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Abnormal fatty alcohol metabolism in cultured keratinocytes from patients with Sjögren-Larsson syndrome

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Abstract Sjögren-Larsson syndrome (SLS) is an inherited neurocutaneous disorder characterized by ichthyosis, mental retardation, spasticity, and deficient activity of fatty aldehyde dehydrogenase (FALDH). FALDH is an enzyme component of fatty alcohol:NAD oxidoreductase (FAO), which is necessary for fatty alcohol metabolism. To better understand the biochemical basis for the cutaneous symptoms in this disease, we investigated lipid metabolism in cultured keratinocytes from SLS patients. Enzyme activities of FALDH and FAO in SLS cells were <10% of normal. SLS keratinocytes accumulated 45-fold more fatty alcohol (hexadecanol, octadecanol, and octadecenol) than normal, whereas wax esters and 1-*O*-alkyl-2,3-diacylglycerols were increased by 5.6-fold and 7.5-fold, respectively. SLS keratinocytes showed a reduced incorporation of radioactive octadecanol into fatty acid (24% of normal) and triglyceride (13% of normal), but incorporation into wax esters and 1-*O*-alkyl-2,3-diacylglycerol was increased by 2.5-fold and 2.8-fold, respectively. Our results indicate that FALDH deficiency in SLS keratinocytes causes the accumulation and diversion of fatty alcohol into alternative biosynthetic pathways. The striking lipid abnormalities in cultured SLS keratinocytes are distinct from those seen in fibroblasts and may be related to the stratum corneum dysfunction and ichthyosis in SLS.—Rizzo, W. B., D. A. Craft, T. Somer, G. Carney, J. Trafrova, and M. Simon. **Abnormal fatty alcohol metabolism in cultured keratinocytes from patients with Sjögren-Larsson syndrome. *J. Lipid Res.* 2008.** 49: **410–419.**

Supplementary key words aldehyde dehydrogenase • alkylglycerol • epidermis • ether glycerolipid • fatty aldehyde • ichthyosis • mental retardation • plasmalogen • stratum corneum • wax ester

Sjögren-Larsson syndrome (SLS) is an inherited autosomal recessive disorder characterized by ichthyosis, mental retardation, and spastic diplegia or tetraplegia (1). The disease is caused by mutations in the *ALDH3A2* gene that encodes fatty aldehyde dehydrogenase (FALDH) (2), an enzyme that oxidizes long-chain aliphatic aldehydes to fatty acids (3, 4). FALDH is necessary for the oxidation of aldehydes derived from fatty alcohol metabolism by interacting with fatty alcohol dehydrogenase as part of the fatty alcohol:NAD oxidoreductase (FAO) enzyme complex. Patients with SLS, therefore, exhibit deficient activity of both FALDH and FAO (1).

The pathogenic mechanisms responsible for cutaneous disease in SLS are not known, but they are thought to involve abnormalities in the lipid composition of the intercellular membranes in the stratum corneum (SC) (5). Most biochemical studies of SLS, however, have focused on cultured fibroblasts, plasma, or noncutaneous cells from patients, in which several lipid abnormalities have been found. Owing to FAO deficiency, patients with SLS accumulate long-chain fatty alcohols in cultured fibroblasts and plasma (6). In addition, fibroblasts from SLS patients have deficient metabolism of fatty aldehydes generated from the catabolism of ether glycerolipids (7). In vitro studies with SLS fibroblasts implicate FALDH in the conversion of phytol, a branched-chain fatty alcohol derived from the diet, to phytanic acid (8) and in the subsequent α-oxidation of phytanic acid that generates a fatty aldehyde product (9). ω-Oxidation of leukotriene B_4 (LTB4) is impaired in SLS leukocytes (10), and SLS patients have increased concentrations of LTB4 and its metabolite, 20-hydroxy-LTB4, in urine (11). In cultured fibroblasts from SLS patients, fatty aldehydes react with phosphatidylethanolamine, forming an unusual lipid

Manuscript received 26 February 2007 and in revised form 17 October 2007.
Published, JLR Papers in Press, October 30, 2007.
DOI 10.1194/jlr.M700469-JLR200

Abbreviations: CH_2Cl_2, dichloromethane; DMA, dimethyl acetal; FALDH, fatty aldehyde dehydrogenase; FAO, fatty alcohol:NAD oxidoreductase; HPTLC, high-performance thin-layer chromatography; LTB4, leukotriene B_4; N-alkyl-PE, *N*-alkyl-phosphatidylethanolamine; PKC, protein kinase C; SC, stratum corneum; SLS, Sjögren-Larsson syndrome; 16:0-OH, hexadecanol; 18:0-OH, octadecanol; 18:1-OH, octadecenol; 22:0-OH, behenyl alcohol.

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adduct, *N*-alkyl-phosphatidylethanolamine (N-alkyl-PE) (12). Other biochemical abnormalities reported in SLS patients include deficiency of serum polyunsaturated fatty acids that are products of δ-6 desaturation (13) and reduced levels of ceramide-1 and ceramide-6 in cutaneous scales (14).

Despite these studies, lipid metabolism has not been investigated in the cells that are directly responsible for the formation of the SC in SLS patients. We now report that cultured keratinocytes from SLS patients have defective fatty alcohol metabolism that results in a distinctive lipid profile characterized by the accumulation of wax esters and ether glycerolipids. These lipid abnormalities, which are unique to keratinocytes, may be implicated in the cutaneous pathogenesis of SLS.

MATERIALS AND METHODS

Human subjects

This research was approved by the Institutional Review Boards of the Medical College of Virginia (where the work was initiated), the University of Nebraska Medical Center, and the State University of New York at Stony Brook. After obtaining informed consent for all participants, skin punch biopsies were taken from the upper arms of four SLS patients and age- and sex-matched control subjects. At the time of skin biopsy, patient 1 was a 2.5 year old female who was homozygous for the c.710G>A mutation in the *ALDH3A2* gene, and patient 2 was a 2 year old female who was a compound heterozygote for c.1094C>T and c.471+2T>G. SLS patient 3 was a 4.5 year old male who carried two mutations, c.798G>C and c.943C>T. Patient 4 was a 9 year old male who was a compound heterozygote for c.386-6A>G and c.554G>C. All of the patients exhibited symptoms of ichthyosis, spastic diplegia, and mild mental retardation.

Materials

Lipid standards, including 1-*O*-hexadecyl-2,3-dipalmitoyl-*rac*-glycerol, 1-*O*-hexadecyl-*rac*-glycerol, and 1-*O*-octadecyl-*rac*-glycerol (batyl alcohol), were obtained from Sigma-Aldrich. [9,10-^{3}H]Octadecanol (7 Ci/mmol) was synthesized from radioactive oleic acid (Amersham) by catalytic hydrogenation of the fatty acid methyl ester with platinum oxide (15), regeneration of the free stearic acid by saponification, and subsequent reduction to the alcohol with lithium aluminum hydride (16). Fatty aldehydes were synthesized from the corresponding fatty alcohols by oxidation with dipyridine chromic anhydride complex and subsequently purified by TLC as described (17). The aldehydes were stored in hexane under a nitrogen atmosphere at −20°C. Standard silica gel G TLC plates (LK6D) and high-performance thin-layer chromatography (HPTLC) plates were obtained from Whatman. All solvents were analytical grade or HPLC grade from Mallinckrodt Chemicals or Fisher Chemical Co. Methanolic HCl was from Supelco, Inc. Mesyl chloride (methanesulfonyl chloride) and L-2,3-*O*-isopropylidene-*sn*-glycerol [(*R*)-(−)-2,2-dimethyl-1,3-dioxolane-4-methanol] were obtained from Sigma-Aldrich. All other chemicals were from Sigma-Aldrich.

Synthesis of 1-*O*-heptadecyl-glycerol

This synthesis was adapted from Zheng et al. (18). Ninety-three milligrams (0.36 mmol) of 1-heptadecanol and 184 mg (1.82 mmol) of triethylamine were dissolved in 5 ml of dichloromethane (CH_2Cl_2) and placed in a test tube with a stirring bar in an ice bath. Sixty-three milligrams (0.55 mmol) of mesyl chloride in 1 ml of CH_2Cl_2 was added dropwise over 2 min, and the solution was stirred for 60 min. The solution was warmed to 40°C, and the heptadecanol mesylate product was concentrated over a stream of nitrogen and then purified by TLC using solvent system A, consisting of CH_2Cl_2/ethyl acetate/triethylamine (95:5.0:0.5). The silica region containing heptadecanol mesylate ($R_f \sim 1$) was collected by scraping and extracted twice with solvent A, and the extract was dried under a stream of nitrogen.

KOH (21 mg, 0.380 mmol) in 63 μl of water was added to 40 ml of toluene in a glass beaker with a stirring bar. Fifty milligrams of L-2,3-*O*-isopropylidene-*sn*-glycerol was then added to the toluene/KOH and heated at ~110°C with stirring reflux for 15 min. After cooling slightly, the heptadecanol mesylate from the previous step was dissolved in 5 ml of toluene and added rapidly dropwise to the toluene solution, which was subsequently heated at reflux (110–115°C) for 3 h. Formation of the 1-*O*-heptadecyl-2,3-*O*-isopropylidene-*sn*-glycerol product was checked by TLC using solvent A. Three spots with R_f values of ~0.9, 0.7, and 0.4 were visualized after spraying the TLC plate with rhodamine G. The R_f = 0.7 spot corresponding to 1-*O*-heptadecyl-2,3-*O*-isopropylidene-*sn*-glycerol gave a prominent peak with a molecular ion at *m/z* 370 upon analysis by chemical ionization-GC-MS.

The remaining toluene solution containing the unpurified 1-*O*-heptadecyl-2,3-*O*-isopropylidene-*sn*-glycerol was then concentrated by boiling to ~15 ml and subsequently extracted by the addition of 20 ml of CH_2Cl_2 and 10 ml of sodium chloride-saturated water. After removal of the aqueous layer, the organic layer was dehydrated over Na_2SO_4 and dried under nitrogen. The dried product was dissolved in 5 ml of warm methanol, after which 0.15 ml of 12 N HCl was added to the solution and incubated at 45°C for 1.5 h. Conversion of the isopropylidene derivative to the 1-*O*-heptadecyl-*sn*-glycerol product ($R_f \sim 0.4$) was checked by TLC using a solvent system consisting of CH_2Cl_2/ethyl acetate (65:35).

To further purify the product from minor contaminants, the methanolic solution containing the 1-*O*-heptadecyl-*sn*-glycerol product was dried under nitrogen and reacted with 1 ml of pyridine and 0.5 ml of acetic anhydride for 1 h at 37°C. The acetylated product was purified by TLC using hexane-ether-acetic acid (60:40:1) as a solvent. The acetylated 1-*O*-heptadecylglycerol product, which comigrated on TLC with acetylated batyl alcohol (1-*O*-octadecyl-glycerol), was recovered by extracting the silica with methanol.

To regenerate the 1-*O*-heptadecyl glycerol, the acetylated product was dried under nitrogen, dissolved in 1.5 ml of 0.3 *N*-ethanolic sodium hydroxide, and heated at 90°C for 2 h. The purified product was then recovered by adding 2 ml of water and extracting twice with 3 ml of hexane. The combined hexane extracts were dried under nitrogen to give a white solid. The yield was ~60%.

Cells

Keratinocytes were routinely grown on lethally irradiated 3T3 feeder cells according to Rheinwald and Green (19) as modified by Randolph and Simon (20). Keratinocytes were grown at 37°C in medium with 5% FBS and harvested by scraping at 7 days after reaching confluence. No attempt was made to remove feeder cells, which accounted for <5–10% of the cells in the postconfluent keratinocyte cultures. Skin fibroblasts were grown in Dulbecco's minimal essential medium with 10% fetal bovine serum, penicillin, and streptomycin.

Enzyme assays

Cells were harvested by scraping and washed twice with PBS. Cell pellets were homogenized in 50 mM Tris-HCl, pH 8.0, and

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0.25 M sucrose using a motor-driven glass-Teflon homogenizer. FALDH was assayed using octadecanal as substrate (3), and FAO activity was determined using [^{3}H]octadecanol as substrate (21). Cell protein was measured according to Lowry et al. (22). Enzyme activities were expressed as pmol/min/mg protein.

Lipid analysis

Cell pellets were collected by scraping and washed twice with PBS. Cell pellets were sonicated in 1 ml of water using a probe sonicator and then homogenized using a motor-driven glass-Teflon homogenizer. An aliquot (0.05 ml) of the cell homogenate was removed for protein determination (22). For most analyses, 0.25 ml of cell homogenate was extracted overnight with 2.5 ml of chloroform-methanol (1:1). After removing insoluble material by centrifugation, lipid extracts were washed according to Folch, Lees, and Sloane Stanley (23) before analysis. The major lipid classes (phospholipids and neutral lipids) were separated by HPTLC on 10 cm plates using a modification of a three-stage solvent system described by Macala, Yu, and Ando (24). The plates were prewashed by development with chloroform-methanol-water (60:35:8) followed by chloroform-methanol-acetic acid-formic acid-water (35:15:6:2:1). Lipids were dissolved in chloroform-methanol (1:1), and 5–10 μl was spotted in a 0.5 cm line at the origin (1 cm above the bottom of the plate). The plate was developed sequentially using the following solvent systems with thorough drying between developments: *1*) chloroform-methanol-acetic acid-formic acid-water (35:15:6:2:1) to 40% up the plate; *2*) hexane-diethyl ether-acetic acid (32.5:17.5:1) to 70% up the plate; and *3*) toluene to the top of the plate. To better separate neutral lipids, the first solvent development was skipped and the plate was developed in the second solvent system to 40% up the plate, followed by toluene to the top. The plate was sprayed with 10% $CuSO_4$ in phosphoric acid, and lipids were visualized by heating at 180°C for 15 min. The plates were scanned and the images were analyzed using SigmaGel software. A mixture of the following standard lipids was cochromatographed: sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, cholesterol, triglyceride, 1-*O*-palmitoyl-2,3-dipalmitoylglycerol, wax ester (palmitoyl palmitate), and cholesterol oleate. Preliminary analyses were done to establish the linearity of detection for each lipid class to ensure that lipids did not exceed the linear range for quantitation. For every plate of cellular lipids, five lanes of varying amounts of lipid standards were simultaneously run to generate standard curves for quantitation. The amount of each cellular lipid was expressed as μg lipid/mg cell protein.

For fatty alcohol analysis, washed cell pellets were sonicated in water as described above. Free fatty alcohols were extracted and measured by gas chromatography according to Rizzo and Craft (6). Phospholipids were isolated by TLC and transmethylated for determination of fatty acid and dimethyl acetal (DMA) composition by gas chromatography (25).

To measure the wax ester content of the cells by gas chromatography, 10 μg of behenyl-arachidonate wax ester was added to the initial cell sonicate as an internal standard. Lipids were extracted according to Bligh and Dyer (26), dried under argon, and spotted on a silica gel TLC plate. The plate was developed sequentially with hexane and toluene. Wax ester regions were localized by exposure to iodine vapor, collected by scraping, and recovered from the silica gel by extracting twice with 2 ml of chloroform. After drying under argon, the wax esters were transmethylated with 1 N methanolic HCl. The fatty acid methyl esters and fatty alcohols were recovered by extracting with hexane, dissolved in CH_2Cl_2, and analyzed by gas chromatography on a Hewlett-Packard 5890 gas chromatograph equipped with a 30 m × 0.32 mm internal diameter HP-INNOWax column and flame ionization detector. The initial oven temperature was 150°C, injection temperature was 220°C, and detector temperature was 275°C. After 1 min, the oven temperature was increased at 15°C/min to 225°C, followed by 5°C/min to 260°C, for a final oven time of 2 min. Fatty alcohols and fatty acid methyl esters were identified according to retention times using appropriate standards. The amount of wax ester was estimated based on the sum of fatty alcohols (C14–C18) recovered using behenyl alcohol (22:0-OH) as the internal standard, and the cell content of wax esters was expressed as μg/mg protein.

Structural analysis of 1-*O*-alkyl-diacylglycerol by GC-MS

Keratinocyte lipids were extracted and separated by TLC as described above. The silica region corresponding to 1-*O*-alkyl-2,3-diacylglycerol was collected by scraping, and lipids were extracted with hexane-diethyl ether (3:1). The lipid extract was dried under a stream of nitrogen and hydrolyzed in 1.5 ml of 1 N methanolic NaOH at 90°C for 2 h. After the addition of 2 ml of water, nonsaponifiable lipids (containing alkylglycerol) were extracted twice into 3 ml of hexane, and saponifiable lipids (containing fatty acids) were extracted into hexane after the addition of 1 ml of 2 N HCl. The fatty acid composition of the saponifiable lipids was determined by GC after conversion to their methyl esters as described (25). The hexane-containing nonsaponifiable lipids and alkylglycerol were back-extracted with 1 ml of water and dried under nitrogen. Isopropylidene derivatives were prepared by dissolving the alkylglycerol in 1 ml of acetone and adding 15 μl of perchloric acid (27). After 15 min at room temperature, 2 ml of water was added and the isopropylidene glycerol derivatives were extracted twice into 2 ml of petroleum ether, combined, dried under nitrogen, and dissolved in 50 μl of hexane for GC-MS analysis. To prepare acetate derivatives, the dried alkylglycerols were treated with 0.5 ml of acetic anhydride and 1 ml of pyridine at room temperature overnight. After the addition of 2 ml of water, the acetate derivatives were extracted into hexane and either analyzed directly by GC-MS or further purified by TLC (R_f = 0.80) using a solvent system consisting of hexane-diethyl ether (90:10). Standards of 1-*O*-hexadecylglycerol and 1-*O*-octadecylglycerol were similarly derivatized.

Alkylglycerol samples were analyzed on an Agilent 6890N gas chromatograph equipped with a HP5-MS capillary column (30 m × 0.25 mm internal diameter) and a 5973 mass selective detector. The injector temperature was 250°C. The initial oven temperature was 120°C; after 2 min, the temperature was increased at 10°C/min to 330°C, which was maintained for 5 min. The mass detector was run in the positive chemical ionization mode using methane as the reagent gas. The ionizing energy was 150 eV, and the source temperature was 250°C.

Measurement of total 1-*O*-alkyl-glycerolipids and 1-*O*-alkyl-diacylglycerols in keratinocytes

To measure total 1-*O*-alkyl-glycerolipids (neutral and phospholipids) in keratinocytes, cells were collected and homogenized in 1 ml of water, and 0.5 ml was extracted overnight with 5 ml of chloroform-methanol (1:1). Five micrograms of 1-*O*-heptadecylglycerol was added as an internal standard. The lipids were washed according to Folch, Lees, and Sloane Stanley (23), dried under nitrogen, and treated with 1.5 ml of 1 N methanolic NaOH for 2 h at 90°C. After cooling to room temperature, 2 ml of water was added, nonsaponifiable lipids (including alkylglycerols) were extracted twice into 2 ml of hexane and back-washed with water, and the combined hexane extracts were dried under a stream of nitrogen. Isopropylidene derivatives of the alkylglycerols were prepared as described above and analyzed by chemical ionization-GC-MS. Alkylglycerols were quantitated against 1-*O*-hepta-

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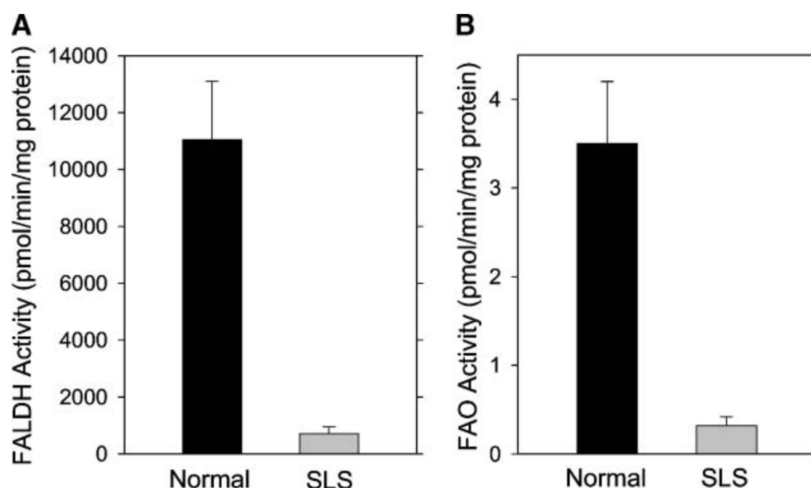

Fig. 1. Enzyme activities in cultured keratinocytes from normal controls and Sjögren-Larsson syndrome (SLS) patients. Data are expressed as means ± SD for four patients and four normal controls. A: Fatty aldehyde dehydrogenase (FALDH) activity. B: Fatty alcohol:NAD oxidoreductase (FAO) activity.

decylglycerol as an internal standard and expressed as ng alkylglycerol/mg cell protein.

To specifically measure 1-*O*-alkyl-diacylglycerol, the Folch-washed lipids were separated on a silica TLC plate using a two-stage solvent system consisting of initial development with hexane-diethyl ether-acetic acid (32.5:17.5:1) to 40% up the plate, followed by a second development with toluene to the top of the plate. 1-*O*-Palmitoyl-2,3-dipalmitoylglycerol was spotted as a standard. The corresponding silica region containing cellular 1-*O*-alkyl-diacylglycerols was visualized under ultraviolet light after spraying the plate with rhodamine G and collected by scraping, and the lipids were recovered by extracting twice with 4 ml of hexane-diethyl ether (3:1). Five micrograms of 1-*O*-heptadecylglycerol was added as an internal standard, and the lipids were dried under nitrogen. Lipids were treated with 1 N methanolic NaOH as described above, and nonsaponifiable lipids containing alkylglycerols were extracted into hexane (carefully avoiding the interface to prevent carryover of NaOH from the lower phase) and dried. Isopropylidene derivatives were prepared as described above and analyzed by chemical ionization-GC-MS. Initial studies showed an overall 80 ± 4% (n = 5) recovery of standard 1-*O*-hexadecyl-2,3-dihexadecylglycerol when corrected for the removal of fatty acids in the hydrolysis step (60.4% of the initial lipid mass). The intra-assay coefficient of variation was 5.2% (n = 5), and the interassay coefficient of variation determined over 5 days was 8.7%. The MS response factors for 1-*O*-hexadecylglycerol and 1-*O*-octadecylglycerol differed by <10% from that of 1-*O*-heptadecylglycerol, so no corrections were made for differences in detector response.

Metabolism of radioactive octadecanol by cultured keratinocytes

Keratinocytes cultured in 80 cm^2 flasks were incubated with 20 ml of medium containing (9,10-^{3}H]octadecanol (50,000 cpm/ml) for 24 h. Cells were washed three times with PBS and collected by scraping. Total cell lipids were extracted as above and separated by TLC using three sequential solvents consisting of *1*) hexane, *2*) toluene, and finally *3*) hexane-diethyl ether-acetic acid (80:20:1) to halfway up the plate. Lipids were visualized with iodine vapor and collected by scraping, and radioactivity was determined.

RESULTS

Activities of FALDH and FAO in cultured keratinocytes from SLS patients were <10% of the mean activities measured in normal controls (**Fig. 1**). Notably, the FALDH activity in normal keratinocytes was similar to that reported in fibroblasts (24), but the FAO activity in keratinocytes was reduced to ∼5–10% of that seen in fibroblasts.

Keratinocyte fatty alcohol content

Analysis of free fatty alcohols in the keratinocytes showed the presence of hexadecanol (16:0-OH), octadecanol (18:0-OH), and octadecenol (18:1-OH). The levels of 16:0-OH and 18:0-OH were greatly increased in SLS (**Table 1**). The cell content of 18:1-OH was more variably increased in the SLS cells. Added together, the total fatty alcohol content of the SLS keratinocytes was 45-fold higher than normal. Other fatty alcohols, including phytol, were not detected (data not shown). Like keratinocytes, fibroblasts from SLS patients also demonstrated a large

TABLE 1. Free fatty alcohol content of keratinocytes from SLS patients and normal controls

	Keratinocytes		Fibroblasts	
Fatty Alcohol	Controls	SLS	Controls	SLS
Hexadecanol (16:0-OH)	3 ± 2	155 ± 62[a]	12 ± 5	105 ± 21[a]
Octadecanol (18:0-OH)	2 ± 3	58 ± 8[a]	6 ± 3	60 ± 23[b]
Octadecenol (18:1-OH)	0.2 ± 0.4	10 ± 13	0.2 ± 0.4	5 ± 6
Total	5 ± 4	223 ± 60[a]	19 ± 8	170 ± 32[a]

SLS, Sjögren-Larsson syndrome. Data are expressed as ng fatty alcohol/mg cell protein (means ± SD) for five to six determinations.
[a] $P < 0.001$ using *t*-test comparing normal and SLS cells.
[b] $P = 0.001$ using *t*-test comparing normal and SLS cells.

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TABLE 2. Lipid content of cultured keratinocytes and fibroblasts determined by high-performance thin-layer chromatography

Lipid	Keratinocytes		Fibroblasts	
	Controls	SLS	Controls	SLS
Sphingomyelin	12 ± 5	17 ± 5	77 ± 13	63 ± 12
Phosphatidylcholine	27 ± 11	38 ± 8	146 ± 31	121 ± 20
Phosphatidylethanolamine + phosphatidylinositol + phosphatidylserine	36 ± 7	38 ± 6	110 ± 17	92 ± 16
Cholesterol	23 ± 4	22 ± 3	49 ± 9	38 ± 5
Triglyceride	6 ± 1	6 ± 1	18 ± 5	16 ± 3
1-*O*-Alkyl-2,3-diacylglycerol	0.4 ± 0.3	3.0 ± 0.6[a]	Not detected	Not detected
Wax esters	0.6 ± 0.4	3.4 ± 1.0[b]	Not detected	Not detected
Cholesteryl esters	5 ± 3	5 ± 2	4 ± 2	7 ± 2

Data are expressed as μg lipid/mg cell protein (means ± SD) for four SLS patients and four normal controls. Note that the three-stage solvent system (see Materials and Methods) used for these experiments did not adequately separate phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine; therefore, they were measured together.

[a] $P < 0.001$ using *t*-test comparing normal and SLS cells.

[b] $P < 0.01$ using *t*-test comparing normal and SLS cells.

increase in fatty alcohol content compared with controls (Table 1).

Keratinocyte lipid analysis by HPTLC

To detect other possible lipid abnormalities in SLS, lipid extracts of normal and SLS keratinocytes and fibroblasts were screened by HPTLC using two solvent systems, one that separated most major phospholipids and neutral lipids (**Table 2**) and a second that better separated neutral lipids. As shown in **Fig. 2**, SLS keratinocytes showed a noticeable accumulation of wax esters and a lipid that comigrated with 1-*O*-alkyl-2,3-diacylglycerol. These two lipids were relatively minor components in normal cells, together accounting for ~1% of the total lipids (Table 2). In SLS keratinocytes, wax esters were increased by 5.6-fold and the putative 1-*O*-alkyl-diacylglycerol was increased by 7.5-fold above normal; together, they constituted 6.4% of the total lipids (Table 2). In addition, an unidentified lipid, perhaps diester wax, that migrated immediately below the wax ester region in keratinocytes, appeared to be increased in the SLS cells (Fig. 2). Other lipid classes, including cholesterol, triglycerides, and phospholipids, were not significantly different from normal cells. Unlike keratinocytes, wax esters and 1-*O*-alkyl-diacylglycerol were not detected in fibroblasts from SLS patients or controls, and the other lipid classes were comparable (Table 2).

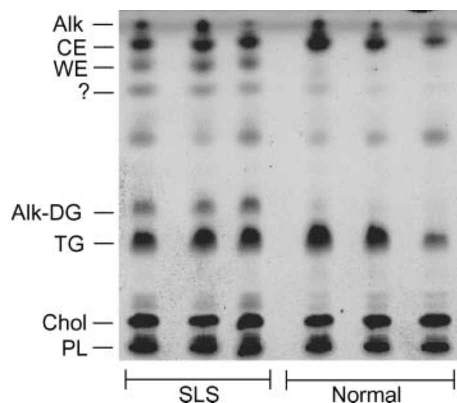

Fig. 2. High-performance thin-layer chromatography analysis of keratinocyte neutral lipids from three normal controls and three SLS patients. Total cellular lipids (corresponding to 1.2–1.9 mg of cell protein) were spotted on each lane. Lipids were separated using a two-step solvent system consisting of hexane-diethyl ether-acetic acid (32:17:1) developed to 40% up the plate, followed by development in toluene to the top. Lipids were visualized by charring. Alk, alkane; CE, cholesteryl ester; WE, wax ester; ?, unidentified lipid; Alk-DG, alkyl-diacylglycerol; TG, triglyceride; Chol, cholesterol; PL, phospholipid.

Keratinocyte wax ester analysis

The fatty alcohol and fatty acid composition of the wax esters purified from SLS keratinocytes was determined by gas chromatography. The fatty alcohols corresponded to those that accumulated as free alcohols (16:0-OH, 18:0-OH, and 18:1-OH) (data not shown). The fatty acids chiefly consisted of 16:1, 16:0, 18:1, and 18:0. Using 22:0-OH derived from behenyl-arachidonate wax ester as an internal standard, the SLS keratinocytes had 9.6-fold more wax esters than did the normal control cells.

Identification of 1-*O*-alkyl-diacylglycerol by GC-MS

The keratinocyte lipid that comigrated on HPTLC with 1-*O*-alkyl-2,3-diacylglycerol was collected and subjected to alkaline hydrolysis to release the fatty acids, and the resulting alkylglycerols were converted to isopropylidene derivatives for subsequent analysis by chemical ionization-GC-MS. As shown in **Fig. 3A, B**, the hydrolyzed lipid products from SLS and normal keratinocytes were identified as 1-*O*-pentadecylglycerol (peak 1), 1-*O*-hexadecylglycerol (peak 3), 1-*O*-octadecylglycerol (peak 7), and two monounsaturated isomers of 1-*O*-octadecenylglycerol (peaks 5 and 6). The identities of peaks 3 and 7 were established by comparison of their retention times and mass spectra with those of authentic standards. As shown in Fig. 3C, the mass spectrum for 1-*O*-hexadecylglycerol (peak 3) had prominent ions at m/z 357 [M+1], m/z 355 [M−1], m/z 341 [M−15], and m/z 299 [M−57], identical to the spectrum of authentic 1-*O*-hexadecylglycerol (data

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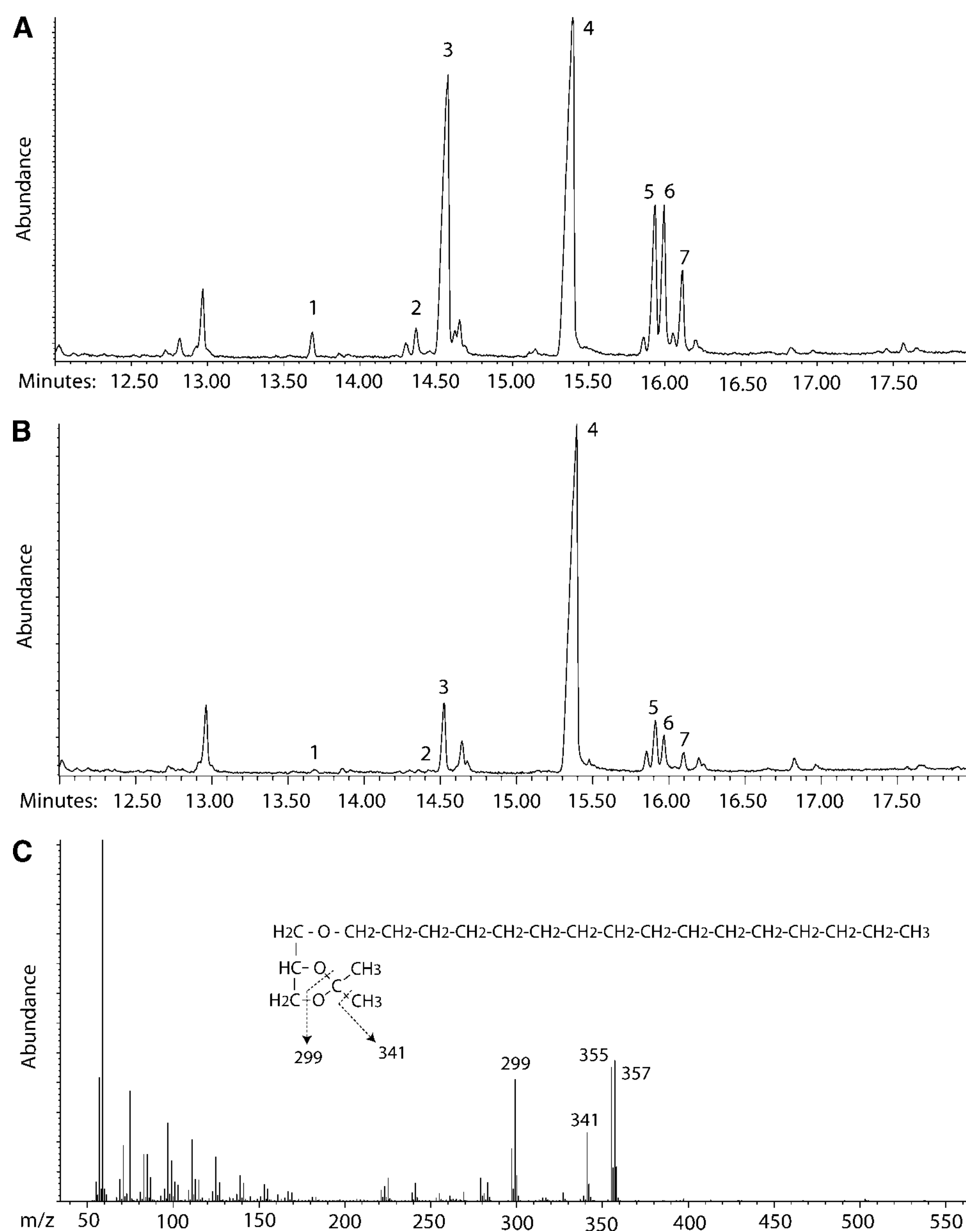

Fig. 3. Identification of 1-*O*-alkyl-2,3-diacylglycerol derived from SLS keratinocytes. The alkyl-diacylglycerols were purified by TLC and subjected to alkaline hydrolysis to produce deacylated 1-*O*-alkylglycerols before the formation of isopropylidine derivatives and analysis by chemical ionization-GC-MS. 1-*O*-Heptadecylglycerol (peak 4) served as an internal standard. A, B: Total ion chromatograms of SLS keratinocyte lipids (A) and normal keratinocyte lipids (B). Peak 1, 1-*O*-pentadecylglycerol; peak 2, 1-*O*-hexadecenylglycerol; peak 3, 1-*O*-hexadecylglycerol; peak 4, 1-*O*-heptadecylglycerol internal standard; peaks 5, 6, 1-*O*-octadecenylglycerol; peak 7, 1-*O*-octadecylglycerol. C: Mass spectrum of peak 3 confirms its structure as 1-*O*-hexadecyl-2,3-isopropylidine-glycerol.

not shown) and characteristic of other 1-*O*-alkylglycerols. The identification of peak 7 as 1-*O*-octadecylglycerol was similarly established by comparison with an authentic standard, whereas peaks 5 and 6 were inferred to be positional double bond isomers of 1-*O*-octadecenylglycerol based on their distinct retention times and identical spectra with prominent [M+1], [M−1], [M−15], and [M−57] ions of 2 mass units less than that seen for peak 7. Peak 2 was similarly identified as 1-*O*-hexadecenylglycerol. We did not, however, determine the position of the double bond in the alkyl chains. The formation of isopropylidene derivatives rules out the possibility that the alkyl chain was in the *sn*-2 position. The identities of these alkylglycerols were further confirmed by GC-MS analysis of their acetate derivatives (data not shown).

The major fatty acids constituting the acyl groups in the 1-*O*-alkyl-diacylglycerol fraction in SLS keratinocytes consisted of 16:1 (4.6–7.4%), 16:0 (12.7–13.9%), 18:2 (10.5–12.3%), 18:1 (30.0–33.8%), and 18:0 (6.4–10.4%). No significant differences were seen in the fatty

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acid composition of this lipid derived from SLS and normal keratinocytes.

Measurement of 1-*O*-alkyl-diacylglycerol lipids by chemical ionization-GC-MS

We measured 1-*O*-alkyl-diacylglycerol lipids in keratinocytes after purification by TLC, alkaline hydrolysis, and conversion of the alkylglycerols to their isopropylidene derivatives. As shown in **Table 3** and Fig. 3, SLS keratinocytes accumulated each of the 1-*O*-alkylglycerol species detected (15:0, 16:1, 16:0, 18:1, and 18:0) compared with normal cells. The sum of these 1-*O*-alkylglycerol species was 240 ± 63 ng/mg protein in normal keratinocytes and 1,378 ± 427 ng/mg protein in SLS cells ($P = 0.01$), which corresponded to a 5.7-fold increase.

To measure the total 1-*O*-alkylglycerol content of the cells, including ether phospholipids and 1-*O*-alkyl-diacylglycerols, total cellular lipid extracts were analyzed without TLC fractionation. The cellular content of total 1-*O*-alkylglycerol lipids in normal keratinocytes was 432 ± 94 ng/mg protein (n = 3), whereas SLS cells had 2,512 ng/mg protein (n = 2).

Metabolism of [^{3}H]18:0-OH in keratinocytes

To investigate the metabolism of fatty alcohol in intact cells, SLS and normal keratinocytes were incubated with [^{3}H]18:0-OH and its incorporation into several lipids was measured (**Fig. 4**). In SLS keratinocytes, there was a significant reduction in the incorporation of radioactivity into fatty acids (24% of normal) and triglycerides (13% of normal), whereas radioactive wax esters and 1-*O*-alkyl-2,3-diacylglycerol were increased by 2.5-fold and 2.8-fold, respectively. This diversion of [^{3}H]18:0-OH away from fatty acid pathways to fatty alcohol biosynthetic pathways was estimated by calculating the ratio of radioactivity incorporated into wax esters + 1-*O*-alkyl-2,3-diacylglycerol compared with fatty acids + triglycerides. This ratio was increased by 16-fold in SLS keratinocytes (7.35 ± 1.16) compared with normal cells (0.46 ± 0.7).

Phospholipid fatty acid and DMA analysis

To search for deficient polyunsaturated fatty acids that are products of δ-6 desaturation (13), we analyzed the fatty acid composition of total phospholipids purified from normal and SLS keratinocytes. No convincing differences were seen in the relative amounts of 18:2(n-6), 18:3(n-6), 20:3(n-6), and 20:4(n-6) (**Table 4**). In these analyses, the ether-linked alkenyl chains of 1-*O*-alk-1-enyl-glycerophospholipids (plasmalogens) are converted to saturated DMA derivatives. Although there was a trend toward increased 16:0-DMA and 18:0-DMA in the phospholipids from the SLS keratinocytes, it did not reach statistical significance.

TABLE 3. 1-*O*-Alkylglycerols derived from 1-*O*-alkyl-diacylglycerol lipids in cultured keratinocytes from SLS patients and normal controls

Alkyl Chain	Controls	SLS	*P*
15:0	8 ± 4	42 ± 8	<0.01
16:1	5 ± 2	51 ± 13	<0.01
16:0	95 ± 28	675 ± 213	<0.01
18:1[a]	57 ± 22	258 ± 71	0.01
18:1[b]	55 ± 13	245 ± 64	<0.01
18:0	21 ± 2	106 ± 64	0.08
Total	240 ± 63	1,378 ± 427	0.01

1-*O*-Alkylglycerols were converted to isopropylidene derivatives and measured by chemical ionization-GC-MS using 1-*O*-heptadecylglycerol as an internal standard. Data are expressed as ng 1-*O*-alkylglycerol/mg cell protein (means ± SD; n = 3). Statistical significance between normal and SLS keratinocytes was determined using a *t*-test.

[a] Peak 5 in the chromatogram in Fig. 3.

[b] Peak 6 in the chromatogram in Fig. 3.

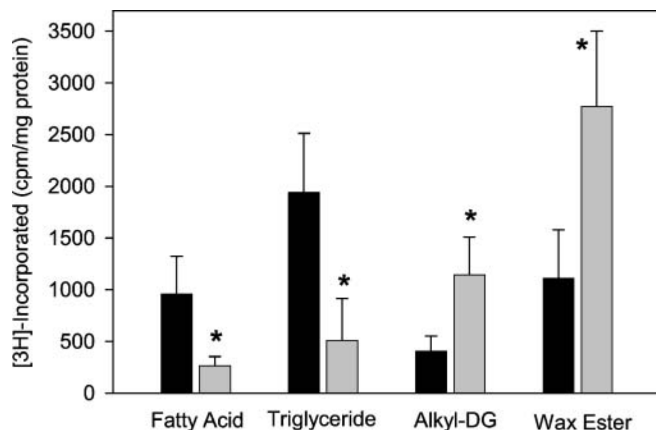

Fig. 4. Incorporation of [^{3}H]octadecanol (18:0-OH) into keratinocyte lipids. Cells were incubated with [^{3}H]18:0-OH for 24 h, after which cellular lipids were extracted and separated by TLC. Black bars, normal control cells; gray bars, SLS cells. Data are expressed as cpm/mg protein (means ± SD) for four determinations from two experiments. * $P \leq 0.01$ using Student's *t*-test. Alkyl-DG, alkyl-diacylglycerol.

DISCUSSION

Cultured keratinocytes from SLS patients show a striking accumulation of fatty alcohols, wax esters, and 1-*O*-alkyl-2,3-diacylglycerol lipids. This lipid profile is distinct from that seen in SLS fibroblasts, which accumulate fatty alcohol only. Despite both cell types having a comparable deficiency of FALDH and FAO, the SLS keratinocytes respond to this enzymatic defect by diverting fatty alcohol into biosynthetic pathways leading to wax esters and neutral alkyl-diacylglycerols (1-*O*-alkyl-2,3-diacylglycerol) (**Fig. 5**). Unlike the neutral lipids, convincing increases were not seen in the plasmalogen phospholipid levels in keratinocytes, as estimated by phospholipid DMA composition. Previous studies have shown that DMA levels in erythrocytes from SLS patients are either normal or mildly increased (21).

FAO deficiency in SLS keratinocytes resulted in a large increase in fatty alcohol content similar to that seen in SLS fibroblasts. Cellular fatty alcohol levels are determined by their biosynthetic rate and subsequent flux through other metabolic pathways (Fig. 5). These pathways function in the form of a "fatty alcohol cycle" in which fatty alcohol is synthesized from fatty acid and used for bio-

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TABLE 4. Fatty acid and DMA composition of keratinocyte phospholipids

Fatty Acid	Controls	SLS
14:0	1.97 ± 0.76	1.65 ± 0.29
15:0	0.52 ± 0.12	0.40 ± 0.06
16:0	19.80 ± 1.45	18.90 ± 0.26
16:1(n-7)	10.62 ± 2.28	8.23 ± 2.21
17:0	0.77 ± 0.14	0.62 ± 0.11
18:0	14.34 ± 2.78	16.94 ± 4.03
18:1(n-9)	28.83 ± 3.63	28.65 ± 2.81
18:2(n-6)	6.39 ± 2.36	6.03 ± 1.95
18:3(n-3)	0.08 ± 0.03	0.08 ± 0.02
18:3(n-6)	0.06 ± 0.03	0.06 ± 0.03
19:0	0.10 ± 0.02	0.09 ± 0.01
20:0	0.36 ± 0.11	0.47 ± 0.17
20:1(n-9)	0.49 ± 0.10	0.80 ± 0.15[a]
20:3(n-6)	0.28 ± 0.17	0.26 ± 0.21
20:4(n-6)	0.76 ± 0.27	0.71 ± 0.24
22:0	0.42 ± 0.13	0.59 ± 0.23
22:1(n-9)	0.41 ± 0.08	0.76 ± 0.29[b]
22:4(n-3)	0.07 ± 0.02	0.14 ± 0.08
22:6(n-3)	0.65 ± 0.28	0.79 ± 0.04
24:0	0.49 ± 0.24	0.49 ± 0.07
24:1(n-9)	0.37 ± 0.11	0.51 ± 0.13
26:0	0.22 ± 0.12	0.34 ± 0.09
26:1	0.13 ± 0.02	0.20 ± 0.08
DMA		
16:0	0.40 ± 0.13	1.01 ± 0.46
18:0	0.10 ± 0.06	0.26 ± 0.15
16:0 + 18:0	0.49 ± 0.19	1.27 ± 0.61

DMA, dimethyl acetal derivatives derived from ether-linked alcohol. Data are expressed as the percentage of total peak area (means ± SD) in cells from four SLS patients and six normal controls.

[a] $P < 0.01$.

[b] $P < 0.05$.

synthetic purposes (ether lipids and wax esters) and excess alcohol is recycled back to fatty acid (16). In cultured fibroblasts, the synthesis and oxidation of fatty alcohol occurs simultaneously. A block in fatty alcohol oxidation in SLS (28) or impaired incorporation of alcohol into ether lipid biosynthesis, as seen in patients with rhizomelic chondrodysplasia punctata (29), results in fatty alcohol accumulation. In genetically mutant cultured Chinese hamster ovary cells, the combination of FAO deficiency and defective ether glycerolipid synthesis leads to a much greater accumulation of fatty alcohol than either metabolic defect alone (30), suggesting that fatty alcohol can be diverted through alternative pathways when one pathway is blocked. This conclusion is supported by our studies demonstrating preferential channeling of radioactive 18:0-OH into wax esters and 1-*O*-alkyl-2,3-diacylglycerol in SLS keratinocytes.

Under normal circumstances, the enzymes responsible for fatty alcohol synthesis and oxidation are regulated to provide adequate lipid substrate for biosynthetic needs. In tumors that have a high rate of ether phospholipid biosynthesis, the activity of the fatty alcohol-synthesizing enzyme, acyl-CoA reductase, tends to be high and FAO activity is low (31). The reverse pattern is seen in tissues, such as liver, that have very low amounts of ether lipids or wax esters. We found that keratinocytes possessed a very low FAO activity compared with fibroblasts, consistent with a high fatty alcohol substrate requirement for lipid biosynthesis. The large increase in wax esters and alkyldiacylglycerols in the SLS keratinocytes suggests that the amount of these lipids is determined in part by alterations in the intracellular fatty alcohol pool size rather than solely by the activity of their respective biosynthetic enzymes.

Ether glycerolipids are widely distributed in mammalian tissues and have been studied extensively. Many ether lipids, such as plasmalogen forms of phosphatidylethanolamine or phosphatidylcholine, have a vinyl ether-linked unsaturated alkyl chain in the *sn*-1 position of the glycerol backbone and are phosphorylated at the *sn*-3 position. Neutral ether glycerolipids lack this phosphate group and are far less abundant in most tissues. The skin, however, may be an exception. Oku et al. (32, 33) found that 1-*O*-alkyl-2,3-diacylglycerol was synthesized by cultured rat keratinocytes and appeared on the skin surface of hairless mice. The function of 1-*O*-alkyl-2,3-diacylglycerol is not known. Plasmalogen ether phospholipids are thought to have antioxidant activity by virtue of their oxygen-sensitive vinyl ether group (34). Reactive oxygen species arising from intracellular oxidative stress cause a cleavage of the vinyl ether bond of the alkyl chain, which is released as fatty aldehyde (35). Increases in neutral vinyl ether glycerolipids in SLS may theoretically offer protection against oxidative stress, but any benefit would probably be offset by a greater production of detrimental fatty aldehydes, which are poorly metabolized because of FALDH deficiency (7).

Hernell et al. (13) and others (36) have reported that SLS patients have deficient serum polyunsaturated fatty

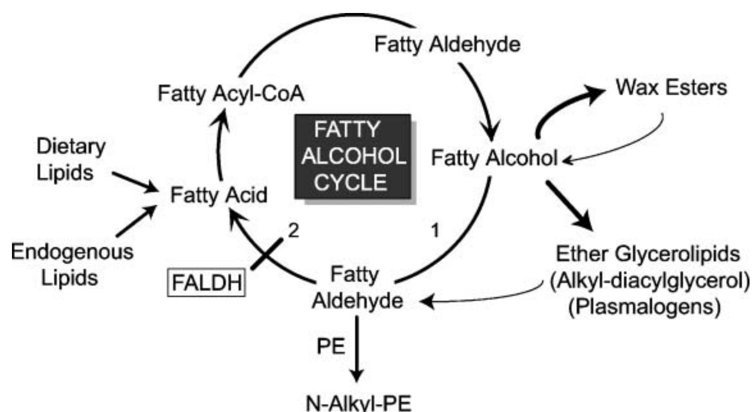

Fig. 5. Fatty alcohol cycle and metabolic pathways altered in SLS keratinocytes. FAO consists of two components, fatty alcohol dehydrogenase (reaction 1) and FALDH (reaction 2), that catalyze the sequential oxidation of fatty alcohol to fatty acid. The line across reaction 2 indicates the metabolic block in SLS. Increased fatty alcohols in SLS are diverted into the biosynthesis of wax esters and ether glycerolipids. PE, phosphatidylethanolamine; N-Alkyl-PE, *N*-alkyl-phosphatidylethanolamine.

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acids that are derived from δ-6 desaturation. They found normal levels of the essential fatty acid linoleic acid, 18:2(n-6), but decreased 18:3(n-6) and 20:3(n-6) in serum phospholipids of patients. However, Avigan et al. (37) found normal desaturase activity and no reduction in polyunsaturated fatty acids in cultured skin fibroblasts from SLS patients, consistent with our keratinocyte fatty acid data. It is possible that the in vivo serum fatty acid deficiencies in SLS reflect a more selective tissue-specific lipid derangement that is not reproduced in keratinocyte or fibroblast cultures.

It is likely that in vivo lipid abnormalities in the keratinocytes of SLS patients are responsible for the cutaneous symptoms of this disease. The pathogenesis of most forms of ichthyosis culminates in a disruption of the epidermal water barrier, resulting in increased transepidermal water loss and dehydration of the skin. The water barrier is critically dependent on the lipid composition and proper formation of the intercellular membranes of the SC (38–40). These membranes are largely composed of ceramides, cholesterol, and free fatty acids. Membranes destined for the SC are synthesized in granular keratinocytes and packaged into cytoplasmic lamellar bodies. These membrane-rich structures subsequently fuse with the apical surface of the cell plasma membrane and extrude their contents into the keratinocyte-SC boundary, where further lipid processing occurs in association with membrane maturation. Ultimately, the membranes are assembled into highly organized stacked lamellar structures in the SC that attach to and interdigitate between the dead corneocytes. Alterations in the lipid composition of these membranes by experimental manipulation or originating from inborn errors of lipid metabolism can disrupt the epidermal water barrier and lead to ichthyosis (39). Ultrastructural studies of the skin in SLS reveal misshapen lamellar bodies, some lacking membranes, abnormal cytoplasmic lamellar inclusions in the granular keratinocytes, and lipid droplets in the SC, indicative of defective in vivo lipid metabolism (41, 42).

Although the composition of the lipid inclusions in SLS skin is not known, our results in cultured keratinocytes suggest that they might be composed of fatty alcohol, wax esters, and/or 1-*O*-alkyl-2,3-diacylglycerol. Fatty alcohols are usually minor lipids and are not known to be present in the SC membranes. Neutral ether glycerolipids (33) and wax esters (43, 44) are prominent lipids on the skin surface, where they are thought to be synthesized in sebaceous glands and secreted onto the skin, but neither lipid is considered a structural component of the SC membranes. If these lipids accumulate in granular keratinocytes of the skin to the extent seen in cultured SLS keratinocytes, they may affect lamellar body membrane formation, alter the lipid composition of the SC membranes, and disrupt the epidermal water barrier. Additional lipid abnormalities, such as increases in N-alkyl-PE (12) and deficiencies of polyunsaturated fatty acids (13) or ceramides (14), might also contribute to the cutaneous lipid defect.

Aside from a direct structural effect on the SC membranes, our results also raise the intriguing possibility that the unique lipid abnormality in SLS keratinocytes may interfere with the normal process of keratinocyte differentiation by perturbing key cell signaling pathways. Keratinocyte differentiation is induced by certain physiologic agents, including cholesterol sulfate (45, 46), 1,25-dihydroxyvitamin D_3 (47), and increased intracellular calcium (48), which stimulate protein kinase C (PKC) activity, either directly or indirectly, through generation of the lipid mediator 1,2-diacylglycerol. Several isoforms of PKC are present in cultured keratinocytes (49), and at least two (PKCη and PKCδ) have been implicated in the process of epidermal cell differentiation (50). Previous studies have shown that rat brain PKC activity is inhibited by 1-*O*-alkylglycerol (51, 52) and stimulated by 1-*O*-alkyl-2-acylglycerol (53), two potential metabolites of 1-*O*-alkyl-2,3-diacylglycerol. Interestingly, medium-chain fatty alcohols (C8–C10) either inhibit or enhance PKCα activity in a complex manner, but the effects of longer chain alcohols, which accumulate in SLS cells, have not been studied (54). Therefore, it is intriguing to speculate that two of the lipids (or their metabolites) that accumulate in SLS keratinocytes might influence PKC activity and interfere with certain steps in cell differentiation. Abnormalities in 12*R*-eicosanoid metabolism and intracellular signaling have also been hypothesized to exist in SLS (55). Studies to investigate the potential role of fatty alcohol-related lipids on cell signaling in SLS keratinocytes may reveal clues about the pathogenic mechanisms causing epidermal dysfunction in this disease.

This research was supported by Grant AR-44552 from the National Institute of Arthritis, Musculoskeletal, and Skin Diseases and by National Institutes of Health Grant P20 RR-016469 from the Institutional Development Award Networks of Biomedical Research Excellence Program of the National Center for Research Resources. The authors thank Rebecca Spieker for technical assistance.

REFERENCES

1. Rizzo, W. B. 2001. Sjögren-Larsson syndrome: fatty aldehyde dehydrogenase deficiency. *In* The Metabolic and Molecular Bases of Inherited Disease. C. R. Scriver, K. Beckman, G. M. Small, and D. Valle, editors. McGraw-Hill, New York. 2239–2258.
2. De Laurenzi, V., G. R. Rogers, D. J. Hamrock, L. N. Marekov, P. M. Steinert, J. G. Compton, N. Markova, and W. B. Rizzo. 1996. Sjögren-Larsson syndrome is caused by mutations in the fatty aldehyde dehydrogenase gene. *Nat. Genet.* **12:** 52–57.
3. Kelson, T. L., J. M. Secor McVoy, and W. B. Rizzo. 1997. Human liver fatty aldehyde dehydrogenase: microsomal localization, purification, and biochemical characterization. *Biochim. Biophys. Acta.* **1335:** 99–110.
4. Rizzo, W. B., and D. A. Craft. 1991. Sjögren-Larsson syndrome. Deficient activity of the fatty aldehyde dehydrogenase component of fatty alcohol:NAD^+ oxidoreductase in cultured fibroblasts. *J. Clin. Invest.* **88:** 1643–1648.
5. Rizzo, W. B. 2007. Sjögren-Larsson syndrome: molecular genetics and biochemical pathogenesis of fatty aldehyde dehydrogenase deficiency. *Mol. Genet. Metab.* **90:** 1–7.
6. Rizzo, W. B., and D. A. Craft. 2000. Sjögren-Larsson syndrome: accumulation of free fatty alcohols in cultured fibroblasts and plasma. *J. Lipid Res.* **41:** 1077–1081.
7. Rizzo, W. B., E. Heinz, M. Simon, and D. A. Craft. 2000. Microsomal fatty aldehyde dehydrogenase catalyzes the oxidation of aliphatic

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aldehyde derived from ether glycerolipid catabolism: implications for Sjögren-Larsson syndrome. *Biochim. Biophys. Acta.* **1535:** 1–9.
8. van den Brink, D. M., J. N. van Miert, G. Dacremont, J. F. Rontani, G. A. Jansen, and R. J. A. Wanders. 2004. Identification of fatty aldehyde dehydrogenase in the breakdown of phytol to phytanic acid. *Mol. Genet. Metab.* **82:** 33–37.
9. Verhoeven, N. M., C. Jakobs, G. Carney, M. P. Somers, R. J. A. Wanders, and W. B. Rizzo. 1998. Involvement of microsomal fatty aldehyde dehydrogenase in the alpha-oxidation of phytanic acid. *FEBS Lett.* **429:** 225–228.
10. Willemsen, M. A., J. J. Rotteveel, J. G. de Jong, R. J. A. Wanders, L. IJlst, G. F. Hoffmann, and E. Mayatepek. 2001. Defective metabolism of leukotriene B4 in the Sjögren-Larsson syndrome. *J. Neurol. Sci.* **183:** 61–67.
11. Willemsen, M. A., J. G. de Jong, P. H. van Domburg, J. J. Rotteveel, R. J. A. Wanders, and E. Mayatepek. 2000. Defective inactivation of leukotriene B4 in patients with Sjögren-Larsson syndrome. *J. Pediatr.* **136:** 258–260.
12. James, P. F., and R. A. Zoeller. 1997. Isolation of animal cell mutants defective in long-chain fatty aldehyde dehydrogenase. Sensitivity to fatty aldehydes and Schiff's base modification of phospholipids: implications for Sjögren-Larsson syndrome. *J. Biol. Chem.* **272:** 23532–23539.
13. Hernell, O., G. Holmgren, S. F. Jagell, S. B. Johnson, and R. T. Holman. 1982. Suspected faulty essential fatty acid metabolism in Sjögren-Larsson syndrome. *Pediatr. Res.* **16:** 45–49.
14. Paige, D. G., N. Morse-Fisher, and J. I. Harper. 1994. Quantification of stratum corneum ceramides and lipid envelope ceramides in the hereditary ichthyoses. *Br. J. Dermatol.* **131:** 23–27.
15. Christie, W. W. 1982. Lipid Analysis. Pergamon Press, Ltd., Oxford, UK.
16. Rizzo, W. B., D. A. Craft, A. L. Dammann, and M. W. Phillips. 1987. Fatty alcohol metabolism in cultured human fibroblasts: evidence for a fatty alcohol cycle. *J. Biol. Chem.* **262:** 17412–17419.
17. Valicenti, A. J., and R. T. Holman. 1976. Oxidation of long-chain alcohols to aldehydes by the dipyridine chromic anhydride complex. *Chem. Phys. Lipids.* **17:** 389–392.
18. Zheng, H., R. I. Duclos, Jr., C. C. Smith, H. W. Farber, and R. A. Zoeller. 2006. Synthesis and biological properties of the fluorescent ether lipid precursor 1-*O*-[9′-(1″-pyrenyl)]nonyl-*sn*-glycerol. *J. Lipid Res.* **47:** 633–642.
19. Rheinwald, J. G., and H. Green. 1975. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell.* **6:** 331–343.
20. Randolph, R. K., and M. Simon. 1993. Characterization of retinol metabolism in cultured human epidermal keratinocytes. *J. Biol. Chem.* **268:** 9198–9205.
21. Rizzo, W. B., A. L. Dammann, D. A. Craft, S. H. Black, A. H. Tilton, D. Africk, E. Chaves-Carballo, G. Holmgren, and S. Jagell. 1989. Sjögren-Larsson syndrome: inherited defect in the fatty alcohol cycle. *J. Pediatr.* **115:** 228–234.
22. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
23. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
24. Macala, L. J., R. K. Yu, and S. Ando. 1983. Analysis of brain lipids by high performance thin-layer chromatography and densitometry. *J. Lipid Res.* **24:** 1243–1250.
25. Rizzo, W. B., M. W. Phillips, A. L. Dammann, R. T. Leshner, S. S. Jennings, J. Avigan, and V. K. Proud. 1987. Adrenoleukodystrophy: dietary oleic acid lowers hexacosanoate levels. *Ann. Neurol.* **21:** 232–239.
26. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
27. Hanahan, D. J., J. Ekholm, and C. M. Jackson. 1963. Studies on the structure of glyceryl ethers and the glyceryl ether phospholipids of bovine erythrocytes. *Biochemistry.* **2:** 630–641.
28. Rizzo, W. B., A. L. Dammann, and D. A. Craft. 1988. Sjögren-Larsson syndrome. Impaired fatty alcohol oxidation in cultured fibroblasts due to deficient fatty alcohol:nicotinamide adenine dinucleotide oxidoreductase activity. *J. Clin. Invest.* **81:** 738–744.
29. Rizzo, W. B., D. A. Craft, L. L. Judd, H. W. Moser, and A. B. Moser. 1993. Fatty alcohol accumulation in the autosomal recessive form of rhizomelic chondrodysplasia punctata. *Biochem. Med. Metab. Biol.* **50:** 93–102.
30. James, P. F., W. B. Rizzo, J. Lee, and R. A. Zoeller. 1990. Isolation and characterization of a Chinese hamster ovary cell line deficient in fatty alcohol:NAD^+ oxidoreductase activity. *Proc. Natl. Acad. Sci. USA.* **87:** 6102–6106.
31. Lee, T. C., V. Fitzgerald, N. Stephens, and F. Snyder. 1980. Activities of enzymes involved in the metabolism of ether-linked lipids in normal and neoplastic tissues of rat. *Biochim. Biophys. Acta.* **619:** 420–423.
32. Oku, H., J. Shudo, J. Nagata, and I. Chinen. 1996. Accumulation of 1-O-alkyl-2,3-diacylglycerols in cultured rat keratinocytes. *Biochim. Biophys. Acta.* **1300:** 35–41.
33. Oku, H., J. Shudo, K. Mimura, A. Haratake, J. Nagata, and I. Chinen. 1995. 1-*O*-Alkyl-2,3-diacylglycerols in the skin surface lipids of the hairless mouse. *Lipids.* **30:** 169–172.
34. Nagan, N., and R. A. Zoeller. 2001. Plasmalogens: biosynthesis and functions. *Prog. Lipid Res.* **40:** 199–229.
35. Stadelmann-Ingrand, S., S. Favreliere, B. Fauconneau, G. Mauco, and C. Tallineau. 2001. Plasmalogen degradation by oxidative stress: production and disappearance of specific fatty aldehydes and fatty α-hydroxyaldehydes. *Free Radic. Biol. Med.* **31:** 1263–1271.
36. Maaswinkel-Mooij, P. D., O. F. Brouwer, and W. B. Rizzo. 1994. Unsuccessful dietary treatment of Sjögren-Larsson syndrome. *J. Pediatr.* **124:** 748–750.
37. Avigan, J., B. D. Campbell, D. A. Yost, O. Hernell, G. Holmgren, and S. F. Jagell. 1985. Sjögren-Larsson syndrome: delta 5- and delta 6-fatty acid desaturases in skin fibroblasts. *Neurology.* **35:** 401–403.
38. Coderch, L., O. Lopez, A. de la Maza, and J. L. Parra. 2003. Ceramides and skin function. *Am. J. Clin. Dermatol.* **4:** 107–129.
39. Madison, K. C. 2003. Barrier function of the skin: "la raison d'etre" of the epidermis. *J. Invest. Dermatol.* **121:** 231–241.
40. Wertz, P. W. 2000. Lipids and barrier function of the skin. *Acta Derm. Venereol. Suppl. (Stockh.).* **208:** 7–11.
41. Ito, M., K. Oguro, and Y. Sato. 1991. Ultrastructural study of the skin in Sjögren-Larsson syndrome. *Arch. Dermatol. Res.* **283:** 141–148.
42. Shibaki, A., M. Akiyama, and H. Shimizu. 2004. Novel ALDH3A2 heterozygous mutations are associated with defective lamellar granule formation in a Japanese family of Sjögren-Larsson syndrome. *J. Invest. Dermatol.* **123:** 1197–1199.
43. Nicolaides, N. 1974. Skin lipids: their biochemical uniqueness. *Science.* **186:** 19–26.
44. Downing, D. T., and J. S. Straus. 1974. Synthesis and composition of surface lipids of human skin. *J. Invest. Dermatol.* **62:** 228–244.
45. Ikuta, T., K. Chida, O. Tajima, Y. Matsuura, M. Iwamori, Y. Ueda, K. Mizuno, S. Ohno, and T. Kuroki. 1994. Cholesterol sulfate, a novel activator for the η isoform of protein kinase C. *Cell Growth Differ.* **5:** 943–947.
46. Denning, M. F., M. G. Kazanietz, P. M. Blumberg, and S. H. Yuspa. 1995. Cholesterol sulfate activates multiple protein kinase C isoenzymes and induces granular cell differentiation in cultured murine keratinocytes. *Cell Growth Differ.* **6:** 1619–1626.
47. Bollag, W. B., and R. J. Bollag. 2001. 1,25-Dihydroxyvitamin D3, phospholipase D and protein kinase C in keratinocyte differentiation. *Mol. Cell. Endocrinol.* **177:** 173–182.
48. Bikle, D. D., D. Ng, C-L. Tu, Y. Oda, and Z. Xie. 2001. Calcium- and vitamin D-regulated keratinocyte differentiation. *Mol. Cell. Endocrinol.* **177:** 161–171.
49. Denning, M. F. 2004. Epidermal keratinocytes: regulation of multiple cell phenotypes by multiple protein kinase C isoforms. *Int. J. Biochem. Cell Biol.* **36:** 1141–1146.
50. Ohba, M., K. Ishino, M. Kashiwagi, S. Kawabe, K. Chida, N-H. Huh, and T. Kuroki. 1998. Induction of differentiation in normal human keratinocytes by adenovirus-mediated introduction of the η and δ isoforms of protein kinase C. *Mol. Cell. Biol.* **18:** 5199–5207.
51. McNeely, T. B., G. Rosen, M. V. Londner, and S. J. Turco. 1989. Inhibitory effects on protein kinase C activity by lipophosphoglycan fragments and glycosylphosphatidylinositol antigens of the protozoan parasite Leishmania. *Biochem. J.* **259:** 601–604.
52. Warne, T. R., F. G. Buchanan, and M. Robinson. 1995. Growth-dependent accumulation of monoalkylglycerol in Madin-Darby canine kidney cells. *J. Biol. Chem.* **270:** 11147–11154.
53. Ford, D. A., R. Miyake, P. E. Glaser, and R. W. Gross. 1989. Activation of protein kinase C by naturally occurring ether-linked diglycerides. *J. Biol. Chem.* **264:** 13818–13824.
54. Slater, S. J., M. B. Kelly, J. D. Larkin, C. Ho, A. Mazurek, F. J. Taddeo, M. D. Yeager, and C. D. Stubbs. 1997. Interaction of alcohols and anesthetics with protein kinase Cα. *J. Biol. Chem.* **272:** 6167–6173.
55. Lefevre, C., B. Bouadjar, V. Ferrand, G. Tadini, A. Megarbane, M. Lathrop, J. F. Prud'homme, and J. Fischer. 2006. Mutations in a new cytochrome P450 gene in lamellar ichthyosis type 3. *Hum. Mol. Genet.* **15:** 767–776.

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