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Journal

Archives of Dermatological Research, 306(8)

ISSN

0340-3696

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Publication Date

2014-10-01

DOI

10.1007/s00403-014-1478-2

Peer reviewed

Published in final edited form as:
Arch Dermatol Res. 2014 October ; 306(8): 731–737. doi:10.1007/s00403-014-1478-2.

Ultrastructure of skin from Refsum disease with emphasis on epidermal lamellar bodies and stratum corneum barrier lipid organization

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Abstract

Classic Refsum disease (RD) is a rare, autosomal recessively-inherited disorder of peroxisome metabolism due to a defect in the initial step in the alpha oxidation of phytanic acid (PA), a C 16 saturated fatty acid with four methyl side groups, which accumulates in plasma and lipid enriched tissues (please see van den Brink, et al. 2006). It has been proposed that the disease complex in RD is in part due to the high affinity of phytanic acid for retinoid X receptors and peroxisome proliferator-activated receptors. Structurally, epidermal hyperplasia, increased numbers of cornified cell layers, presence of cells with lipid droplets in stratum basale and reduction of granular layer to a single layer have been reported by Blanchet-Bardon et al (1978). However, lamellar body (LB) density and secretion were reportedly normal. We recently examined biopsies from 4 unrelated patients, using both OsO_4 and RuO_4 post-fixation to evaluate the barrier lipid structural organization. Although lamellar body density appeared normal, individual organelles often had distorted shape, or had non-lamellar domains interspersed with lamellar structures. Some of the organelles seemed to lack lamellar contents altogether, showing instead uniformly electron-dense contents. In addition, we also observed mitochondrial abnormalities in the nucleated epidermis. Stratum granulosum-stratum corneum junctions also showed co-existence of non-lamellar and lamellar domains, indicative of lipid phase separation. Also, partial detachment or complete absence of corneocyte lipid envelopes (CLE) was seen in the stratum corneum of all RD patients. In conclusion, abnormal LB contents, resulting in defective lamellar bilayers, as well as reduced CLEs, likely lead to impaired barrier function in RD.

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Authors state no conflicts of interest.

This content is solely the responsibility of the authors and does not necessarily represent the office views of either the National Institutes of Health or the Department of Veterans Affairs. The invaluable editorial assistance of Ms. Joan Wakefield throughout the preparation of the manuscript is also greatly appreciated.

Keywords

Refsum disease; abnormal lamellar bodies; barrier defects; lysosome-related organelles; ultrastructure

INTRODUCTION

Classic *Refsum disease* (RD) was first described by Sigvald Refsum in 1946. RD is a rare, autosomal, recessively-inherited disorder of peroxisome metabolism due to a defect in the initial step in the alfa-oxidation of phytanic acid, a C16 saturated fatty acid with four methyl side groups (at the C3, 7, 11, and 15 positions) [13, 27]. Humans obtain PA only from dietary sources, such as diary and ruminant fat [14, 25, 28]. Phytol, the precursor of PA, is a component of chlorophyll, but gut fermentation of chlorophyll in the ruminant stomach also produces phytol, which then is converted into PA and stored in fats. It has become evident that the 3-methyl group in PA prevents its beta oxidation, while alfa oxidation of PA results in the formation of pristanic acid, which then can undergo beta oxidation. Watkins, et al (1994) showed that it is phytanoyl CoA (and not PA) that is the true substrate for alfa oxidation. Mihalik et. al (1995) discovered the enzyme phytanoyl CoA hydroxylase (PHYH), which converts phytanoyl Co A to 2-hydroxyphytanoyl CoA, and that the enzyme localizes to peroxisomes. Soon thereafter, it was established that deficiency of PHYH is the molecular basis of adult Refsum disease [12], which results in accumulation of PA – plasma levels of which are an excellent marker for the disease.

The initial symptom of classic RD is often night blindness, which can progress to severe visual impairment. Mild scaling usually begins later, typically during adolescence, but even as late as the 4^{th} or 5^{th} decade [2]. The cutaneous phenotype resembles *ichthyosis vulgaris*, with flexural sparing and no erythema. Within an appropriate clinical setting, the biochemical diagnosis of RD can be made by finding elevated phytanic acid levels in plasma.

Late-onset or classic *Refsum disease* (RD) (OMIM #256500) is distinguished from infantile RD, a complex disorder of peroxisomal biogenesis, in which peroxisomes fail to form, resulting in loss-of-function of multiple enzymes. Ichthyosis is not a feature of infantile RD, but in classic RD, ichthyosis occurs along with neurologic features, including peripheral neuropathy and retinitis pigmentosa. Although severely-affected patients can die in childhood, the onset is often insidious, becoming symptomatic only in adolescence, from a disease complex that can include deafness, cerebellar ataxia, and anosmia [13]. Because neurologic features do not develop until the second decade of life or still later, the diagnosis is often delayed. Earlier recognition (e.g., by ophthalmologic exam and/or assessment of plasma phytanic acid levels) would facilitate preventive dietary interventions, which can reduce the severity of the largely-irreversible neurological damage [13, 27]. Cardiac arrhythmias may be fatal in RD, but these, as well as other disease symptoms, improve with implementation of a phytol-free diet.

Since ichthyosis can be the harbinger of RD, the possibility that timely skin biopsies could allow early initiation of a phytol-free diet seems plausible, but observations to date on the

histologic and ultrastructural features of the epidermis in classic RD are limited. Known features include acanthosis, spongiosis, hyperkeratosis, and hypergranulosis, as well as lipid inclusions in basal and suprabasal cells of the epidermis [1–3], to the best of our knowledge, there is little information about the pathogenesis of the dermatitis in this disorder. Activation of peroxisome proliferator activated receptors (PPARs) are known to improve epidermal barrier function [24], through enhanced production of epidermal lipids in mouse models. Yet, while phytanic acid is an agonist for both the retinoid X receptor [17, 19, 31] and PPARs [8], its accumulation in Refsum patients instead leads to barrier dysfunction. Thus, the pathogenesis of skin lesions in RD likely reflects phytanic acid (PA) accumulation, rather than the downstream consequences of phytanic acid-induced PPAR signaling. To assess the potential impact of excess PA on epidermal structure and function, we examined here the ultrastructure of epidermis in four RD patients, searching for the structural basis for the putative barrier abnormality using both OsO_4 and RuO_4 post fixation. Since the phenotype in all of the ichthyoses represents a response to the barrier abnormality, we focused on the structure and internal organization of epidermal lamellar bodies and their secreted contents, as well as downstream alterations in SC lamellar bilayer structure.

Patient Materials and Methods

Biopsies were obtained from the inguinal region of 4 female (55 to 73 years of age) patients with informed consent from the Regensberg University Hospital. All patients were recruited with the help of the German Association of Refsum Patient Support Group. Diagnoses was made according to clinical presentation and molecular genetics (Suppl. Table 1), and confirmed by sequencing of the PHYH and PEX7 genes from genomic DNA obtained from blood leukocytes. All 4 patients were compound heterozygotes for previously published mutations of *PHYH* gene [12, 13], leading to loss-of-function of the phytanoyl-CoA hydroxylase, and were on a long-term, special diet (restriction of dairy and ruminant fat products). Patients fasted for 12 hours before skin biopsies were taken. Samples were immediately fixed in modified Karnovsky's fixative, cut into small pieces, and subsequently washed in 0.1 M sodium cacodylate buffer, and post fixed using either OsO_4 (for general ultrastructural evaluation) or RuO_4 (for examining the barrier lipid structures), and processed routinely for transmission electron microscopy. Samples were embedded in a low viscosity Epon-Epoxy mixture [10]. Ultrathin sections were double-stained with uranyl acetate and lead citrate, and examined in a Zeiss 10A Electron microscope operated at 60 KV.

OBSERVATIONS

RD Epidermis Displays Epidermal Hyperplasia and Hyperkeratosis

In survey (low-magnification) electron micrographs, epidermal hyperplasia and hyperkeratosis, as well as spongiosis; i.e., widening of the intercellular spaces within the basal and spinous layers of epidermis, were apparent. These non-specific features also are seen in psoriasis and other hyperproliferative dermatoses (Fig. 1A). But in contrast to other hyperproliferative dermatoses, flocculent material often was evident in these enlarged extracellular spaces. Finally, many cells in the stratum granulosum (SG) appear deficient in keratohyalin granules (KHGs) (Fig. 1B), while other SG cells showed normal quantities of

KHGs. We did not observe lipid droplets in basal cells. Though what appeared to be vacuoles in some basal cells on light microscopy, appeared instead to be empty extracellular areas, abutting cells that appeared in cross sections (Fig. 1A).

Abnormalities in Lamellar Bodies in RD

Above the spinous (SS) layer, the large gaps between adjacent nucleated cells become unapparent. Secreted contents of lamellar bodies instead are seen between adjacent granular cells, as well as at the SG-SC interface. But, focal areas at the SG-SC interface are devoid of LB contents; instead appearing electron-lucent (Figs. 4A&B). In a similar area from another patient, such gaps are even more pronounced, and more or less continuous (not shown), apparently due to an even more severe LB abnormality. Lamellar bodies with perfectly normal contents, but with a 'triangular' shape, also are occasionally encountered in RD (Fig. 2; insert).

Several ovoid organelles in the size-range of epidermal lamellar bodies can be seen in the SS and SG. While the majority of these organelles display lamellar contents typical of epidermal lamellar bodies, some contain a mixture of lamellar and non-lamellar contents, while still others display electron-lucent microvesicular structures (Fig. 2). Also seen are membrane-bound organelles with a core of electron dense material, within a less dense matrix (Suppl. Fig. 1), resembling 'core-granules' in oral mucosa [29].

Another organelle, with similar dimensions to lamellar bodies, but with uniformly dark contents, also can be seen with RuO_4 post-fixation (Fig. 3). These membrane-bound vesicles sometimes appear in close proximity to mitochondria, or connected to and/or budding off a membrane system (Fig. 3C; arrow), likely representing the trans-Golgi network [4]. Mitochondria often are swollen in appearance, displaying a paucity of cristae, unlike those seen in normal human skin (Suppl. Fig. 2).

Abnormal LB Secretion and Post-Secretory Processing in RD

The secreted contents of LBs at the SG-SC interface appear non-uniform, with some differences among individual RD patients. While the profile can be normal, some areas appear deficient in LB contents (Fig. 4). We also frequently noted abnormal foci of non-lamellar amorphous material both with OsO_4 (Fig. 4A) and RuO_4 (Fig. 4B) post-fixation. Above the SG-SC interface, the SC extracellular lamellar bilayers are not drastically different from those of normal human SC. However, bilayers in RD appear to be more loosely packed than in normal SC. Additionally, large non-lamellar domains, embedded within the SC extracellular matrix, often are noticeable (Fig. 5).

Absent Corneocyte Lipid Envelopes in RD

Another striking feature in RD is the apparent lack of a tightly-adherent, corneocyte lipid envelope (CLE) external to the cornified envelope. Whether all patients lack a CLE due to failure of CLE formation, or whether loss of the CLE could reflect peeling or delamination from the corneocyte surface (as suggested by Fig. 6, arrow), could not be determined.

DISCUSSION

To the best of our knowledge, this is the first detailed report on abnormalities of epidermal barrier-related structures in RD, building upon earlier reports that noted cytosolic vacuoles and non-membrane bound lipid droplets in the basal epidermal cells of involved epidermis. [3]. Using more current post-fixation and staining methods, we have identified several distinctive ultrastructural features of RD epidermis. The most striking changes in morphology in the epidermis of RD patients occur in epidermal lamellar bodies, reflecting likely qualitative changes in their biochemical composition. Although there are some normal appearing LBs, the majority of these organelles displayed only partial lamellar contents. Despite the possibility that apparent pleomorphisms of lamellar bodies could reflect plane of sectioning, there is ample reason to attribute this observation to variations in their contents (cargo). Immunoelectron microscopic studies of Ishida-Yamamoto and colleagues have shown [11] that corneodesmosin is present in a subset, but not in all lamellar bodies. Lamellar bodies in many skin diseases appear to have deficient disk contents, which correspond to downstream defects within the SC lamellar structures, including non-lamellar/lamellar domain separation [6]. Some of the organelles shown here correspond with the dimensions and distribution range of normal lamellar bodies, but they contain either completely electron-lucent contents or amorphous, electron-dense material with no visible lamellar structures. Although the latter types of organelles display morphological features classically associated with lysosomes (personal communication, J. Reddy), it is interesting to note that typical lysosomes, exhibiting ultrastructurally identifiable morphology, normally do not occur in the outer epidermis [16]. Yet, epidermal lamellar bodies are indeed ‘lysosome-related organelles’ (LROs) that contain several lysosomal hydrolases [16]. Lysosomal storage disorders (such as Gaucher and Niemann Pick disease) are known to affect lamellar body contents, and to alter the post-secretory processing of barrier lipid precursors [9]. As a result, these disorders show functional alterations that include increased transepidermal water loss (TEWL) [9, 22]. Under normal physiological conditions, the main function of lamellar bodies is to supply extracellular domains with specialized lipid and non-lipid components related to multiple SC functions [21]. In the case of RD patients, the lysosome-like organelles (in all probability abnormal lamellar bodies with accumulated phytanic acid), once secreted could be expected to alter the molar ratio of SC lipids focally, resulting in sites with ‘domain separation’ within the SC extracellular matrix. Our results suggest that the putative defect in the permeability barrier, as well as the abnormal desquamation in RD, can be attributed to structural and functional alterations in lamellar body contents and post-secretory processing [18].

Since it is known that phytanic acid accumulates in the epidermis of RD patients, especially the phospholipids [20], it is conceivable that PA is sequestered preferentially with lamellar bodies. There, phytanic acid could replace linoleic acid in acylglucosylceramides, leading to barrier defects similar to those seen in essential fatty acid deficiency (EFAD) [7] and certain other ichthyosis that lack a CLE (i.e., ALOX mutations [LOX 12 and eLOX [30]]), and neutral lipid storage disease [26]. PA, if substituted for linoleic acid (LA) in the acyl group of acylglycosylceramides likely would serve as a substrate for the lipase that generates the ω-acyl Cer component for the CLE. Omega-hydroxylation of PA, although not specifically

reported for epidermis, has been shown to occur in liver microsomes [15], and it is feasible that this process occurs in the epidermis, as well. If so, this abnormality would result in a defective or absent CLE, and/or render this monolayer more susceptible to delamination from the corneocyte surface, as appeared to occur in some of our subjects. An alternate possibility is that the CLE fails to adhere tightly to the corneocyte surface; and hence, shows various degrees of separation, providing an appearance of peeling away from the cornified envelope.

Although we have not found any published data on TEWL levels in RD patients, the severe morphological abnormalities of the barrier lipids clearly speak to a permeability barrier defect – mostly through defects in the 'mortar' lipids. These alterations in the structure and functions of the lamellar body secretory system underscore the extent of metabolic defects in this rare, inherited disease, caused by defective α-oxidation of phytanic acid (present in a wide range of food - esp. diary, meat and fish), with its accumulation in the epidermis. Mutations causing the loss of phytanoyl-co enzyme A hydroxylase (which catalyzes the second step in breakdown of phytanic acid to pristanic acid) as the causative factor is now well-established as the cause of RD. All of the inherited ichthyoses studied to date, while of completely different causation, exhibit prominent permeability barrier abnormalities [5, 6, 23]. We identified two classes of structural abnormalities that either separately or together could account for the putative permeability barrier abnormality in RD: First, abnormal epidermal lamellar body structure and content could alter the organization of the barrier lipids [6], and such abnormalities in extracellular lipid organization could form the primary structural basis for the putative barrier defect in RD. Second, loss of the CLE, either by a failure of assembly and/or delamination, could contribute to the barrier abnormality as well. Loss of the CLE would impair the scaffold functions of individual corneocytes, as shown in EFAD, ALOX mutants and neutral lipid storage disease (NLSDI) that also affect the CLE. In this disease, lamellar bilayers become disorganized, rendering the extracellular matrix of SC 'porous' to water. The defect in the CLE likely can be attributed to accumulated phytanic acid which would replace linoleic acid in acylceramides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully appreciate the exceptional help from the German Association of Refsum Patient Support Group which made this work possible. Research reported in this publication was supported by the National Institutes of Health Grant R01 AR061106, with additional resources provide by the Veterans Affairs Medical Center, San Francisco, CA.

Abbreviations

CLE	corneocyte lipid envelope
EFAD	essential fatty acid deficiency
KHG	keratohyalin granules

LA	linoleic acid
LB	lamellar body
LRO	lysosome-related organelle
PA	phytanic acid
PPAR	peroxisome proliferator-activated receptor
RCDP	rhizomelic chondrodysplasia punctate
RD	Refsum disease
SC	stratum corneum
SG	stratum granulosum
TEWL	transepidermal water loss

References

1. Anton-Lamprecht I, Kahlke W. Ultrastructure of inborn errors of keratinization. V. Ichthyosis in Refsum's syndrome (heredopathia atactica polyneuritiformis) (author's transl). Arch Dermatol Forsch. 1974; 250:185–206. [PubMed: 4138779]
2. Blanchet-Bardon, C.; Anton-Lamprecht, I.; Puissant, A.; Schnyder, U. Ultrastructural features of ichthyotic skin in Refsum's syndrome. In: Marks, R.; Dykes, P., editors. The Ichthyoses. SP Medical & Scientific Books; 1978. p. 65-69.
3. Davies MG, Marks R, Dykes PJ, Reynolds D. Epidermal abnormalities in Refsum's disease. Br J Dermatol. 1977; 97:401–406. [PubMed: 73380]
4. Elias PM, Cullander C, Mauro T, Rassner U, Komuves L, Brown BE, Menon GK. The secretory granular cell: the outermost granular cell as a specialized secretory cell. J Invest Dermatol Symp Proc. 1998; 3:87–100.
5. Elias PM, Williams ML, Holleran WM, Jiang YJ, Schmuth M. Pathogenesis of permeability barrier abnormalities in the ichthyoses: inherited disorders of lipid metabolism. J Lipid Res. 2008; 49:697–714. [PubMed: 18245815]
6. Elias, PM.; Williams, ML.; Crumrine, D.; Schmuth, M. Ichthyoses - clinical, biochemical, pathogenic, and diagnostic assessment. S. Kargar AG; Basel: 2010.
7. Elias PM, Gruber R, Crumrine D, Menon G, Williams ML, Wakefield JS, Holleran WM, Uchida Y. Formation and functions of the corneocyte lipid envelope (CLE). Biochim Biophys Acta. 2014; 1841:314–318. [PubMed: 24076475]
8. Heim M, Johnson J, Boess F, Bendik I, Weber P, Hunziker W, Fluhmann B. Phytanic acid, a natural peroxisome proliferator-activated receptor (PPAR) agonist, regulates glucose metabolism in rat primary hepatocytes. FASEB J. 2002; 16:718–720. [PubMed: 11923221]
9. Holleran WM, Ginns EI, Menon GK, Grundmann JU, Fartasch M, McKinney CE, Elias PM, Sidransky E. Consequences of beta-glucocerebrosidase deficiency in epidermis. Ultrastructure and permeability barrier alterations in Gaucher disease. J Clin Invest. 1994; 93:1756–1764. [PubMed: 8163674]
10. Hou SY, Mitra AK, White SH, Menon GK, Ghadially R, Elias PM. Membrane structures in normal and essential fatty acid-deficient stratum corneum: characterization by ruthenium tetroxide staining and x-ray diffraction. J Invest Dermatol. 1991; 96:215–223. [PubMed: 1991982]
11. Ishida-Yamamoto A, Simon M, Kishibe M, Miyauchi Y, Takahashi H, Yoshida S, O'Brien TJ, Serre G, Iizuka H. Epidermal lamellar granules transport different cargoes as distinct aggregates. J Invest Dermatol. 2004; 122:1137–1144. [PubMed: 15140216]

12. Jansen GA, Ofman R, Ferdinandusse S, Ijlst L, Muijsers AO, Skjeldal OH, Stokke O, Jakobs C, Besley GT, Wraith JE, Wanders RJ. Refsum disease is caused by mutations in the phytanoyl-CoA hydroxylase gene. Nat Genet. 1997; 17:190–193. [PubMed: 9326940]
13. Jansen GA, Ferdinandusse S, Hogenhout EM, Verhoeven NM, Jakobs C, Wanders RJ. Phytanoyl-CoA hydroxylase deficiency. Enzymological and molecular basis of classical Refsum disease. Adv Exp Med Biol. 1999; 466:371–376. [PubMed: 10709665]
14. Kim J, Cho Y. Gromwell (Lithospermum erythrorhizon) supplementation enhances epidermal levels of ceramides, glucosylceramides, beta-glucocerebrosidase, and acidic sphingomyelinase in NC/Nga mice. Journal of medicinal food. 2013; 16:927–933. [PubMed: 24074295]
15. Komen JC, Duran M, Wanders RJ. Omega-hydroxylation of phytanic acid in rat liver microsomes: implications for Refsum disease. J Lipid Res. 2004; 45:1341–1346. [PubMed: 15102880]
16. Lazarus GS, Hatcher VB, Levine N. Lysosomes and the skin. J Invest Dermatol. 1975; 65:259–271. [PubMed: 808573]
17. Lemotte PK, Keidel S, Apfel CM. Phytanic acid is a retinoid X receptor ligand. Eur J Biochem. 1996; 236:328–333. [PubMed: 8617282]
18. Menon GK, Ghadially R, Williams ML, Elias PM. Lamellar bodies as delivery systems of hydrolytic enzymes: implications for normal and abnormal desquamation. Br J Dermatol. 1992; 126:337–345. [PubMed: 1571254]
19. Radominska-Pandya A, Chen G. Photoaffinity labeling of human retinoid X receptor beta (RXRbeta) with 9-cis-retinoic acid: identification of phytanic acid, docosahexaenoic acid, and lithocholic acid as ligands for RXRbeta. Biochemistry. 2002; 41:4883–4890. [PubMed: 11939783]
20. Reynolds DJ, Marks R, Davies MG, Dykes PJ. The fatty acid composition of skin and plasma lipids in Refsum's disease. Clin Chim Acta. 1978; 90:171–177. [PubMed: 82497]
21. Schmitz G, Muller G. Structure and function of lamellar bodies, lipid-protein complexes involved in storage and secretion of cellular lipids. J Lipid Res. 1991; 32:1539–1570. [PubMed: 1797938]
22. Schmuth M, Man MQ, Weber F, Gao W, Feingold KR, Fritsch P, Elias PM, Holleran WM. Permeability barrier disorder in Niemann-Pick disease: sphingomyelin-ceramide processing required for normal barrier homeostasis. J Invest Dermatol. 2000; 115:459–466. [PubMed: 10951284]
23. Schmuth M, Gruber R, PME, Williams M. Ichthyosis update: towards a function-driven model of pathogenesis of the disorders of cornification and the role of corneocyte proteins in these disorders. Adv Dermatol. 2007; 23:231–256. [PubMed: 18159904]
24. Schmuth M, Jiang YJ, Dubrac S, Elias PM, Feingold KR. Thematic Review Series: Skin Lipids. Peroxisome proliferator-activated receptors and liver X receptors in epidermal biology. J Lipid Res. 2008; 49:499–509. [PubMed: 18182682]
25. Tsuji K, Mitsutake S, Ishikawa J, Takagi Y, Akiyama M, Shimizu H, Tomiyama T, Igarashi Y. Dietary glucosylceramide improves skin barrier function in hairless mice. J Dermatol Sci. 2006; 44:101–107. [PubMed: 17000082]
26. Uchida Y, Cho Y, Moradian S, Kim J, Nakajima K, Crumrine D, Park K, Ujihara M, Akiyama M, Shimizu H, Holleran WM, Sano S, Elias PM. Neutral lipid storage leads to acylceramide deficiency, likely contributing to the pathogenesis of Dorfman-Chanarin syndrome. J Invest Dermatol. 2010; 130:2497–2499. [PubMed: 20520629]
27. van den Brink DM, Wanders RJ. Phytanic acid: production from phytol, its breakdown and role in human disease. Cell Mol Life Sci. 2006; 63:1752–1765. [PubMed: 16799769]
28. Wanders RJ, Jansen GA, Skjeldal OH. Refsum disease, peroxisomes and phytanic acid oxidation: a review. Journal of neuropathology and experimental neurology. 2001; 60:1021–1031. [PubMed: 11706932]
29. Wertz PW, Swartzendruber DC, Squier CA. Regional variation in the structure and permeability of oral mucosa and skin. Adv Drug Deliv Rev. 1993; 12:1–12.
30. Zheng Y, Yin H, Boeglin WE, Elias PM, Crumrine D, Beier DR, Brash AR. Lipoxygenases mediate the effect of essential fatty acid in skin barrier formation: a proposed role in releasing omega-hydroxyceramide for construction of the corneocyte lipid envelope. J Biol Chem. 2011; 286:24046–24056. [PubMed: 21558561]

31. Zomer AW, van Der Burg B, Jansen GA, Wanders RJ, Poll-The BT, van Der Saag PT. Pristanic acid and phytanic acid: naturally occurring ligands for the nuclear receptor peroxisome proliferator-activated receptor alpha. J Lipid Res. 2000; 41:1801–1807. [PubMed: 11060349]

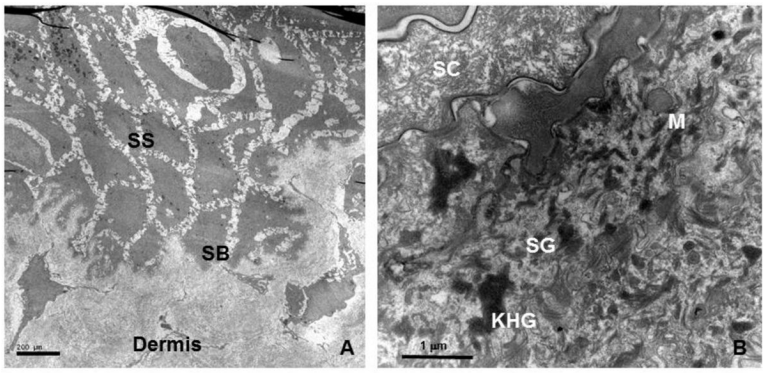

Fig. 1. A. Low magnification electron micrograph of RD epidermis, showing prominent acanthosis

B. SG and lower SC from a RD patient. Note paucity of keratohyalin granules compared to normal epidermis. While the nucleated epidermal layers do not show prominent intercellular spaces, the SG-SC interface shows such gaps. KHG, keratohyalin granules; M, mitochondria; SB, stratum basale; SG, stratum granulosum; SS, stratum spinosum. OsO_4 post fixation.

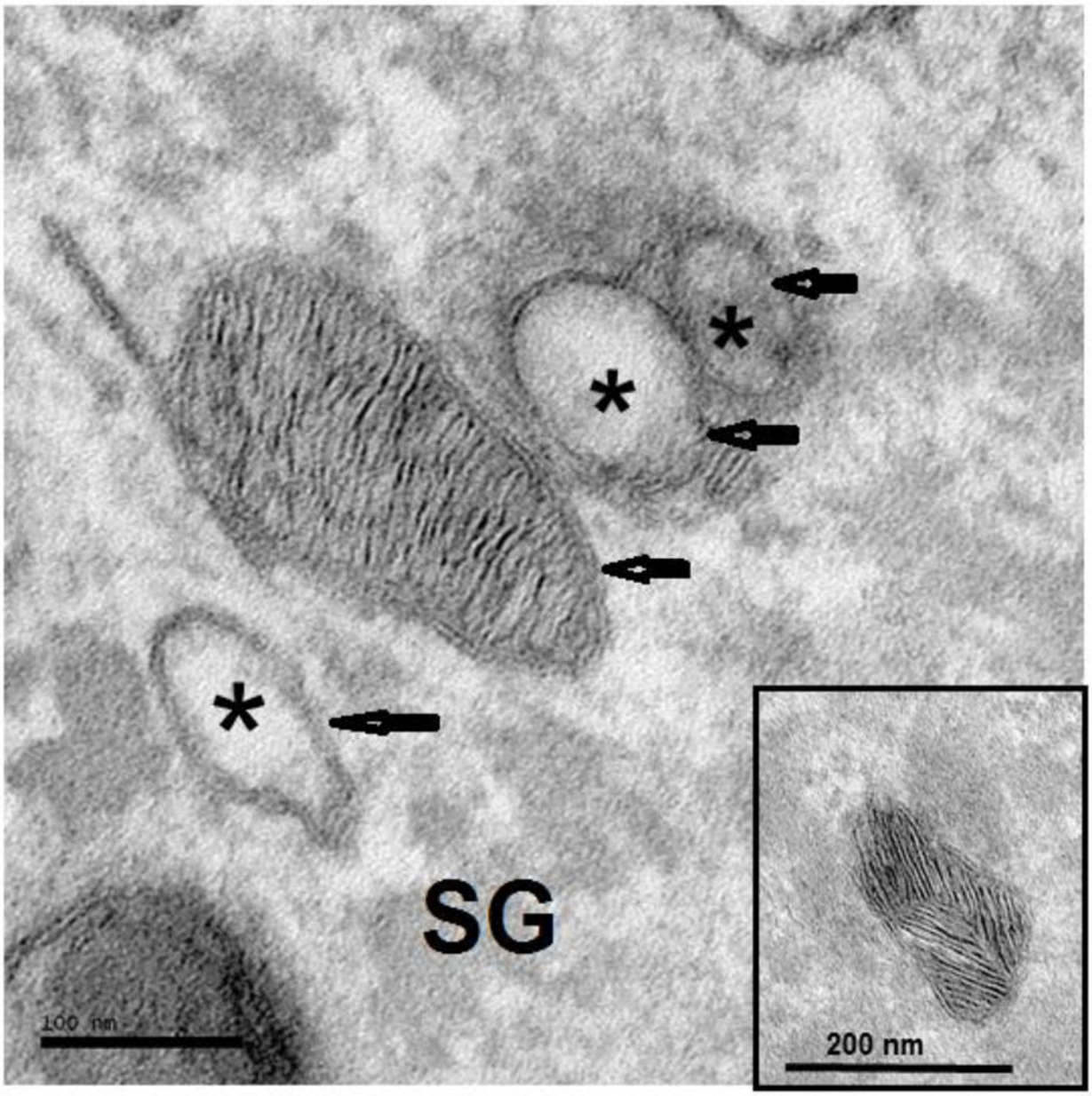

Fig. 2. High magnification views of lamellar bodies in SG layer, documenting the heterogeneous population of normal and abnormal appearing LBs (arrows), including membrane-bound organelles with non-lamellar microvesicular contents (asterisks)

Inset: A lamellar body with unusual shape, but with normal lamellar contents. LB, lamellar body; SG, stratum granulosum. OsO_4 post-fixation.

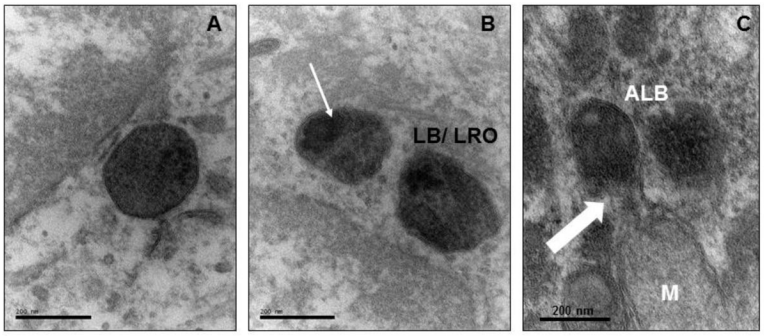

Fig. 3. Other organelles with similar size range as LBs, but more uniformly filled with non-lamellar electron dense contents

A&B. Organelles with a morphology that closely resembles classic lysosomes (LRO). RuO_4 post fixation. **C.** Micrograph illustrating the close association of (abnormal) LB with mitochondria. ALB, abnormal lamellar body; LB/LRO, lamellar body/lysosome-like organelle; M, mitochondria. RuO_4 post fixation.

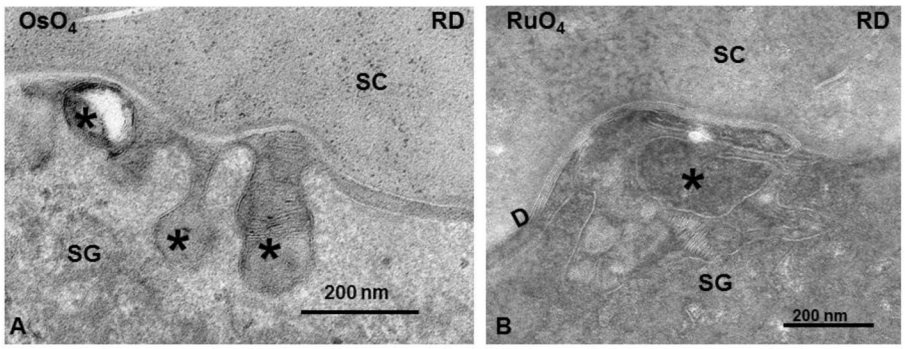

Fig. 4. Electron micrographs showing the SG-SC interface with normal and abnormal secreted contents

A. Electron lucent areas (asterisks) denote domain separation between lamellar and non-lamellar contents. OsO_4 post fixation. **B**. Another area of SG-SC interface with lamellar and non-lamellar (asterisk) domains. D, desmosome; SC, stratum corneum; SG, stratum granulosum. RuO_4 post fixation.

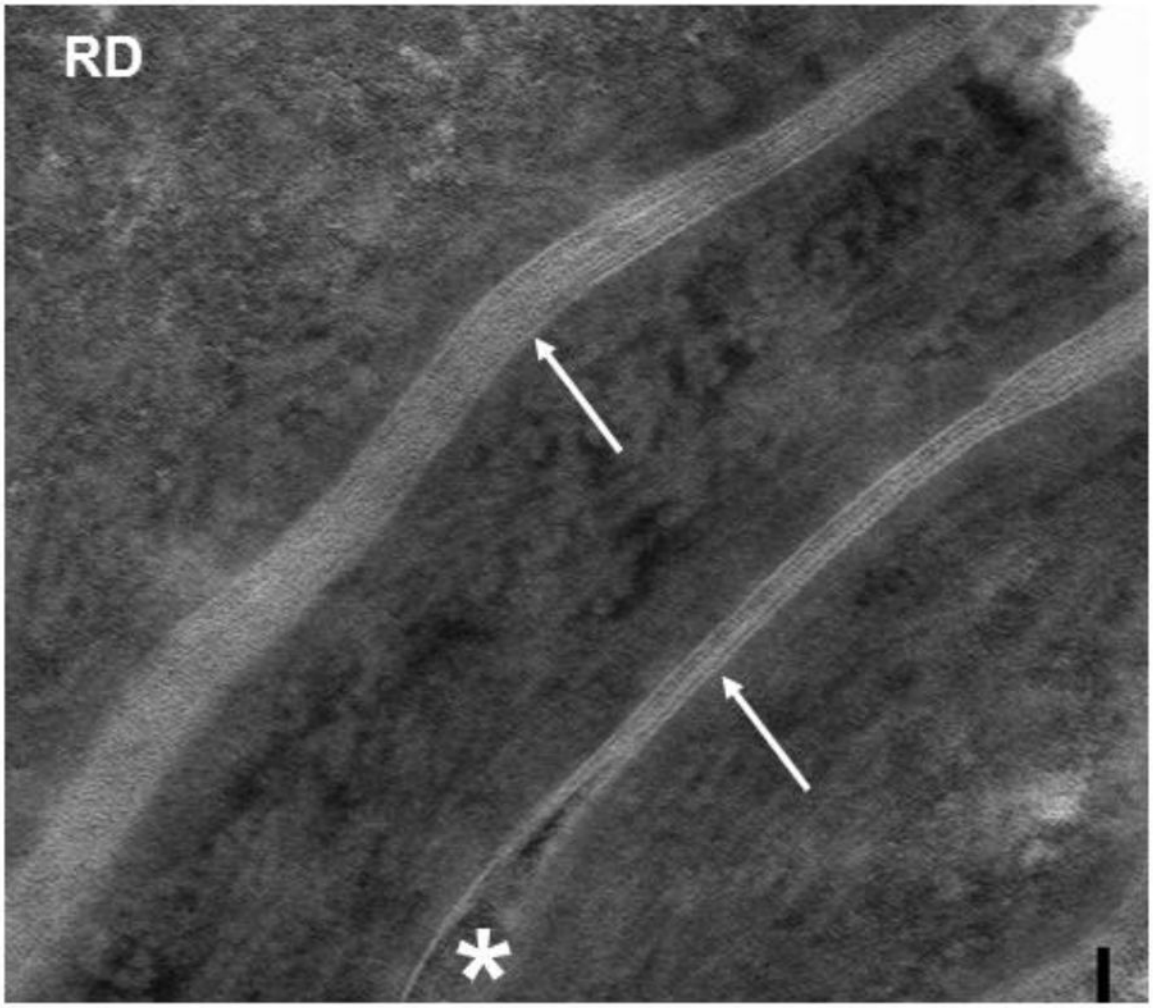

Fig. 5. Near normal appearance of the extracellular lamellae in RD

Note focal areas of lamellar/non-lamellar domain separation (asterisks). RuO_4 post fixation.

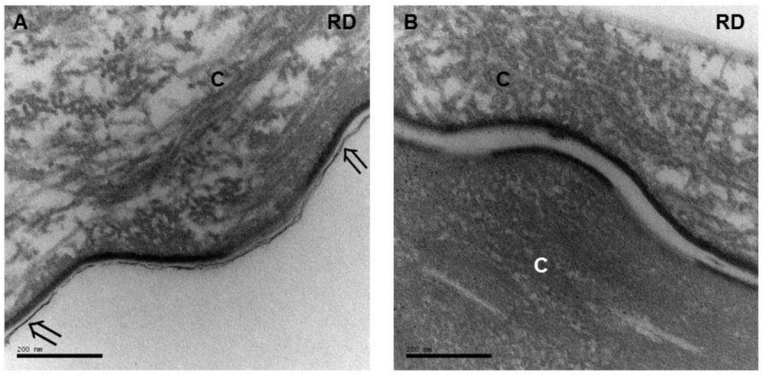

Fig. 6.

A. Electron micrograph showing loose attachment (‘delamination’) of the CLE external to the corneocyte envelope (open arrows). **B**. Micrograph showing absence of CLE along the corneocyte surface. C = corneocyte. OsO_4 post fixation.