Abnormal fatty acid composition of erythrocyte glycosphingolipids in congenital dyserythropoietic anemia type II

Jean-François Bouhours, Danièle Bouhours, and Jean Delaunay

Laboratoire de Physiologie Générale et Comparée, Université Claude-Bernard, 69622 Villeurbanne Cedex and Groupe de Recherche sur le Globule Rouge, Faculté de Médecine Grange-Blanche, 69373 Lyon Cedex 08, France

Abstract Glycosphingolipids were isolated from the erythrocytes of three siblings clinically affected with congenital dyserythropoietic anemia type II (CDA-II) as well as from the erythrocytes of their parents and of normal individuals. Glycolipids were analyzed by thin-layer chromatography of their native and O-acetylated form and by high performance liquid chromatography of their O-acetyl-N-*p*-nitrobenzoyl derivatives. The glycolipid content of the CDA-II erythrocytes was 2.5 to 3 times higher than normal. This abnormal concentration resulted from a moderate accumulation of all the glycolipids present in normal erythrocytes and from a strong increase (at least 10 times over normal level) of lactotriaosyl- and lactoneotetraosylceramide. Glycolipids contained higher amounts of long chain fatty acids (C_{22}–C_{24}) in CDA-II patients than in controls. Free ceramide content of CDA-II erythrocytes was two times greater than the control value and the fatty acid composition was also altered. —**Bouhours, J-F., D. Bouhours, and J. Delaunay.** Abnormal fatty acid composition of erythrocyte glycosphingolipids in congenital dyserythropoietic anemia type II. *J. Lipid Res.* 1985. **26:** 435–441.

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Congenital dyserythropoietic anemia type II (CDA-II) is a rare refractory disease inherited as an autosomal recessive defect. The anemia is believed to result from an abnormal maturation of the erythrocytes in the bone marrow and a shortened life span of the red blood cells when released into the peripheral circulation (1). The observation of erythroblasts with multiple nuclei and the abnormal lysis of the erythrocytes by an allo-antibody present in many normal sera are responsible for the other name of the disease: hereditary erythroblastic multinuclearity associated with a positive acidified-serum test or HEMPAS (2).

Electron microscopic studies of the bone marrow have shown that most erythroblasts and some erythrocytes display a duplicated peripheral membrane forming cisternae under the cell surface (3, 4). These abnormalities are correlated by the presence of membrane material in excess. Joseph, Gockerman, and Alving (5) have found in a study of two clinically affected siblings that the glycolipid content of the erythrocytes is higher than normal. The glycolipid composition is disturbed with abnormally elevated amounts of glycolipids containing N-acetylglucosamine (6).

In order to assess the constant abnormalities linked to the disease, we studied the glycolipid composition of the erythrocytes of a family of three sisters clinically affected with CDA-II and their non-affected parents. The present study not only confirmed the elevation of the glycolipid content, which was 2.5 to 3 times higher than normal in all three patients with accumulation of lactotriaosyl- and lactotetraosylceramide, but new observations were brought to light. HPLC produced CDA-II glycolipid profiles easily distinguishable from controls. In addition, free ceramide, recently characterized in human erythrocytes (7), was 2 times more concentrated in the erythrocytes of the CDA-II patients and their parents than in the controls. Finally, analysis of the fatty acid composition demonstrated a preferential accumulation of glycolipids with long chain fatty acids.

METHODS

Red blood cells

Blood was drawn from five members of a family from the Jura mountains, in the east of France. The father

Abbreviations: GLC, gas-liquid chromatography; HPLC, high performance liquid chromatography; TLC, thin-layer chromatography; HPTLC, high performance thin-layer chromatography; CDA-II, congenital dyserythropoietic anemia type II. Glycosphingolipids were designated according to the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (22).

(subject 1) and the mother (subject 2) were not consanguineous and were clinically and hematologically normal. Their daughters (subjects 3, 4, and 5) were born, respectively, in 1967, 1970, and 1975. The diagnosis of CDA-II was sustained by the appropriate clinical and laboratory data (4). The erythrocytes of three healthy individuals were analyzed as controls (subjects 6, 7, and 8). Blood was maintained and processed at 4°C until lipid extraction. Within 5 hr after being drawn, blood was centrifuged at 1000 *g* for 10 min. The plasma and buffy coat were removed. Erythrocytes were washed in two volumes of saline and centrifuged. After two additional washings under the same conditions, packed erythrocytes were ready for lipid extraction.

Lipid extraction

Seven ml of cold methanol (−20°C) per ml of packed erythrocytes was added and the suspension was allowed to warm at room temperature under stirring. Seven ml of chloroform was added and the extraction proceeded under stirring for 16 hr. The suspension was centrifuged at 1500 *g* for 10 min. The supernatant was decanted. The pellet was homogenized in 10 ml of chloroform–methanol 1:1 (v/v) per ml of packed erythrocytes. After 1 hr of stirring, the suspension was centrifuged. The supernatant was decanted and the pellet was homogenized in 5 ml of chloroform–methanol 1:2 (v/v) per ml of packed erythrocytes. After extraction and centrifugation under the same conditions as above, the pooled supernatants were dried in a rotary evaporator, taken up in chloroform–methanol 2:1 (v/v) and submitted to partition according to Folch, Lees, and Sloane Stanley (8). The glycolipids of the upper phase were recovered by chromatography on a Sep-Pak C_{18} cartridge (Waters Associates, Inc., Milford, MA) (9) and added to the lipids of the lower phase. The resultant extract was dried repeatedly with addition of isopropanol in order to eliminate all traces of water. The dry lipid residue was then acetylated with pyridine–acetic anhydride 3:2 (v/v) for 16 hr under nitrogen.

Separation of neutral lipids and glycosphingolipids

Acetylated neutral lipids and glycosphingolipids were separated by chromatography on a Florisil column (10). Glycosphingolipids were deacetylated with sodium methoxide. They were suspended in the theoretical upper phase of Folch et al. (8) by sonication and purified from water-soluble contaminants by chromatography on a Sep-Pak cartridge (9).

Chemical assays

Sphingosine content was measured by the fluorimetric method of Naoi, Lee, and Roseman (11). Cholesterol content was measured by the method of Zlatkis, Zak, and Boyle (12) on the neutral lipid fraction obtained by Florisil column chromatography. Total phosphorus was assayed by the method of Bartlett (13) on the lower phase lipids after partition (8).

Thin-layer chromatography (TLC)

TLC was performed on regular and high-performance silica gel thin-layer plates (10 cm high) (E. Merck, Darmstadt, West Germany). Native glycolipids were analyzed in solvent A: chloroform–methanol–water 60:35:8 (v/v). Acetylated glycolipids were analyzed in solvent B: chloroform–methanol 98:2 (v/v). Glycolipids were made visible by successive sprayings of a 2% ethanolic solution of 1-naphthol and a 50% aqueous solution of concentrated sulfuric acid. Plates were heated 5 min at 120°C.

Derivatization of purified glycolipids

The isolated glycolipids or aliquots of the total purified glycolipids were dried for 2 hr in vacuum in the presence of phosphorus pentoxide. They were acetylated overnight in 0.1 ml of pyridine–acetic anhydride 1:1 (v/v). Then acetic anhydride was destroyed with 0.5 ml of water and the acetylated glycolipids were extracted in 0.5 ml of chloroform. The chloroform phase was washed successively with 0.2 ml of a 10% Na_2CO_3 solution and twice with 0.2 ml of water. It was dried under nitrogen and dissolved in the suitable amount of solvent.

The O-acetylated-N-*p*-nitrobenzoylated glycolipids were prepared as described by Suzuki, Kundu, and Marcus (14). The glycolipid derivatives were quantified by taking into account that N-acetylhexosamine-containing glycolipids, namely lactotriaosylceramide and the two tetraosylceramides, contained two acylamide groups substituted with *p*-nitrobenzoyl, while the other glycolipids contained one substituted acylamide group on ceramide.

High-performance liquid chromatography (HPLC)

The O-acetylated-N-*p*-nitrobenzoylated glycolipids were analyzed on a 125-4 RT column (Merck) filled with 5-μm particles of Si 60 silica. The solvent delivery system was from Gilson Medical Electronics and the column eluate was monitored at 260 nm with a Philips PU 4020 spectrophotometer. Analysis was initiated isocratically in hexane–dichloromethane 2:1 (v/v) containing 1% isopropanol at a flow rate of 0.5 ml/min. After 5 min, the gradient was established. The concentration of isopropanol in hexane–dichloromethane 2:1 was raised from 1% at 5 min to 3.5% at 55 min and 8% at 90 min. The column was recycled to the initial conditions in 10 min with a linear gradient of 8 to 1% isopropanol in hexane–dichloromethane 2:1. The column was stabilized for 15 min in the initial solvent before starting a new analysis.

Gas–liquid chromatography (GLC) of fatty acid methyl esters

Individual glycolipids isolated by TLC were methanolyzed in 0.8 N anhydrous methanolic HCl at 80°C for 18

hr. Fatty acid methyl esters were extracted in n-hexane and analyzed by GLC on a glass column packed with Anachrom ABS coated with 3% OV-1 (w/w) (Analabs, North Haven, CT) (15). GLC was performed on a Hewlett-Packard Model 5710 apparatus and the signal of the flame ionization was recorded on a Hewlett-Packard Model 3380 integrator.

RESULTS

The cholesterol and phospholipid contents of CDA-II erythrocytes were within normal values (**Table 1**). The glycosphingolipid content however was 2.5 to 3 times more elevated in the patients and 1.5 times higher in their parents than in the controls. In normal individuals there was 20 μmol of glycosphingolipids per 100 ml of packed erythrocytes. This quantity included free ceramide (7). Free ceramide migrated between the solvent front and the glucosylceramide position when glycosphingolipids were analyzed by TLC (**Fig. 1**) (16). It was present in all the samples under study and accounted for 20 to 32% of the glycolipids fractions. The erythrocytes of the CDA-II patients and their parents had the same enrichment in free ceramide as in total glycosphingolipids.

Among the glycolipids, there was a considerable accumulation of dihexosyl-, triaosyl-, and tetraosylceramide in CDA-II erythrocytes. Analysis of the native and peracetylated glycolipids by TLC revealed that CDA-II erythrocytes contained two triaosyl- and two tetraosylceramides. Sequential degradation by exoglycosidases and gas–liquid chromatography of the component sugars (not shown) indicated that CDA-II erythrocytes had a large amount of lactotriaosyl- and neolactotetraosylceramide in addition to globotriaosyl- and globotetraosylceramide which are commonly found in human erythrocytes. These results confirmed similar findings by Joseph and Gockerman (6). The two tetraosylceramides were resolved into two bands by HPTLC in solvent A (Fig. 1). The upper band contained the major glycolipid of all

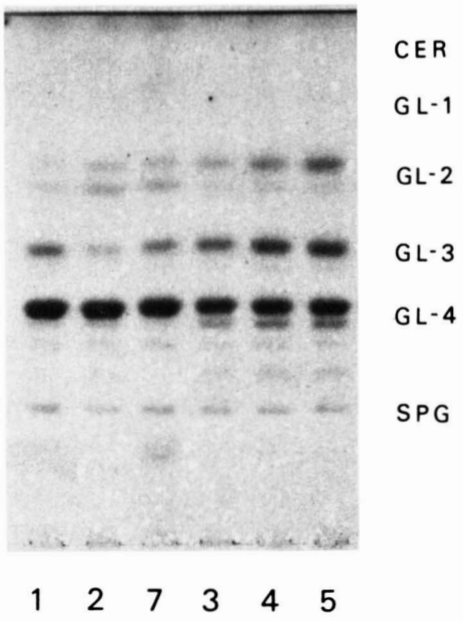

Fig. 1 Glycosphingolipids of CDA-II erythrocytes. Glycosphingolipids were analyzed by HPTLC in solvent A. They were visualized with the 1-naphthol reagent. Numbering of the lanes refers to numbering of individuals: 1, father; 2, mother; 3, 4, 5, CDA-II patients; 7, control. CER, free ceramide; GL-1 to 4 refer to monohexosyl- to tetraosylceramide; SPG, sialosylparagloboside.

erythrocytes under study, globotetraosylceramide, commonly designated as globoside. The lower band which contained neolactotetraosylceramide, commonly designated as paragloboside (17), had a very weak intensity in the controls and parents. It was markedly enhanced in the three CDA-II patients. The two triaosylceramides, however, gave a single spot in this chromatographic system and were separated on thin-layer plates only after acetylation. In order to make accurate quantitative determination of individual glycolipids in a single run, we used high performance liquid chromatography.

Quantitation of neutral glycolipids by HPLC

O-acetylated-N-*p*-nitrobenzoylated glycolipids were

TABLE 1. Lipid composition of erythrocytes of CDA-II patients, parents, and controls

	Unaffected		CDA-II Affected Daughters			Controls		
	Father	Mother						
	1	2	3	4	5	6	7	8
	µmol/100 ml packed erythrocytes							
Cholesterol	278	257	290	308	315	285	283	276
Phospholipids	339	313	290	323	325	328	317	312
Sphingolipids	30.5	32.7	50.1	62.8	61.4	23.0	19.8	21.2
Free ceramide	8.6	12.1	10.4	13.4	13.7	5.8	5.7	4.6

Sphingolipids refer to total sphingolipids except sphingomyelin. Sphingolipids and free ceramide were measured with the sphingosine assay. Numbers represent mean values of triplicate assays of each sample. Standard deviation was within 7% on cholesterol and phospholipid determinations and within 12% on sphingosine determinations.

obtained and analyzed essentially as described by Suzuki et al. (14). HPLC profiles of glycolipids (**Fig. 2**) were similar in the controls and parents. The glucosylceramide concentration remained low at the control level in CDA-II erythrocytes (**Table 2**) while there was accumulation of lactosylceramide (peaks 2, 3, and 4), lactotriaosylceramide (peak 5) and neolactotetraosylceramide (peak 7).

Lactosylceramide and globotriaosylceramide occurred in similar quantities in CDA-II erythrocytes. These quantities were higher than in controls. The most remarkable peculiarity of the glycolipid composition of CDA-II erythrocytes was the abnormally high concentration of lactotriaosyl- and neolactotetraosylceramide which was 10 times above the control value. Lactotriaosylceramide accounted for 42–43% of total triaosylceramides. The level of lactotriaosylceramide was 0.25 and 0.13 μmol/100 ml of packed erythrocytes in controls 6 and 7, respectively. The father had a higher amount (0.68 μmol) and the concentration in the mother's erythrocytes was in the normal range (0.32 μmol/100 ml of packed cells). Both parents had much less lactotriaosylceramide than their affected daughters. Paragloboside accounted for 2–3% of total tetraosylceramides in the controls, whereas it reached 14 to 18% in CDA-II. The father and mother had normal levels of paragloboside.

Fatty acid composition of glycosphingolipids

Lactosylceramide was resolved by HPTLC into two bands of equal intensity in normal erythrocytes, whereas the upper band was more intense than the lower one in CDA-II (Fig. 1). Lactosylceramide was resolved into three peaks (Fig. 2, peaks 2, 3, and 4) by HPLC. Peak 3 was much higher than peak 4 in CDA-II while in normal erythrocytes it was the opposite. The abnormal chromatographic behavior of lactosylceramide prompted us to investigate possible modifications of the fatty acid composition of CDA-II glycosphingolipids.

None of the glycosphingolipids contained 2-hydroxylated fatty acids. The two bands of lactosylceramide seen on TLC (Fig. 1) were due to segregation of molecular species according to the chain length of their fatty acids (**Table 3**). The upper band was a glycolipid with a larger amount of long chain fatty acids and the lower band was a glycolipid containing mainly medium chain fatty acids.

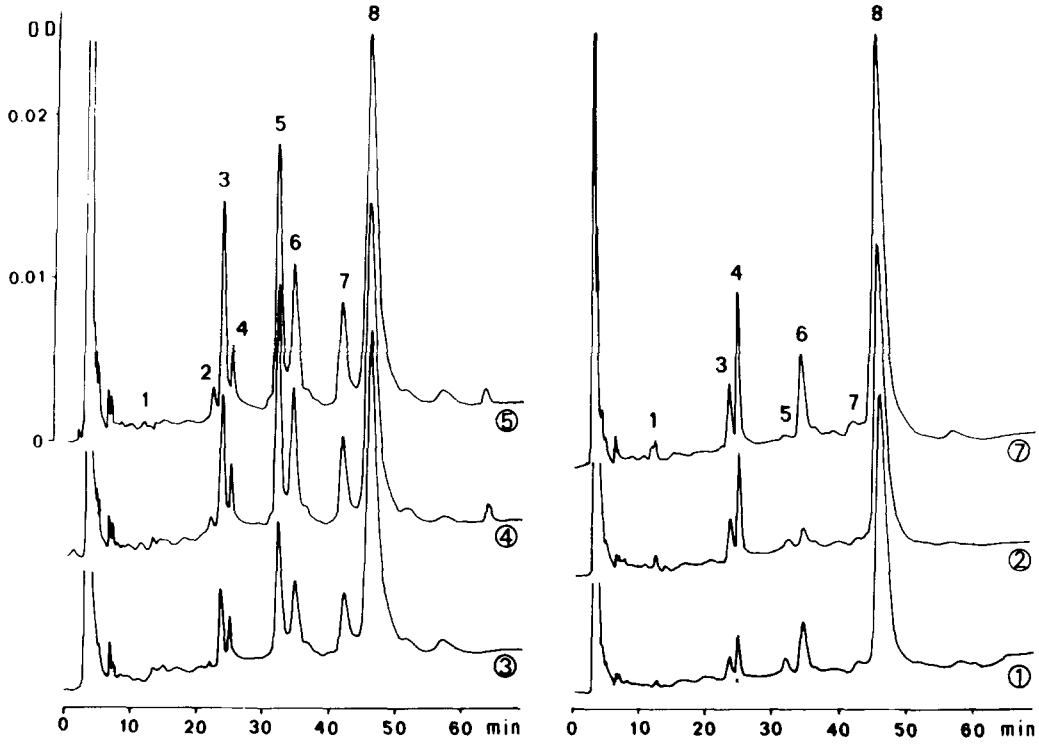

Fig. 2 HPLC analyses of the glycolipids of erythrocytes. Glycosphingolipids were analyzed as their O-acetyl-N-*p*-nitrobenzoyl derivatives and detected by ultraviolet absorbance at 260 nm. The column filled with 5-μm silica particles was eluted with a gradient of isopropanol concentration in hexane–dichloromethane 2:1 (see Methods). Profiles are numbered according to the numbers assigned to individuals. On the left side: patients 3, 4, 5. On the right side: 1, father; 2, mother; 7, control. Peak numbering: 1, glucosylceramide; 2, 3, 4, lactosylceramide; 5, lactotriaosylceramide; 6, globotriaosylceramide; 7, neolactotetraosylceramide; 8, globotetraosylceramide.

TABLE 2. Glycosphingolipid composition of erythrocytes of CDA-II patients and parents

Glycolipids	HPLC Peak No	Unaffected		CDA-II Affected			Controls	
		Father	Mother	Daughters				
		1	2	3	4	5	6	7
		μmol/100 ml packed erythrocytes						
GlcCer	1	0.26	0.93		0.82	0.40	0.77	0.31
LacCer	2-3-4	2.83	4.29	5.80	9.54	11.32	2.24	2.77
$LcOse_3Cer$	5	0.68	0.32	4.26	6.68	6.08	0.25	0.13
$GbOse_3Cer$	6	3.66	1.06	5.65	8.94	8.30	1.37	1.80
$nLcOse_4Cer$	7	0.46	0.30	3.01	3.18	3.42	0.28	0.23
$GbOse_4Cer$	8	13.91	12.08	17.98	16.92	14.87	9.87	7.58

Total neutral glycolipid concentrations were measured with the sphingosine assay and the distribution of individual glycolipids was established according to the peak area of each glycolipid separated by HPLC as in Fig. 2.

The different intensities of the two TLC bands of CDA-II lactosylceramide (Fig. 1) as well as the abnormal height of peaks 2 and 3 on HPLC profiles (Fig. 2) were the result of a higher percentage of long chain fatty acids than in normal individuals where more than 80% of the fatty acids were C_{16} and C_{18} fatty acids.

The fatty acid composition of the two triaosylceramides was established (**Table 4**). Both glycolipids had a fatty acid composition very close to that of CDA-II lactosylceramide. This composition differed only slightly from that of normal triaosylceramide by a 10% excess of $C_{24:0}$ at the expense of $C_{16:0}$ fatty acid.

Globoside of normal individuals had a higher percentage of long chain fatty acids than paragloboside and other glycolipids (**Table 5**). The fatty acid composition of CDA-II globoside was the same as in controls. However, CDA-II paragloboside was different and showed a higher content of long chain fatty acids than normal.

The fatty acid composition of free ceramide was also established (**Table 6**). There was an increased proportion of C_{22} and C_{26} fatty acids in the CDA-II patients and their parents. Furthermore, there was much more $C_{24:0}$ than $C_{24:1}$ fatty acid. In normal individuals these two fatty acids occur in close proportion, with the unsaturated slightly more abundant than the saturated C_{24} fatty acid (7). Thus the enrichment of the erythrocyte membrane with free ceramide was accompanied by a modification of the long chain fatty acid composition.

DISCUSSION

The glycosphingolipid content of intact erythrocytes was determined by direct sphingosine assay and calculated per 100 ml of packed erythrocytes (Table 1). Our data on individual glycolipids of normal subjects compared favorably with previous reports (5, 18–20). Our results showed that the glycosphingolipid content was 2.5 to 3 times higher in the CDA-II patients than in the controls whether it was expressed per 100 ml of packed erythrocytes, per mol of cholesterol, or per mol of phospholipids. This is in agreement with data published by Joseph et al. (5) concerning two CDA-II patients and their unaffected sibling. In the present study, the CDA-II patients had a highly elevated concentration of all glyco-

TABLE 3. Fatty acid composition of CDA-II lactosylceramide (GL-2)

Fatty Acid	Patient 3 Total GL-2	Patient 4 Total GL-2	Patient 5			Control 6 Total GL-2
			Upper Band	Lower Band	Total GL-2	
16:0	26.6	21.5	10.9	41.9	17.6	43.4
16:1	0.3	0.2	4.7	9.3	3.6	11.9
18:0	5.9	7.4	6.4	15.9	8.2	12.5
18:1	5.5	2.7	9.6	21.4	11.3	20.2
20:0	1.5	2.1	1.5	2.3	1.0	1.2
22:0	14.8	21.2	13.1	1.5	13.6	1.4
23:0	1.8	3.5	3.5	0.5	1.7	2.0
24:0	30.0	34.9	35.0	5.8	30.6	3.0
24:1	13.5	6.1	15.2	1.3	12.4	4.4

Glycolipids were isolated by HPTLC in solvent A as in Fig. 1. The two bands of lactosylceramide were either pooled or separated (patient 5) for fatty acid analysis. Results are expressed as percentage by weight of fatty acid methyl esters determined by GLC. Standard deviation was within 2% of individual values.

TABLE 4.. Fatty acid composition of the two GL-3 species of CDA-II patients

Fatty Acid	Patient 5		Control 6
	$GbOse_3Cer$	$LcOse_3Cer$	GL-3
16:0	13.4	16.7	26.3
16:1	4.3	7.1	5.0
18:0	6.7	6.4	7.6
18:1	10.7	12.6	11.2
20:0	0.7	0.7	1.0
22:0	12.6	10.0	10.2
23:0	2.4	1.5	1.5
24:0	36.0	32.3	23.1
24:1	13.2	12.7	14.1

Triaosylceramide (GL-3) was isolated by HPTLC in solvent A (Fig. 1), acetylated, and separated by TLC in solvent B into two compounds: globotriaosylceramide ($GbOse_3Cer$) and lactotriaosylceramide ($LcOse_3Cer$). Results are expressed as percentage by weight of fatty acid methyl esters determined by GLC.

lipids except glucosylceramide. They contained 4.2 to 6.7 μmol of lactotriaosylceramide and 3 to 3.4 μmol of neolactotetraosylceramide per 100 ml of packed erythrocytes. These values are in the same range as the values reported by Joseph and Gockerman (6) for their two cases of CDA-II: 5 μmol of lactotriaosylceramide and 3 to 4 μmol of neolactotetraosylceramide. We can conclude that the erythrocytes of patients affected with CDA-II display a remarkable glycolipid content with only small individual variations.

The present study showed that HPTLC of erythrocyte glycolipids displayed two features specific to CDA-II: the increased intensity of the upper spot of lactosylceramide and of the lower spot of tetraosylceramide (Fig. 1). However, the glycolipid composition characteristic of CDA-II erythrocytes appeared more clearly upon HPLC of the O-acetyl-N-*p*-nitrobenzoylated glycolipids according to Suzuki et al. (14). Two minor modifications were introduced: a shorter column with smaller silica particles

TABLE 5. Fatty acid composition of CDA-II tetraosylceramides

Fatty Acid	CDA-II Patient 5		Control 6	
	$GbOse_4Cer$	$nLcOse_4Cer$	$GbOse_4Cer$	$nLcOse_4Cer$
16:0	12.0	27.8	12.7	36.2
16:1	2.4	3.3	2.3	7.0
18:0	5.2	13.9	5.5	13.8
18:1	6.5	11.2	6.4	22.3
20:0	0.8	0.9		1.7
22:0	17.2	12.8	17.4	10.6
23:0	2.7	3.7	2.4	2.5
24:0	35.5	17.6	36.6	3.5
24:1	17.7	8.7	16.6	2.4

Globotetraosylceramide ($GbOse_4Cer$) and lactoneotetraosylceramide ($nLcOse_4Cer$) were separated by HPTLC in solvent A. Results are expressed as percentage by weight of fatty acid methyl esters determined by GLC.

TABLE 6. Fatty acid composition of free ceramide from CDA-II erythrocytes

Fatty Acid	Parents	CDA-II patients			Controls
	1-2	3	4	5	
16:0	23.4	10.6	12.5	10.2	12.7 ± 0.5
16:1	2.2	1.2	1.6	1.5	0.7 ± 0.4
18:0	12.9	7.7	9.6	8.1	11.3 ± 1.3
18:1	6.5	4.9	6.8	5.6	10.2 ± 1.3
20:0	2.6	3.2	4.0	3.4	1.7 ± 0.3
21:0		0.5	0.6	0.4	0.8 ± 0.5
22:0	15.4	13.6	14.1	14.7	9.9 ± 1.8
23:0	2.2	1.9	1.6	1.7	1.7 ± 0.1
23:1		0.8	0.7	0.5	0.5 ± 0.4
24:0	21.3	31.2	26.5	29.8	21.4 ± 1.6
24:1	5.2	12.3	11.9	13.0	24.4 ± 0.9
25:0	1.3	0.5	1.6	1.0	0.7 ± 0.2
25:1	0.7	1.8	0.8	0.8	0.5 ± 0.5
26:0	6.5	6.4	5.4	6.1	2.2 ± 0.2
26:1		3.4	2.3	3.2	1.3 ± 0.4

Free ceramide from parent erythrocytes were pooled before methanolysis. Results are expressed as percentage by weight of fatty acid methyl esters determined by GLC. Controls are expressed as mean ± standard deviation on four normal individuals (from 7).

and the replacement of dichloroethane by dichloromethane. These solvents have similar eluotropic properties but dichloromethane is available in HPLC-grade more easily and at a lower cost than dichloroethane. This HPLC method allowed a good resolution of lactotriaosyl- and neolactotetraosylceramide in the presence of much larger quantities of globotriaosyl- and globotetraosylceramide (Table 2). It must be noted that not all silica columns of similar geometry resolve such differences. However, with an appropriate column, HPLC was the method of choice to detect the abnormal glycolipid profile of CDA-II erythrocytes, while erythrocyte glycosphingolipids of patients affected with congenital dyserythropoietic anemia type I (4) displayed a normal pattern of composition when analyzed either by TLC or HPLC.

A new insight into the disease was brought to light by the study of free ceramide. This sphingolipid, which is isolated with the glycosphingolipid fraction obtained by acetylation and Florisil column chromatography, is an important constituent of the sphingolipids of the normal erythrocyte membrane (7). Its concentration was noticeably increased in CDA-II erythrocytes and reached a level about twice that of the control. The parents of CDA-II patients had also a higher ceramide level than the controls. Otherwise their glycolipid pattern as detected by TLC or HPLC was normal.

The modification of the chromatographic profile of lactosylceramide of CDA-II erythrocytes was found to be a result of an increased percentage of long chain fatty acids. Marcus, Naiki, and Kundu (21) have reported a similar observation in studies of individuals with the rare p phenotype who accumulate lactosylceramide abnormal-

ly in their erythrocytes. In the present study, it was found that triaosylceramides, neolactotetraosylceramide, as well as free ceramide, had also an altered fatty acid composition in CDA-II erythrocytes. Therefore, besides an accumulation of glycosphingolipids and free ceramide, the patients affected by congenital diserythropoietic anemia type-II displayed, in addition, a specific alteration of the lipid portion of these components of the erythrocyte plasma membrane.

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