Glycolipid accumulation in bronchoalveolar space in adult respiratory distress syndrome

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Abstract Surfactant lipids in the alveolar space are believed to play an important role in normal respiratory function. Although the surface-active phospholipids have been extensively studied, the possible role of glycolipids in the surfactant remains to be explored. We have studied the glycolipid composition of cell-free bronchoalveolar lavage from healthy subjects and from adult patients with respiratory distress syndrome. Glycolipids were barely detectable in bronchoalveolar lavage from healthy subjects. However, in adult respiratory distress syndrome, the amount of glycolipid relative to phospholipid was increased by more than twenty times. These lipids, identified as lactosylceramide (galactose-glucose-ceramide) and paragloboside (galactose-N-acetylglucosamine-galactose-glucose-ceramide), may prove to be sensitive markers of lung injury. Since the glycolipids decreased the surface activity of surfactant in vitro, their potential role in the pathogenesis of adult respiratory distress syndrome should be considered.—**Rauvala, H., and M. Hallman.** Glycolipid accumulation in bronchoalveolar space in adult respiratory distress syndrome. *J. Lipid Res.* 1984. **25:** 1257–1262.

Supplementary key words lung surfactant • lactosylceramide • paragloboside

Sudden respiratory failure with diffuse lung involvement, known as adult respiratory distress syndrome (ARDS), may occur among individuals who sustain systemic or pulmonary insults that cause diffuse lung injury. Although a variety of insults, such as sepsis, aspiration, toxins, emboli, circulatory collapse, metabolic, neurologic, or hematologic disorders precede ARDS, the resulting severe lung injury has rather uniform features. The pathogenesis involves an early damage of alveolar-capillary membranes, increased capillary permeability, and abnormalities in the surfactant system (1–4). Despite intensive respiratory support, the mortality of this condition (150,000 annual cases in the U.S.A. alone) is reportedly more than 50% (5).

In order to get insight into the pathogenesis of the respiratory distress syndrome, we have studied the composition of membrane lipids in the bronchoalveolar lavage. Besides the surfactant phospholipids, we have identified another class of membrane lipids, the glycolipids, in bronchoalveolar lavage from ARDS patients. Traces, if any, of these lipids are present in bronchoalveolar lavage from healthy persons. The glycolipids appear during lung injury, and they are able to inhibit the surfactant system in vitro. Therefore, the finding of glycolipid accumulation in ARDS may contribute to our understanding on the pathobiochemistry of this severe disorder.

MATERIALS AND METHODS

Patients

Altogether, 225 bronchoalveolar lavage specimens from 176 patients were studied. The diagnostic categories of these patients have been described previously (6). The 26 cases of ARDS (65 lavage specimens) were defined as acute respiratory failure (i.e., requirement of mechanical ventilation and 50% or more of oxygen within 7 days of the insult) with panlobar infiltrates on the chest radiographs, and with pulmonary artery wedge pressure of <18 mm Hg. There were ten additional cases of respiratory failure; these differed from ARDS owing to the non-acute development of the respiratory failure or to the cardiac failure that was associated with the respiratory failure. The patients with respiratory failure were subjected to bronchoalveolar lavage several times during the course. The lavages were recovered from 0 to 38 days after the onset of the respiratory failure (6). There were 12 individuals with no evidence of active lung disease. Altogether, 128 other patients with various pulmonary diseases (without respiratory failure) were lavaged. The bronchoalveolar lavage glycolipids were analyzed in detail in eight cases. The four patients had ARDS associated with drowning, chest trauma, sepsis, or hypovolemic shock. Of the four controls three subjects had no lung disease, and one subject had local fibrosis in the left upper lobe.

Abbreviation: ARDS, adult respiratory distress syndrome.

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Bronchoalveolar lavage

Bronchoalveolar lavage was recovered through a fiberoptic bronchoscope that was wedged to a lower lobe segment. Lavage was performed using 20 ml of 0.9% saline of which 30–50% was recovered. The lavage return was then centrifuged at 140 *g* for 10 min at 4°C. The pellet was analyzed for differential cell count, and the supernatant was subjected to lipid analysis. There were no detectable differences in the quality or quantity of cells recovered from the ARDS cases as compared to the normal controls (6). The proteins in the lavage samples of ARDS patients were compared with those in plasma in order to determine whether the lavage fluid had sampled the zone of exudative proteins. Using sodium dodecyl sulfate polyacrylamide gel electrophoresis, the plasma and lavage proteins were found to be similar, if not identical (7).

Glycolipid analysis

Standard gangliosides containing one, two, or three sialic acid residues per molecule were isolated from pig brain according to a previously described procedure (8). Trihexosylceramide was isolated from a patient with Fabry disease. Globoside was kindly donated by Dr. S. Hakomori (Seattle, WA) and digalactosylceramide by Dr. C. C. Sweeley (East Lansing, MI). The glycolipids were isolated from human kidney or prepared by weak acid hydrolysis from the isolated gangliosides as described previously (9).

Qualitative glycolipid analysis was carried out on high-performance thin-layer plates (HPTLC plates of Merck) using chloroform–methanol–2.5 M NH_4OH 60:30:8 (v/v/v) as the solvent, or on laboratory-made silica gel H plates (6). The plates were stained for hexose with orcinol in H_2SO_4 and for neuraminic acid with resorcinol in HCl. Glycolipids were isolated on 0.1-mm-thick thin-layer plates, which were prepared from silica gel G. The plates were developed twice in chloroform–methanol–2.5 M NH OH_4 60:30:8 (v/v/v). The glycolipids were detected with iodine and eluted from the plates with chloroform–methanol–water 5:4:1 (v/v/v).

Analysis of monosaccharides and long-chain bases (sphingosines) of the glycolipids degraded in hydrochloric acid–methanol was carried out by gas–liquid chromatography as previously described (10). Linkages between the monosaccharide units were analyzed with permethylation (11). The glycolipids were permethylated with potassium butoxide–dimethyl sulfoxide (12) as previously described. Partially methylated monosaccharides obtained from the permethylated glycolipids in acid degradation were analyzed by gas–liquid chromatography–mass spectrometry using single ion monitoring for the detection of sugars (13).

Measurement of surface activity

The effect of the glycolipids on the surface properties of the natural human surfactant from the amniotic fluid (14) was studied in vitro. The glycolipid was added to a conical test tube and the organic solvent was evaporated to dryness. An aqueous suspension of human surfactant was added to the tube. The mixture was vortexed and freeze-dried. Thereafter, the dry powder was dispersed by vortexing in 150 mM NaCl–1.2 mM $CaCl_2$ to a concentration of 0.4 μmol of glycolipid/ml and 2.0 μmol surfactant phospholipid/ml. The surface activity was analyzed using the pulsating bubble sufactometer (Surfactometer International, Toronto) as described by Enhörning (15). The stroke volume of the pulsator unit was 0.43 μl, and the bubble was pulsating between the diameters of 1.1 and 0.8 mm at a rate of 30 rpm. The temperature during the assay was 37°C. The minimum and maximum surface tensions were constant between 15 and 120 sec after creation of the bubble.

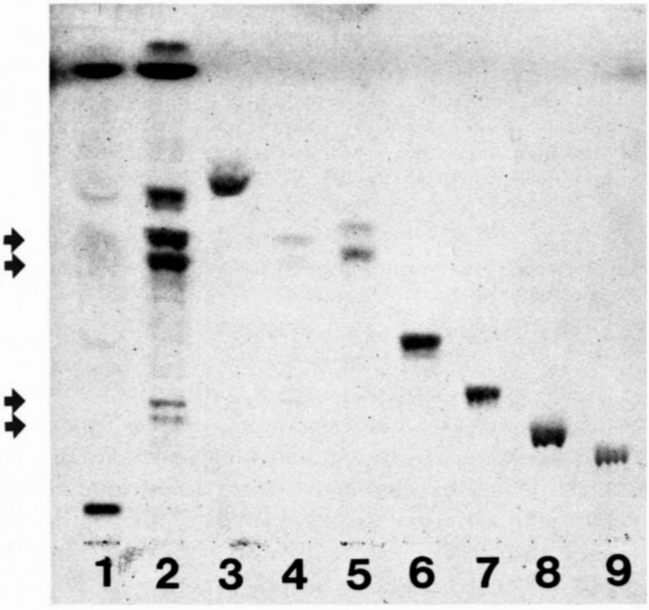

Fig. 1. Thin-layer chromatography of glycolipids from bronchoalveolar lavages. The high performance thin-layer plates were developed in chloroform–methanol–2.5 M NH_4OH 60:30:8 (v/v/v). 1, Control sample from a healthy subject (250 nmol of phospholipid phosphate); 2, sample from an ARDS patient (25 nmol of phospholipid phosphate). Standard glycolipids were run on lanes 3–9; 3, sulfatide (SO_4-Gal-Cer); 4, lactosyl ceramide (Gal-Glc-Cer); 5, digalactosylceramide (Gal-Gal-Cer); 6, trihexosylceramide (Gal-Gal-Glc-Cer); 7, globoside (GalNAc-Gal-Gal-Glc-Cer); 8, monosialoganglioside GM_1; 9, disialoganglioside GD_{1A}. Location of the hexose-positive stain in the lavage samples is marked by arrows. The other bands are due to charring, and are not hexose-positive. Note the intense carbohydrate-containing bands found in the ARDS sample (lane 2) but not in the control sample (lane 1) containing a ten times higher amount of lavage material (as phospholipid phosphate) than the ARDS sample. The gangliosides are designated according to Svennerholm (28).

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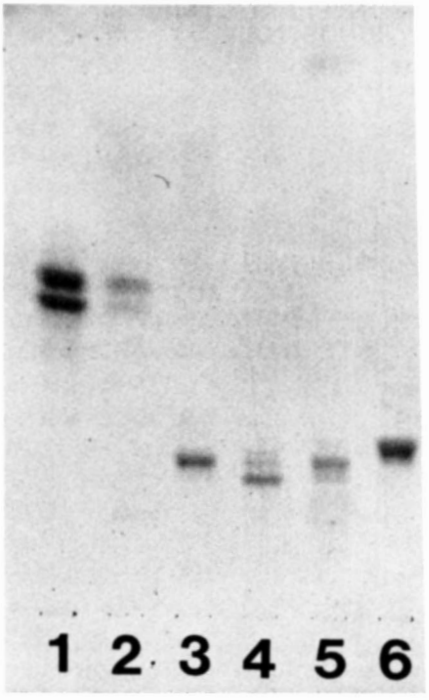

Fig. 2. Thin-layer chromatography of glycolipids isolated from a lavage sample of an ARDS patient. 1, Fast-moving material; 2, standard lactosylceramide (Gal-Glc-Cer); 3, upper band of the slow-moving material; 4, lower band of the slow-moving material; 5, standard paragloboside (Gal-GlcNAc-Gal-Glc-Cer); 6, standard globoside (GalNAc-Gal-Gal-Glc-Cer).

RESULTS

Detection of glycolipids in bronchoalveolar lavage

Thin-layer chromatography of bronchoalveolar lavages from healthy subjects or from patients having lung diseases without respiratory failure revealed only low or nondetectable levels of glycolipids. In contrast, a similar analysis of a total of 78 bronchoalveolar lavages from 36 cases of respiratory failure, including the 26 cases of ARDS, suggested strongly increased amounts of hexose-staining substances that contained no phosphorus. These specimens were recovered 0 to 38 days from the onset of respiratory failure. The carbohydrate-containing material from ARDS migrated on thin-layer chromatography as two doublet bands (**Fig. 1**). The fast-moving doublet corresponded to lactosylceramide, and the slow-moving doublet migrated between globoside and the monosialoganglioside GM_1 (Fig. 1).

Isolation and structure of the glycolipids

The fast-moving lipid was isolated by preparative thin-layer chromatography as a doublet characteristic of lactosylceramide from extraneural tissues (**Fig. 2**). Gas–liquid chromatographic analysis of the sugars and long-chain bases liberated by acid degradation (10) from the fast-moving material revealed galactose and glucose as the monosaccharide components in an approximately equimolar ratio (**Table 1**). The ratio of long-chain base (sphingosine) to glucose was 1:1 by gas–liquid chromatographic analysis. Permethylation, acid degradation, and the identification of the partially methylated monosaccharides (13), revealed a terminal galactose and a 4-O-substituted glucose in the approximate ratio of 1:1. Thus, thin-layer chromatography and analysis of the components liberated in the degradation of the fast-moving material indicated a lactosylceramide structure (galactose-β1, 4-glucose-ceramide).

The slow-moving material that stained for hexose was isolated as two closely moving fractions. These fractions corresponded to the paragloboside bands (Fig. 2). Analysis of the sugar composition of the fractions revealed galactose, glucose, and N-acetylglucosamine in the approximate ratio 2:1:1 (Table 1). Long-chain base (sphingosine) was found in an equimolar ratio to glucose. Methylation analysis of the upper fraction, which was obtained in a higher amount, revealed the presence of a terminal galactose, a 3-O-substituted galactose and a 4-O-substituted glucose in the approximate ratio of 1:1:1. This analysis of the slow-moving material agrees with the finding on thin-layer chromatography and suggests a paragloboside structure (galactose-β1, 4-N-acetylglucosamine-β1, 3-galactose-β1, 4-glucose-ceramide).

Analysis of fatty acids of the lactosylceramide and the main paragloboside fraction revealed 16:0, 18:0, and 24:1 as main components in both glycolipids (**Table 2**).

TABLE 1. Ratio of the monosaccharides in the glycolipids accumulating in bronchoalveolar space in ARDS

Material Isolated	Galactose	Glucose	Glucosamine
Diglycosylceramide area (fast-moving material)	1.07	1.00	N.D.
Upper band of the paragloboside area (slow-moving material)	2.05	1.00	1.17
Lower band of the paragloboside area (slow-moving material)	2.05	1.00	1.07

Monosaccharide composition of the glycolipids isolated from an ARDS patient was determined by gas–liquid chromatography (10) after acid methanolysis; N.D., not detectable.

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TABLE 2. Fatty acid composition of the glycolipids isolated from ARDS patients

Fatty Acid	Lactosylceramide	Paragloboside
14:0[a]	1.2	3.1
16:0	39.2	25.6
16:1	0.5	1.9
18:0	9.1	27.4
18:1	6.7	8.1
22:0	6.5	3.7
22:1	4.9	8.7
24:0	8.1	6.2
24:1	23.7	15.1

Fatty acids were analyzed as methyl esters on a 2.2% SE-30 column programmed from 160°C to 260°C. The data represent percentages based on peak areas.

[a] Number of carbon atoms: number of double bonds.

No hydroxy fatty acids were detected in samples analyzed directly or after trimethylsilylation.

Quantitative determination of the glycolipids

In agreement with the above data, high amounts of galactose and glucose were detected when a sample of lipid-soluble lavage material was degraded and analyzed with gas–liquid chromatography (10). In contrast, it was difficult to detect any sugar peaks from normal lavage material (**Fig. 3**). Thus, gas–liquid chromatography gives a result similar to the screening with thin-layer chromatography (Fig. 1). Quantitative analysis of glycolipid from the lavage material was carried out by measuring the amount of lipid-bound galactose, which gives the approximate molar amount of glycolipid (one mole of galactose per one mole of the main glycolipid). According to this analysis, the molar amount of glycolipid in ARDS is 10 to 20% of the total phospholipid (**Table 3**). The corresponding percentage is 0.5 or less in the control subjects (Table 3).

Effect of glycolipids on lung surfactant

Since the glycolipids could be recovered from the airways in ARDS, we studied whether they alter the surface activity of a natural human surfactant in vitro. In these preliminary experiments we used glycolipids from the brain as model components. As shown in **Table 4,** these glycolipids increased the surface tension obtainable at minimum bubble size. The minimum surface tensions of the glycolipid dispersions (0.4 μmol/ml) were more than 50 mN/m (data not shown).

DISCUSSION

In the present study we have detected two glycolipids that accumulate in the airways in acute, severe respiratory failure (ARDS). They are barely detectable in healthy subjects or in lung disease without respiratory failure (Figs. 1 and 3, Table 3). Comparison of these compounds to standards on thin-layer chromatography as well as isolation and analysis of the sugar and lipid components

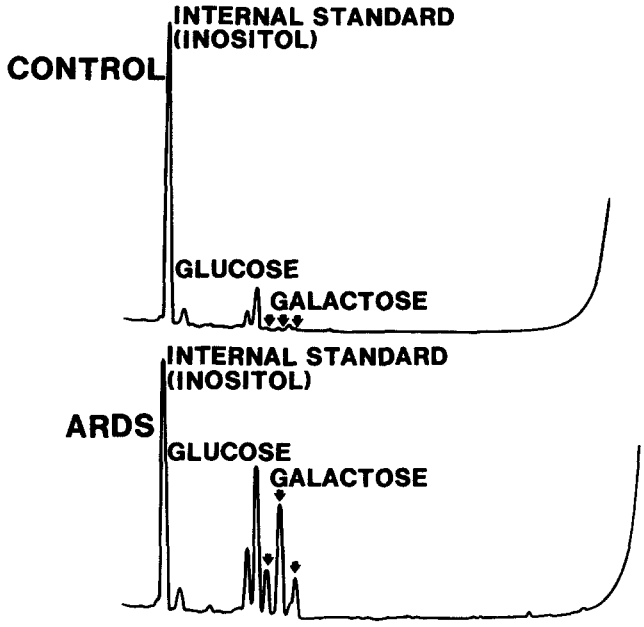

Fig. 3. Gas–liquid chromatography of monosaccharides from the bronchoalveolar lavages. Samples of lavage material were dissolved in chloroform–methanol 2:1 (vol/vol). Aliquots of the lipid-soluble material (82 nmol and 59 nmol of phospholipid phosphate of control and ARDS samples, respectively) containing 10 nmol of inositol (added as an internal standard) were methanolyzed and analyzed for monosaccharides as trimethylsilyl ethers (9). Some glucose could be detected in all samples (upper trace), including the blanks containing the solvents and reagents without any lavage material. Note the intense patterns of the three peaks characteristic of galactose (arrows) and the two peaks of glucose in the ARDS sample (lower trace).

TABLE 3. Lipid-bound galactose and phospholipid phosphate in bronchoalveolar lavage from four patients with ARDS and from four controls

	Lipid-Bound Galactose	Phospholipid Phosphate	Molar Ratio of Glycolipid to Phospholipid[a]
	nmol	*nmol*	$\times 10^2$
Patient No.			
1	84	650	12.9
2	30	297	10.1
3	224	1176	19.0
4	196	1124	17.4
Control No.			
1	N.D.[b] (<1)	758	<0.1
2	N.D. (<1)	1438	<0.1
3	N.D. (<1)	820	<0.1
4	8	1704	0.5

[a] Molar ratio of glycolipid to phospholipid was calculated assuming one mole of galactose per mole of glycolipid and one mole of phosphate per mole of phospholipid.

[b] N.D., not detectable.

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TABLE 4. Effect of the glycolipids on the surface properties of natural human surfactant (HS)

Material (n = 4)	Surface Tension (mN/m)	
	Minimum Bubble Size	Maximum Bubble Size
HS	2 ± 0.1	29.0 ± 0.9
HS + Lact-Cer	11.3 ± 2.0[a]	27.4 ± 2.7
HS + GM_1	6.9 ± 0.8[a]	34.7 ± 2.4
HS + GD_{1A}	10.2 ± 1.7[a]	21.3 ± 2.8[a]

The results are given as mean values ± SD of four independent experiments. The glycolipids were recovered from the brain. Their major fatty acid moiety was stearate (18:0). Lact-Cer, lactosylceramide; GM_1, monosialoganglioside; GD_{1A}, disialoganglioside.

[a] $P < 0.025$ as compared to HS.

(Figs. 1 and 2, Table 1) identified the glycolipids as lactosylceramide (Gal-β1,4-Glc-Cer) and paragloboside (Gal-β1,4-GlcNAc-β1,3-Gal-β1,4-Glc-Cer).

There are no previous reports on abnormal glycolipids in respiratory failure (16, 17). Slomiany, Smith, and Slomiany (18) have detected neutral glyceroglucolipids in alveolar lavage from healthy rabbits. We did not detect these lipids in our material. Instead, the major carbohydrate-containing lipids in the respiratory failure were identified as sphingolipids.

The origin of the abnormal glycolipids in bronchoalveolar space is not known at present. The presence of blood in the airways does not explain their occurrence, since no globoside could be detected as an indicator of red cells or plasma (19). Neutrophils are a possible source of glycolipids (20). They aggregate on pulmonary microvasculature or appear on alveolar spaces as a result of an inflammatory response to tissue injury, releasing superoxide radicals and proteolytic enzymes (21, 22). Both lactosylceramide and paragloboside have been identified in the neutrophils (20). However, the fatty acid composition of the neutrophil glycolipids is quite different from that found in the present study. Especially, the relative amount of the C16:0 fatty acid is much higher in the glycolipids isolated in this study (Table 2) than in the lactosylceramide and paragloboside of the neutrophils (20). Thus, the glycolipids of ARDS may derive from some other cells than the neutrophils. Another possible source of the glycolipids is some cell type of the lung tissue. Both lactosylceramide and paragloboside have been detected in lung (23, 24), from which the glycolipids could accumulate in the alveolar space in ARDS. We propose that the glycolipidosis in bronchoalveolar space indicates a membrane damage typical of ARDS irrespective of its etiology.

Phospholipids are thought to be mainly responsible for the surfactant activity essential for normal respiratory function (25). Previous studies have suggested that the amount of phospholipid in bronchoalveolar lavage return is not significantly different in ARDS from that in normal controls, but the phospholipid composition is abnormal. For example, the lecithin/sphingomyelin ratio is low (6). Furthermore, the lipid-protein complexes isolated from bronchoalveolar lavage of ARDS patients lack the normal surface activity (6, 26).

The present finding of strikingly increased amounts of alveolar glycolipids, approaching or exceeding those of individual surfactant phospholipids, adds a novel aspect to be considered in the pathogenesis of ARDS. Since the normal bilayer structure of phospholipid vesicles is disturbed or even disrupted in the presence of high amounts of glycolipids (27), excess glycolipids on alveolar lining may prove to be deleterious to the surfactant. We have found, using a pulsating bubble surfactometer, that model glycolipids increase the surface tension of natural human surfactant obtained at minimum bubble size corresponding to end-expiration (Table 4). The increase in surface tension took place when the phospholipid-glycolipid ratio was similar to that in ARDS. However, it is unknown whether the glycolipids recovered from the airways in ARDS inhibit the surfactant system in a healthy lung. Therefore, the potential role of the glycolipids as surfactant inhibitors remains unclear, and the significance of the present finding of a strikingly increased glycolipid in a cell-free bronchoalveolar lavage in respiratory failure remains to be further evaluated in terms of current hypotheses on the pathogenesis of this life-threatening disorder (3–5).

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