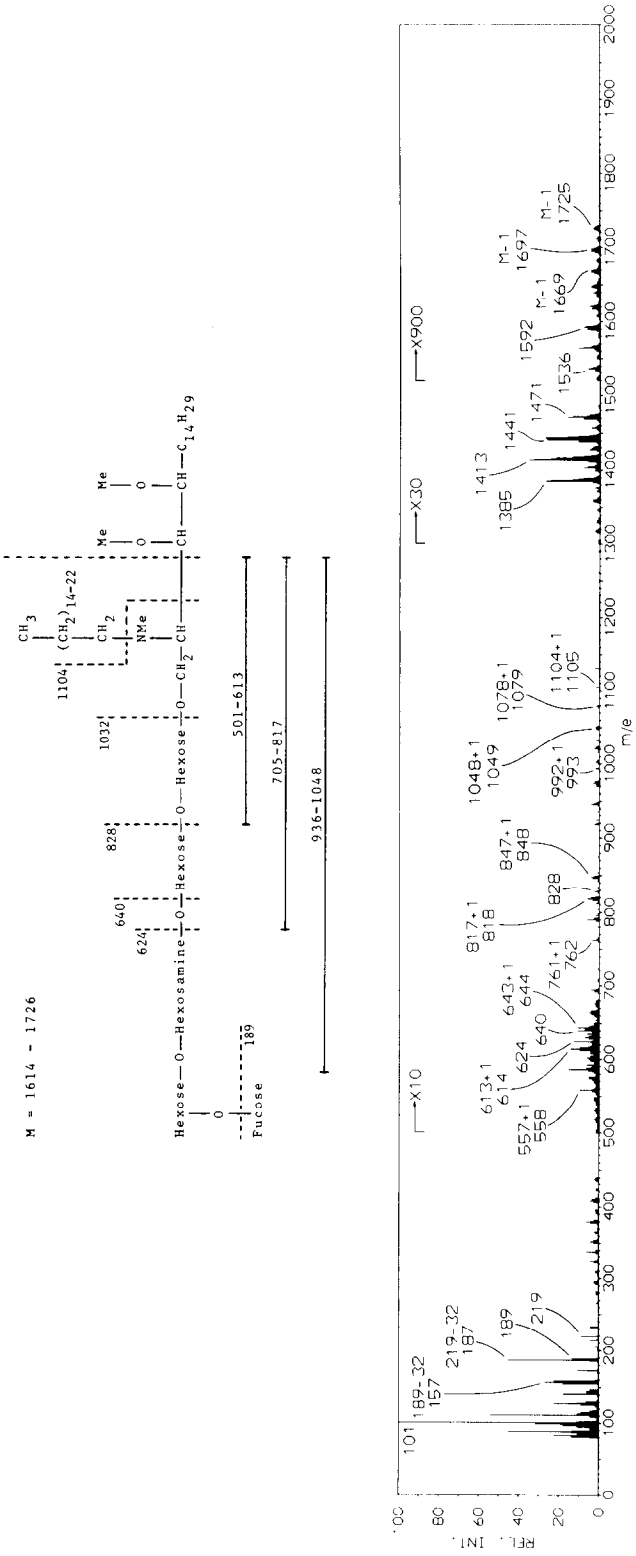

Fig.3. Mass spectrum of the permethylated-reduced derivative of fraction W_3 of fig.1 (white strain). Sample amount 120 μg, electron energy 38 eV, acceleration voltage 4 kV, trap current 500 μA, ion source temperature 300°C and probe temperature 295°C.

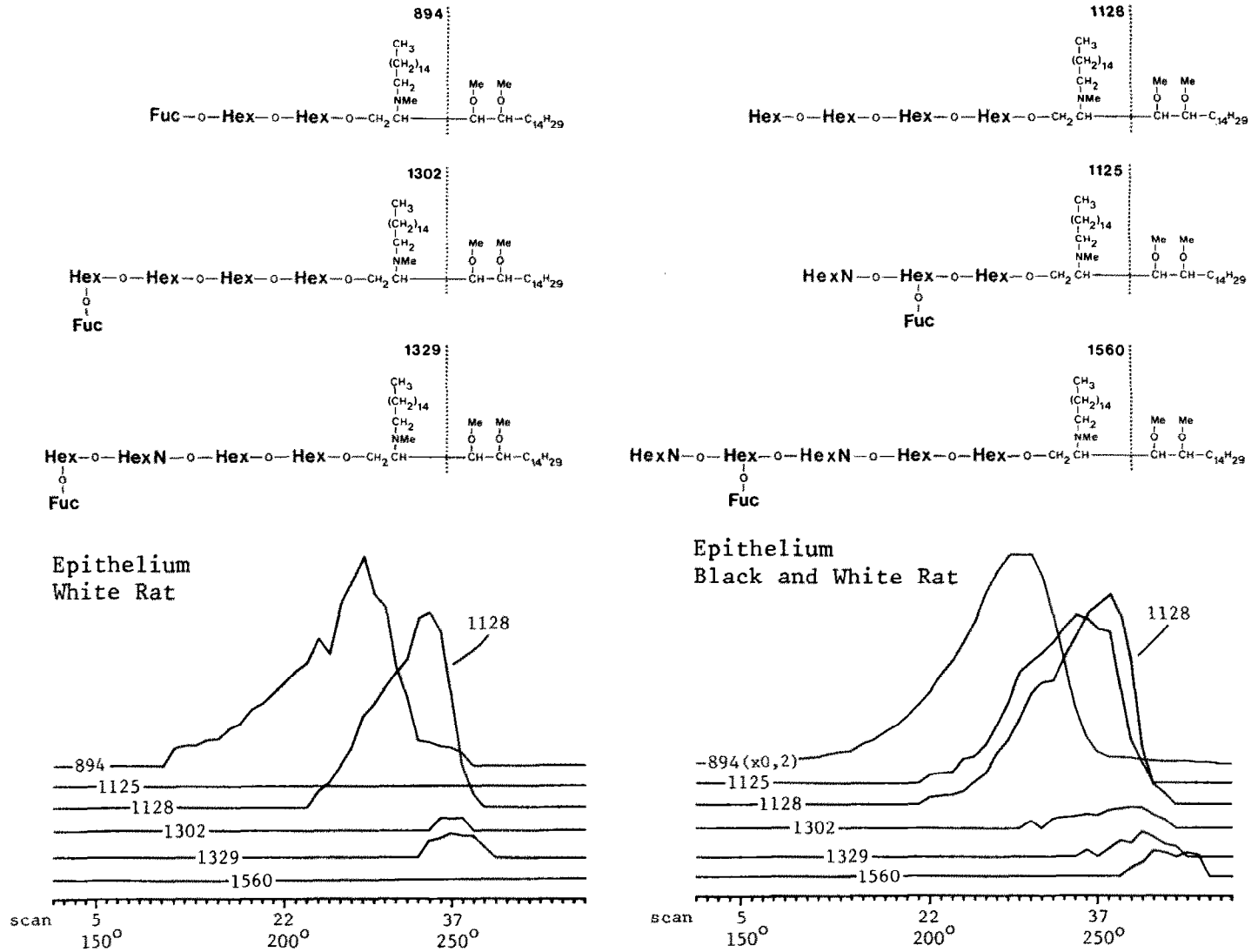

Fig.4. Selected ion monitoring from mass spectrometry of permethylated-reduced glycolipids. Total non-acid glycolipids were prepared from epithelial cells of small intestine of a white (left) and a black-white rat strain. Curves for the major glycolipids with one and three sugars, respectively, are not shown. The sample amounts used were 200 μg, the electron energy was 38 eV, accelerating voltage 4 kV, trap current 500 μA and ion source temperature 290°C. The probe was heated 5°C/min and spectra were recorded every 38 s. The instrument used was an AEI MS 902 mass spectrometer equipped with an interface and a computer system (Instem-Kratos Ltd., England).

case no fragments for an A-type sequence as for fraction B_6 was found. Also, no blood group A activity was present (table 1). Instead one may interpret an H-type pentaglycosylceramide as explained by the top formula.

The localization of the fucolipids to the epithelial cells was shown by mass spectrometry of glycolipids of prepared cells from the two strains (fig.4). By a continuous recording of selected peaks (see top formulas) during heating of the sample from 150° to about 300°C, the black-white strain revealed ions from A-type sequences with four (*m/e* 1125) and six (*m/e* 1560) sugars which were absent in the white strain. H-type sequences with three (*m/e* 894) and five (*m/e* 1302 and *m/e* 1329, respectively) sugars were, however, detected in both samples.

In all cases the conclusions drawn were supported by a parallel analysis also of non-reduced derivatives, which yield better information concerning terminal saccharide arrangements [1].

4. Discussion

The existence of human blood group A active material in gastrointestinal tissue of several animals is well established [7–10]. However, as far as known to us the present report is the first on an A-negative strian of rat, a finding of possible importance for future immunological studies. Similar to the situation for many other species except man [8,10] the blood group A activity found in small intestine of the black-white rat was not detected on erythrocytes or in glycolipids prepared from these. Human blood group B activity as shown for rat before [7] was present in small intestinal glycolipids (table 1) as well as on erythrocytes of both strains. This B activity is probably based on a galactosamine-containing glycolipid recently shown to be a receptor for the macrophage migration inhibition factor [11].

The novel technique used in this paper to reveal the partial chemical identity of blood group fucolipids even when present in mixtures has opened up new possibilities in the study of species, individual and tissue specificity of blood group antigens.

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