Consequences of β-Glucocerebrosidase Deficiency in Epidermis

Ultrastructure and Permeability Barrier Alterations in Gaucher Disease

Walter M. Holleran, Edward I. Ginns, Gopinathan K. Menon, Jens-Uwe Grundmann, Manigé Fartasch, Cindy E. McKinney, Peter M. Elias, and Ellen Sidransky

Department of Dermatology, University of California School of Medicine, San Francisco, California 94143; Dermatology Service, Veterans Administration Medical Center, San Francisco, California 94121; and Section on Molecular Neurogenetics, Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, Maryland 20892

Abstract

Hydrolysis of glucosylceramide by β-glucocerebrosidase results in ceramide, a critical component of the intercellular lamellae that mediate the epidermal permeability barrier. A subset of type 2 Gaucher patients displays ichthyosiform skin abnormalities, as do transgenic Gaucher mice homozygous for a null allele. To investigate the relationship between glucocerebrosidase deficiency and epidermal permeability barrier function, we compared the stratum corneum (SC) ultrastructure, lipid content, and barrier function of Gaucher mice to carrier and normal mice, and to hairless mice treated topically with bromoconduritol B epoxide (BrCBE), an irreversible inhibitor of glucocerebrosidase. Both Gaucher mice and BrCBE-treated mice revealed abnormal, incompletely processed, lamellar body–derived sheets throughout the SC interstices, while transgenic carrier mice displayed normal bilayers. The SC of a severely affected type 2 Gaucher's disease infant revealed similarly abnormal ultrastructure. Furthermore, the Gaucher mice demonstrated markedly elevated transepidermal water loss (4.2 ± 0.6 vs < 0.10 g/m^2 per h). The electron-dense tracer, colloidal lanthanum, percolated between the incompletely processed lamellar body–derived sheets in the SC interstices of Gaucher mice only, demonstrating altered permeability barrier function. Gaucher and BrCBE-treated mice showed < 1% and < 5% of normal epidermal glucocerebrosidase activity, respectively, and the epidermis/SC of Gaucher mice demonstrated elevated glucosylceramide (5- to 10-fold), with diminished ceramide content. Thus, the skin changes observed in Gaucher mice and infants may result from the formation of incompetent intercellular lamellar bilayers due to a decreased hydrolysis of glucosylceramide to ceramide. Glucocerebrosidase therefore appears necessary for the generation of membranes of sufficient functional competence for epidermal barrier function. (*J. Clin. Invest.* 1994. 93:1756–1764.) Key words: epidermis • Gaucher disease • ichthyosis • barrier • stratum corneum

Introduction

Epidermal permeability barrier function is mediated by a mixture of nonpolar lipids, enriched in ceramides (1), which are localized to the intercellular spaces of the stratum corneum (SC)[1] where they form characteristic lamellar bilayers (2–4). During the final stages of epidermal differentiation, a sequence of membrane transitions occurs within the SC extracellular domains (2). Extrusion of lamellar body contents (at the stratum granulosum/SC interface) is followed sequentially by unfurling, elongation, and processing into mature lamellar bilayer unit structures (5–7). Concurrently, marked alterations in lipid composition occur, including the elimination of glucosylceramides and the accumulation of ceramides in the SC (1, 2, 8, 9). Recent studies have shown abundant levels of β-glucocerebrosidase in murine epidermis, with the highest levels of enzyme activity occurring in the SC (10). In contrast, in the SC of mucosal epithelia which display less stringent barrier requirements, endogenous β-glucosidase levels are low (11), glycosylceramides predominate over ceramides (12), untransformed lamellar body contents are retained into the outer SC (12), and mature membrane structures do not form (13). Thus, hydrolysis of lipid precursors, including glucosylceramides to ceramides, could account for the maturational changes in membrane structures that establish a permeability barrier of sufficient competence for terrestrial existence.

Three recent developments allow delineation of the relationship between normal epidermal function and glucocerebrosidase activity. First, the conduritol B epoxides, covalent inhibitors of glucocerebrosidase (14), which produce an inhibitor-based animal model of Gaucher disease (GD) (15, 16), increase epidermal glucosylceramide content and alter permeability barrier function after topical applications (17). However, significant inhibition of enzyme activity (> 90%) was required before the barrier abnormality emerged (17), consistent with the unusually high glucocerebrosidase activity present in the outer layers of normal epidermis (10). Second, visualization of the SC intercellular domains using RuO_4 after fixation (6) has provided new insights into the structural basis of the skin lesions in several inherited and acquired metabolic abnormalities associated with abnormal desquamation (18, 19). Third, glucocerebrosidase-deficient mice (Gaucher mice) have been developed by targeted gene disruption (20, 21).

GD is an autosomal recessive disorder due to a deficiency in β-glucocerebrosidase (EC 3.2.1.45, β-D-glucosyl-N-acylsphingosine glucohydrolase) (22–24). Since this enzyme normally catalyzes the hydrolysis of glucosylceramides to ceramides, excess glucosylceramide accumulates in the lysosomes of reticuloendothelial cells, resulting in splenic, hepatic, bone, and central nervous system involvement (24–27). Although

Address correspondence to Walter M. Holleran, Pharm.D., Dermatology Service (190), Department of Veterans Affairs Medical Center, 4150 Clement Street, San Francisco, CA 94121. Dr. Manige Fartasch's current address is Department of Dermatology, University of Erlangen, Hartmannstrasse 14, D-91052 Erlangen, Germany.

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1. *Abbreviations used in this paper:* BrCBE, bromoconduritol B epoxide; GD, Gaucher disease; HPTLC, high performance TLC; 4MU, 4-methylumbelliferone; SC, stratum corneum; TEWL, transepidermal water loss.

skin involvement in GD had previously been considered uncommon and usually limited to pigmentary changes (28), a subgroup of type 2 Gaucher patients that displays concurrent scaling abnormalities (collodion baby) has been described recently (29–32). The lack of skin manifestations in most patients with GD may relate to differences in residual enzyme activity, since ichthyotic skin involvement in type 2 Gaucher patients is associated with severe disease and very low residual enzyme levels in the extracutaneous tissues of these patients (29). The Gaucher mice created with a null allele are analogous to the most severely affected type 2 Gaucher neonates. These mice have no significant enzyme activity and a severe phenotype, including an ichthyotic skin condition (29). The availability of this severely glucocerebrosidase-deficient transgenic mouse model allows a novel approach for the assessment of glucosylceramide to ceramide hydrolysis in both epidermal permeability barrier function and the pathogenesis of type 2 GD.

To determine further the role of glucocerebrosidase in the epidermis, we compared the permeability barrier function, ultrastructure, and epidermal sphingolipid composition of Gaucher mice with heterozygous littermates, BrCBE-treated animals, and normals. We also studied the epidermal ultrastructure in an infant with GD and ichthyotic skin (29, 30). These studies demonstrate that the skin lesions in both Gaucher mice and humans can be attributed to extracellular membrane structural abnormalities which result from failure of processing of glucosylceramides to ceramides within the SC interstices. Furthermore, the formation of the membrane structures required for a competent epidermal permeability barrier involves glucosylceramide to ceramide processing.

Methods

Materials. 4-Methylumbelliferone (4MU), 4MU-β-D-glucopyranoside, alpha-hydroxy and nonhydroxy ceramides (IV and III, respectively), alpha-hydroxy and nonhydroxy galactocerebrosides (I and II, respectively), and sodium taurocholate were obtained from Sigma Chemical Co. (St. Louis, MO). Bromoconduritol B epoxide (BrCBE) was provided by Dr. Günter Legler (Koln, Germany) and was prepared as described previously (33). Bio-Rad and bicinchoninic acid (BCA) protein assay kits and BSA standards were obtained from Bio-Rad Laboratories (Richmond, CA) and Pierce Chemical Co. (Rockford, IL), respectively. High performance thin-layer chromatography (HPTLC) plates (silica gel 60) were obtained from Merck (Darmstadt, Germany). All solvents were of reagent or HPLC grade.

Animals, topical protocols, and functional studies. Gaucher mice were generated by targeted disruption of the murine glucocerebrosidase gene (20, 21). A null allele was generated in embryonic stem cells through gene targeting, and these genetically modified cells were used to establish a mouse strain carrying the mutation (34, 35). Genotyping of the mice was performed on mouse tail DNA by Southern blotting, as described previously (36).

Hairless male mice (hr/hr) were purchased from Simonsen Laboratories (Gilroy, CA) and fed mouse diet (Ralston Purina Co., St. Louis, MO) and water ad lib. Animals were 8–12-wk old at the time of study. A single topical dose of BrCBE (15 μl of a 25 nmol/μl solution) in vehicle (propylene glycol:ethanol, 70:30, vol/vol) or vehicle alone was applied daily to a 5-cm^2 area on each flank.

Permeability barrier function was assessed as transepidermal water loss (TEWL) and was measured with an electrolytic moisture analyzer (Meeco, Inc., Warrington, PA), as described in detail previously (37, 38). Water loss measurements were obtained over a small area of skin (0.5 cm^2), recorded in ppm/0.5 cm^2 per h over background, and converted to g/m^2 per h.

Preparation of epidermal homogenates and β-glucocerebrosidase assay. Epidermal sheets were obtained from newborn Gaucher mice by first removing whole skin samples from euthanized animals and submerging these samples in calcium- and magnesium-free Dulbecco's PBS containing 10 mM EDTA (pH 7.4) for 35–45 min (37°C), followed by gentle scraping with a scalpel blade. All subsequent steps were performed at 4°C, unless otherwise noted. Epidermal sheets were homogenized in 300 μl of extraction buffer (60 mM potassium phosphate containing 0.1% Triton X-100 by volume, pH 5.96) with a Potter-Elvehjem grinder (Baxter, McGaw Pk, IL). The extracts were sonicated (80%; 2 × 10 s), and cell debris was pelleted in a microfuge at 10,000 *g* for 5 min. Aliquots of epidermal homogenates from each transgenic and BrCBE-treated mouse were assayed for 1 h at 37°C in citrate-phosphate buffer (pH 5.6), using 4MU-β-D-glucopyranoside as the substrate, as described previously (39). Assays of BrCBE-treated epidermis were performed as described previously (17). Protein determinations were performed with either the BCA protein assay kit or the Bradford procedure (40), using BSA as the standard. The specific activity was determined for each sample using a 4MU standard curve and the protein concentration.

Human skin samples. Human skin samples from a type 2 GD patient with a collodion baby phenotype previously described (30) were provided by Dr. Sherer and Dr. Metlay (University of Rochester, Rochester, NY). This and normal skin biopsies were obtained at autopsy and preserved in 10% formaldehyde before subsequent postfixation and preparation for ultrastructural analysis, as described below.

Transmission electron microscopy. Full-thickness skin biopsies were obtained from euthanized Gaucher mice and normal animals, minced to < 0.5 mm^3, and fixed in modified Karnovsky's fixative overnight. Human tissue samples were fixed further in modified Karnovsky's fixative. All samples were then split and postfixed in the dark in either 0.5% RuO_4 or 2% aqueous OsO_4, both containing 1.5% potassium ferrocyanide (6). Lanthanum penetration, as a marker for sites of water permeability, was used to further measure the functional integrity of the intercellular membrane domains in the SC of Gaucher vs heterozygous mice, as described previously (41). Fresh biopsy samples were submerged immediately (30 min, 25°C) in 4% lanthanum nitrate (Electron Microscopy Sciences, Fort Washington, PA) in 10 mM Tris-HCl buffer containing 4% sucrose, pH 7.4 to 7.6, transferred to half-strength Karnovsky's fixative, and prepared for electron microscopy as described above. After fixation for 2 h at room temperature, all samples were dehydrated in graded ethanol solutions and embedded in an Epon-epoxy mixture (42). Thin sections were examined, with or without further contrasting with lead citrate, in an electron microscope (10A; Carl Zeiss, Inc., Thornwood, NY) operating at 60 kV.

Preparation of SC sheets. Samples of whole murine skin were excised and floated dermis-side downward (to minimize contact with the proteolytic enzyme) for 1.0–1.5 h at 25°C in 0.5% trypsin solution (in calcium- and magnesium-free Dulbecco's PBS), at which point the trypsin solution was changed. After a 30-min incubation at 37°C, the tissue was gently sonicated for 10 min to remove the dermis and subjacent nucleated epidermal cells. The residual sheets were rinsed with distilled water, and the area of each sample was determined.

Lipid extraction, fractionation, and quantitation. Lipids were extracted from epidermal and SC sheets, prepared as described above, by a modification of the Bligh-Dyer method (8), dried, weighed, and stored in chloroform at −70°C until used. For separation and quantitation of individual sphingolipid species by HPTLC, we used a modification of the method of Ponec et al. (43), as described recently (37, 38). After the final solvent development, the dried plates were dipped in charring solution, dried again (40°C, 10 min), and charred at 180°C for 5 min as described previously (17). The plates were scanned with a variable wavelength scanning densitometer (Camag, Muttenz, Switzerland), and the lipids were quantitated by comparison to known standards simultaneously run in duplicate for each fraction. Integration was performed using software (CATS II; Camag). Parallel samples

were separated by TLC on silica gel 60 plates and stained for visualization of hexose moieties as previously described (0.2% orcinol in 1 M sulfuric acid) (44).

Results

Epidermal β-glucocerebrosidase activity in animal models

To assess the extent of the biochemical abnormality in type 2 Gaucher mouse SC, we first measured epidermal glucocerebrosidase activity in affected vs phenotypically normal littermates and compared these with enzyme activity in BrCBE-treated epidermis. While glucocerebrosidase activity was reduced by ~ 50% in heterozygous epidermis (88 vs 207 nmol/h per mg cell protein), homozygous animals displayed no significant enzyme activity (Fig. 1). A single topical dose of BrCBE (375 nmol/5 cm^2) was sufficient to inhibit epidermal glucocerebrosidase activity in normal mice by > 95% at 1 h after treatment (Fig. 1) (17). Southern blot analysis confirmed that the homozygous and heterozygous null genotypes corresponded with the absence and reduction of epidermal glucocerebrosidase activity, respectively (data not shown). These results demonstrate the absence of glucocerebrosidase activity in the epidermis of Gaucher mice and the extent of residual activity in heterozygous and BrCBE-treated animals.

Permeability barrier function in animal models

TEWL. We next evaluated whether the absence of glucocerebrosidase activity in Gaucher mice alters permeability barrier function. TEWL measurements were performed on neonates delivered by Cesarean section at or near term (i.e., day 19 or 20 of gestation). The Gaucher mice demonstrated a 10- to 50-fold increase in TEWL over both normal and unaffected littermates (Fig. 2). Moreover, heterozygous animals, which do not present with phenotypic skin abnormalities (29), had no measurable increase in TEWL, suggesting that partial glucocerebrosidase activity is sufficient for normal structural and functional maturation of the murine epidermal barrier. Furthermore, the permeability abnormality was confirmed by an independent method, lanthanum-penetration (see below). In support of this observation, profound inhibition of epidermal β-glucocerebrosidase activity by topical application of BrCBE was necessary to induce a significant increase in TEWL (Fig. 2) (17). These results demonstrate the importance of glucosylceramide to ceramide hydrolysis for the maintenance of epidermal permeability barrier homeostasis.

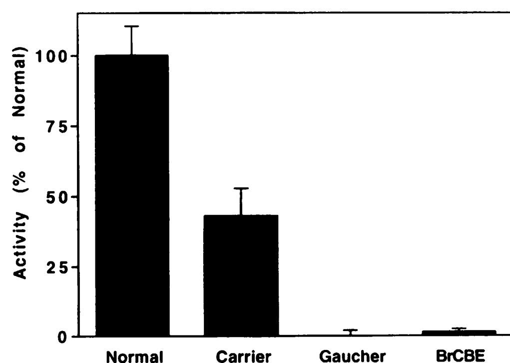


Figure 1. Epidermal glucocerebrosidase activity. Activity of glucocerebrosidase was measured in epidermal homogenates from normal, heterozygous carrier, and homozygous (Gaucher) littermates (see Methods). Activity of BrCBE-treated epidermis was measured 1 h after a single topical dose (375 nmol/5 cm^2), as described previously (17). Results are presented as percentage of normal epidermal enzyme activity. Carrier epidermis contained ~ 50% of normal activity, while Gaucher mice demonstrated no significant glucocerebrosidase activity ($P < 0.001$). BrCBE-treatment also resulted in > 95% inhibition of epidermal glucocerebrosidase activity ($P < 0.001$). Data represent the mean ±SEM for each group ($n > 5$ animals for each group).

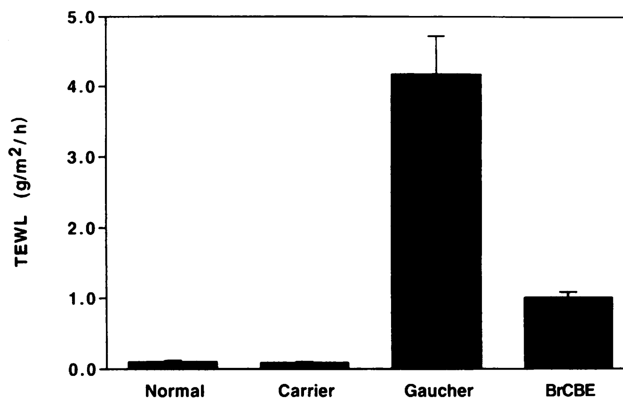


Figure 2. Barrier function in animal models. BrCBE-treated animals (greater than or equal to four determinations on each of three animals) were measured in a separate experiment after 5 d of topical inhibitor application (375 nmol/5 cm^2), as previously described (17). Whereas BrCBE-treated epidermis displayed an intermediate level of permeability barrier abrogation, the type 2 Gaucher mice demonstrated marked elevations in TEWL over both heterozygous carrier and normal animals ($P \leq 0.001$).

Sphingolipid content of affected vs unaffected murine epidermis

The deficiency of epidermal glucocerebrosidase activity could produce an altered distribution of sphingolipids in the epidermis and SC of Gaucher vs heterozygous mice. In both the epidermis and the SC, very little glucosylceramide and a predominance of ceramide are present in heterozygotes, while Gaucher mice display abundant glucosylceramides (Fig. 3, *A* and *B*; only SC data shown). Densitometric analyses confirmed the presence of a high ratio of ceramides to glucosylceramides in both the epidermis and the SC of heterozygote tissue (Fig. 4; SC data only). In contrast, the glucosylceramide content of both the epidermis (data not shown) and the SC of Gaucher mice was 10- to 15-fold ($P \leq 0.001$) higher than normal and/or heterozygous mouse samples (Fig. 4). While the increase in glucosylceramide content of the SC was similar to that described in BrCBE-treated animals, ceramide levels were lower in Gaucher mouse SC than in BrCBE-treated animals (17). These results demonstrate that the absence of epidermal glucocerebrosidase results in an accumulation of glucosylceramides and a depletion of ceramides in the SC of Gaucher mice.

Ultrastructure of transgenic β-glucocerebrosidase–deficient and BrCBE-treated mouse epidermis

The effects of targeted disruption of the murine glucocerebrosidase gene on SC extracellular lamellar bilayer structure were also studied. Electron microscopic evaluation of the Gaucher mouse samples, postfixed in RuO_4, revealed partially pro-

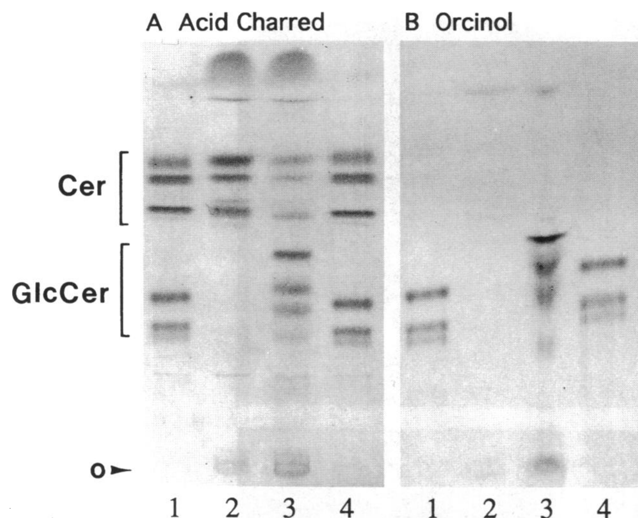


Figure 3. Identification of sphingolipid species. HPTLC separation of representative whole lipid extracts, isolated from both heterozygous carrier (*A* and *B*, lane *2*) and homozygous (Gaucher) (*A* and *B*, lane *3*) SC (see Methods). Ceramide (*Cer*) and glucosylceramide (*GlcCer*) standards were applied in lanes *1* and *4*. *A* shows that SC from carrier mice contains primarily ceramide species, with glucosylceramide species being very low or absent (*A*, lane *2*), similar to that observed in normal mice (17). In contrast, the SC of Gaucher mice shows an abundance of glucosylceramides with an apparent reduction of ceramides (lane *3*). The presence of increased hexose residues in Gaucher mouse SC is confirmed by the simultaneous orcinol stain (*B*, lane *3*). In contrast, little to no orcinol-positive species are evident in carrier SC (*B*, lane *2*).

cessed lamellar body–derived sheets extending throughout the lower (Fig. 5 *A*) and mid to outer (Fig. 5 *B*) SC interstices. These abnormalities in the Gaucher mouse were indistinguishable from the alterations in the SC of animals treated with BrCBE (Fig. 5 *C*) (17). Epidermal lamellar bodies and their secreted contents at the stratum granulosum/SC interface in

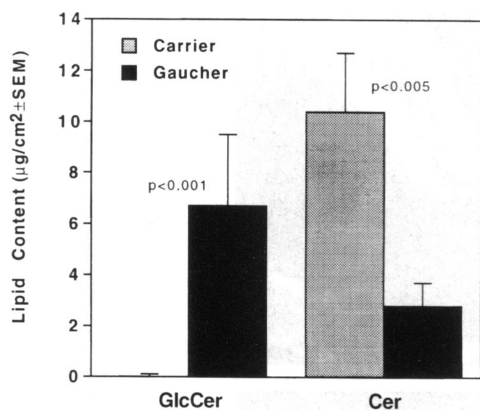


Figure 4. Quantification of SC sphingolipids. SC lipids were extracted from both heterozygous carrier and Gaucher animals, fractionated by TLC, and quantitated by scanning densitometry (see Methods). The content of total glucosylceramides (*GlcCer*) was significantly increased in Gaucher vs carrier SC ($P < 0.001$), while ceramides (*Cer*) were significantly reduced ($P < 0.005$). Each value represents the mean ±SEM from three or more separate animals (duplicate determinations).

the Gaucher mice appeared normal, as occurred in BrCBE-treated animals (17). Finally, unaffected heterozygous littermates showed normal, mature basic lamellar unit structures throughout the SC interstices (Fig. 5 *D*). These studies show that both the Gaucher mice and the BrCBE-treated animals display similar membrane structural abnormalities, namely a failure to form mature extracellular lamellae.

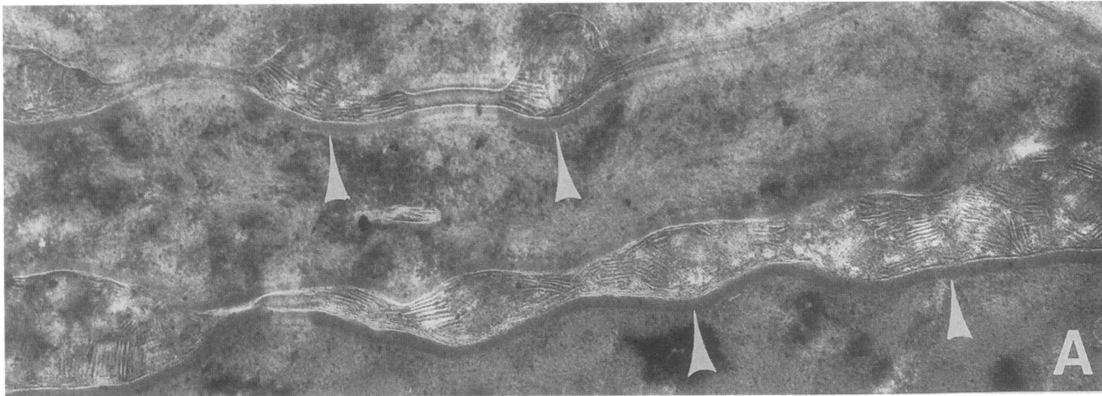
Ultrastructure of epidermis in human GD

In the skin samples from the type 2 Gaucher neonate, the normal lamellar bilayer unit structure of the SC extracellular domains was replaced by arrays of loosely packed, unprocessed lamellar body–derived sheets (Fig. 6, *A* and *B*). These findings closely resembled those of both the Gaucher mouse and the BrCBE-treated models described above. In contrast, normal human skin demonstrates a normal lamellar bilayer unit pattern (Fig. 6 *C*). The structure and contents of lamellar bodies appeared normal in the epidermis from the type 2 Gaucher neonate (not shown) as well as in the animal models. These results demonstrate that absence or depletion of glucocerebrosidase activity in human epidermis is associated with characteristic alterations in the ultrastructure of SC extracellular lamellar structures, without an apparent alteration of either lamellar body contents or delivery.

Lanthanum penetration. Penetration of electron-dense, colloidal lanthanum ions has been used not only to assess water permeation through paracellular pathways but also as an indicator of the integrity of lamellar bilayers for resisting water movement through the SC interstices (41, 45). We used this technique to investigate further the potential role of the abnormal intercellular membranes in the pathogenesis of the skin lesions in the Gaucher mouse and in the development of abnormal barrier function. In both normal and heterozygous transgenic mice, lanthanum ion was excluded from the outer layers of the SC as well as from sites apical to the stratum granulosum/SC interface (Fig. 7 *A;* heterozygous transgenic mouse SC shown). In contrast, lanthanum penetrated via the interstices to all levels of the SC in the Gaucher mice (Fig. 7 *B*), demonstrating an alteration in the integrity of the SC extracellular domains. Moreover, the colloidal lanthanum was interspersed between the incompletely processed, extracellular lamellar structures in the SC of the Gaucher mice (Fig. 7 *B*, *inset*). These results show that the altered membrane structures in the SC interstices of type 2 Gaucher mice display abnormal functional integrity.

Discussion

We have demonstrated previously that inhibition of epidermal β-glucocerebrosidase activity by topical applications of BrCBE, a specific and covalent inhibitor of this enzyme (14), induces an abnormality in permeability barrier function in association with a marked increase in epidermal glucosylceramide content and significant alterations in SC lamellar bilayer structure (17). In this study, we have demonstrated that an absence of epidermal glucocerebrosidase activity in type 2 Gaucher mice leads to abnormal barrier function in association with accumulation of glucosylceramides and depletion of ceramides in the SC (Table I). Moreover, we have shown that failure of conversion of glucosylceramide to ceramide results in persistence of "immature" extracellular membrane structures throughout the SC interstices. Identical ultrastructural changes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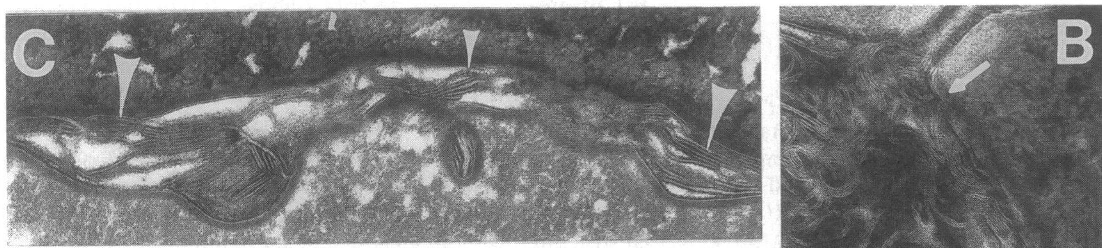
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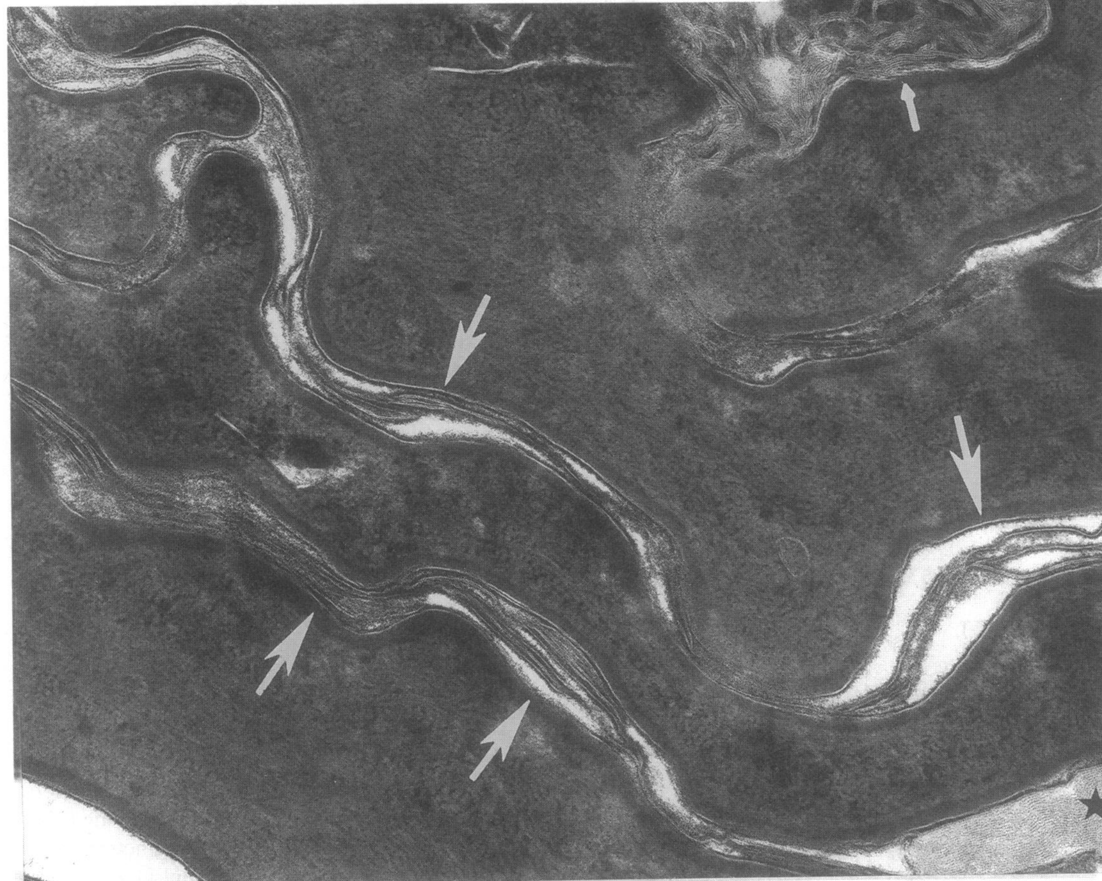
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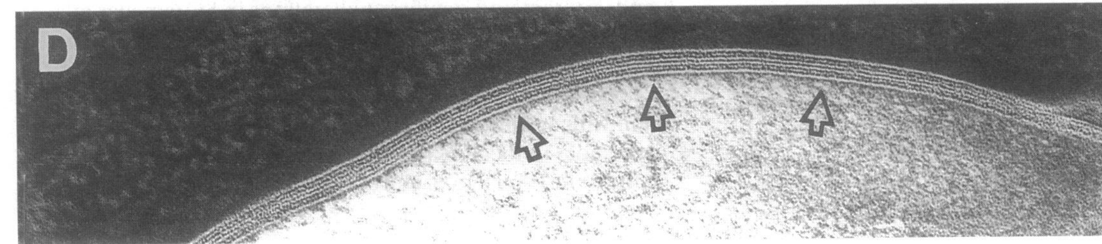
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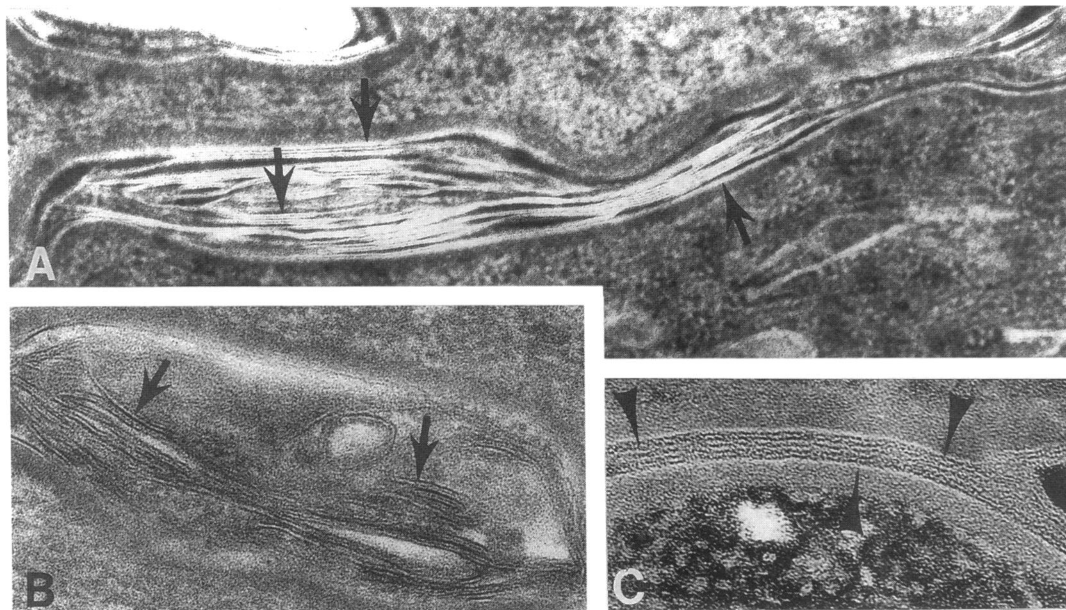

Figure 6. SC ultrastructure in human type 2 GD. In the mid to outer SC of a patient with type 2 GD with ichthyotic skin lesions (*A* and *B*), loosely packed membrane leaflets extend throughout the SC interstices (*arrows*). Conventional OsO_4 staining, which does not delineate interstitial membrane regions in normal SC (not shown), further demonstrates altered intercellular membrane leaflets in the outer SC of type 2 GD (*B*). In normal human epidermis (*C*), intercellular lamellar structures appear normal at all levels of the SC, and normal appearing lamellar basic unit structures are formed (*arrowheads*). *A,* ×67,000 (RuO_4 postfixed); *B,* ×80,000 (OsO_4 postfixed); *C,* ×211,000 (RuO_4 postfixed).

were observed with topical BrCBE treatment, but in this model glucosylceramides accumulate while ceramide levels remain almost normal (17). Establishment of a competent epidermal permeability barrier, therefore, requires conversion of glucosylceramides to ceramides.

Epidermal lipids, in addition to their established role as mediators of the permeability barrier, also influence desquamation (46). For example, inborn errors of lipid metabolism appear to play a role in the pathogenesis of cutaneous lesions in recessive X-linked ichthyosis (47), Refsum disease (48), Sjögren-Larsson syndrome (49), and neutral lipid storage disease (50). The presence of ichthyosiform skin changes in both the glucocerebrosidase-deficient transgenic mice and in some type 2 Gaucher patients suggests that blockade of glucosylceramide hydrolysis, with failure to form normal lamellar bilayer unit structures, not only compromises the integrity of the epidermal permeability barrier, as shown by the increased TEWL and the lanthanum perfusion studies, but also provokes an alteration of normal desquamation.

Two biochemical mechanisms could be responsible for the altered lamellar bilayer structures seen in both the Gaucher mouse and the type 2 Gaucher patient. It is possible that the increase in glucosylceramide could inhibit the formation of normal lamellar bilayers in the SC interstices by interference with acyl chain packing. A comparison of the membrane structures that result from glucosylceramide deposition within Gaucher macrophages vs those described here in Gaucher mouse SC reveals both similarities and differences. The intracellular glucosylceramide-enriched deposits in Gaucher macrophages consist of aggregates of twisted bilayers, with a bilayer width of 60 Å (51). In contrast, x-ray diffraction and ultrastructural studies have demonstrated that the normal lamellar spacing in normal murine and human SC is formed by two back-to-back leaflets measuring ≅ 130 Å (single leaflet = 65 Å) (4, 52). Whereas normal mature lamellar bilayers lack glucosylceramides (1, 2), the increased content of extracellular glucosylceramides which occurs in both BrCBE-treated skin and in Gaucher mouse SC, may prevent formation of this normal repeating bilayer pattern. The altered lamellar bilayers could result from either steric constraints imposed by the additional bulk of the glucose residue and/or the increased hydrogen-bonding capacities provided by the hydrophilic hexose residue (53, 54). Thus, although increased glucose moieties could result in a loss of barrier integrity because of an alteration of lamellar bilayer structures and increased perfusion of water, a concomitant increase in the cohesiveness of corneocytes, due to increased hydrogen bonding, could lead to the abnormal SC retention, i.e., the excess scale seen in Gaucher mice and in certain type 2 Gaucher patients.

Alternatively, abnormal lamellar structures could result from the depletion of SC ceramide as a result of diminished glucosylceramide hydrolysis. We have shown in this study that the ceramide content of Gaucher mouse SC is reduced significantly. Ceramides, along with cholesterol and fatty acids, are known to be important components of the extracellular lipid mixture that mediates barrier function (1). Thus, the lack of sufficient ceramides, even in the presence of increased glucosylceramides, could result in dysfunctional membrane structures. The exact correlation between the level of ceramide deficiency and diminished barrier function is difficult to assess as other SC lipids are also important determinants of the barrier. Recent studies have shown the importance of a correct molar

Figure 5. Ultrastructure of SC of murine animal models. Immature (unprocessed) lamellar body–derived sheets were present in the lower SC interstices (*A, arrows*) and extended throughout the mid to outer SC interstices of homozygous Gaucher mice (*B, arrows*). Topical BrCBE treatment results in similar alterations in the SC lamellar bilayer system (*C, arrows*). In contrast, unaffected heterozygous carrier littermates showed normal, mature basic lamellar unit structures throughout the SC (*D, open arrows*). *A,* ×64,000; *B,* ×75,000; *C,* ×120,000; *D,* ×76,000.

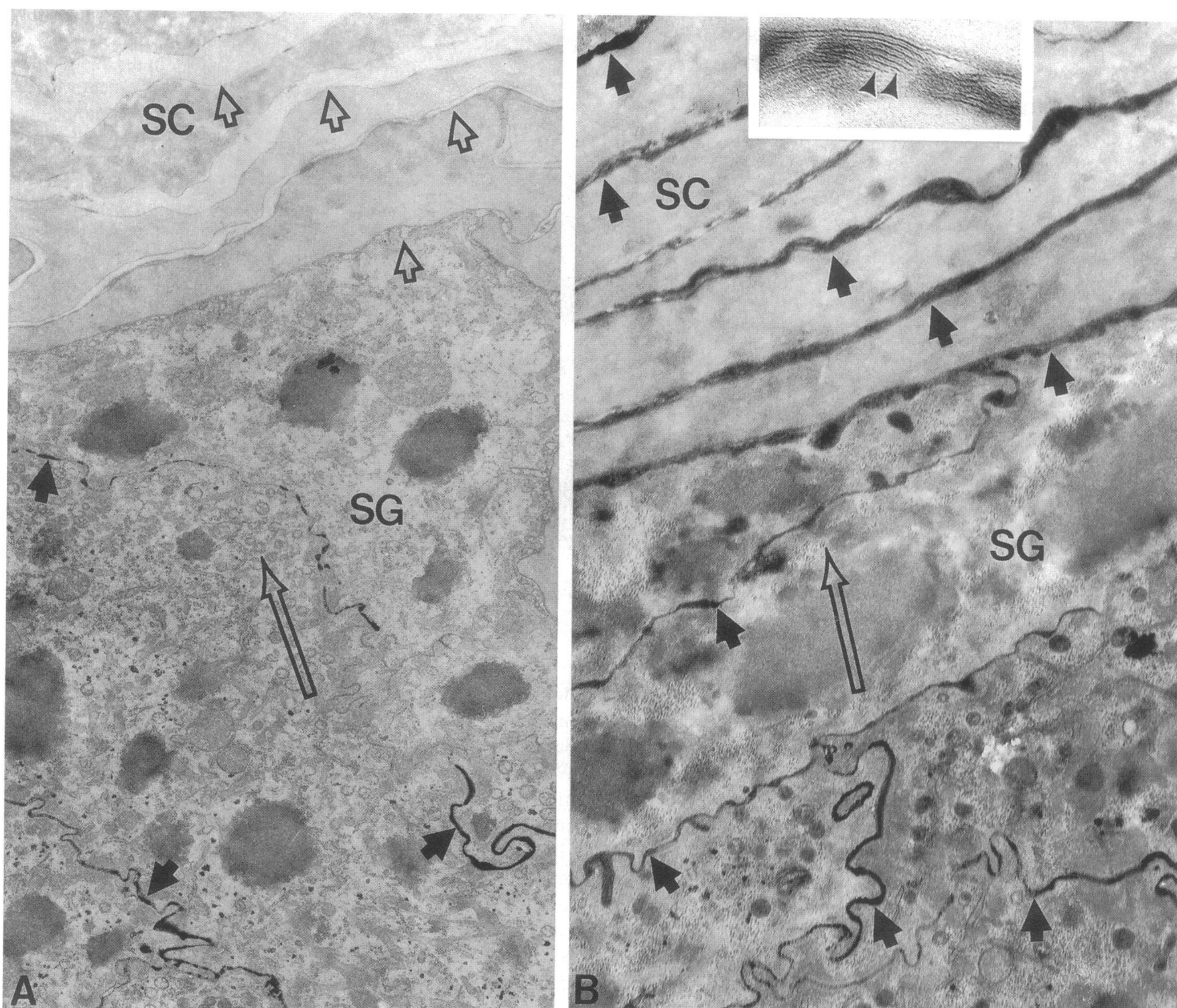

Figure 7. Permeability pathways and membrane integrity. Whole murine skin biopsies were bathed in buffered colloidal lanthanum solution (pH 7.4–7.6) for 30 min, fixed, and prepared for electron microscopy. The approximate direction of water flux within the epidermis is indicated by the large open arrows (*A* and *B*). In carrier epidermis (*A*), electron-dense precipitates of lanthanum remain restricted to the intercellular spaces between cells of the stratum granulosum (*SG*) (*small, closed arrows*) and excluded from all areas above the stratum granulosum SC interface (*small, open arrows*). In contrast, Gaucher epidermis (*B*) shows lanthanum penetration not only within the stratum granulosum interstices but also throughout the intercellular spaces of the SC (*closed arrows*), consistent with an intercellular route for the increased water loss in these animals (compare Fig. 2). Furthermore, lanthanum precipitates outline the immature lamellar sheets in the Gaucher SC interstices (*B, inset, arrows*). *A*, ×15,000; *B*, ×18,900.

ratio of ceramides, cholesterol, and free fatty acids for the recovery of permeability barrier function after acute barrier disruption (55). However, topical coapplications of ceramides in the BrCBE model did not prevent the deleterious effects of this inhibitor on either barrier function or SC lamellar bilayer structure (17). Furthermore, recovery of permeability barrier function after extraction of SC lipids is delayed by BrCBE, and this effect is not altered by coapplications of ceramide (56). Thus, the persistence of glucosylceramides, rather than diminished ceramides, is more likely to be the principle cause of the membrane structural abnormalities leading both to the skin lesions in type 2 Gaucher disease and the permeability barrier defects in the Gaucher mouse.

The lipid composition of Gaucher mouse SC suggests that a majority of ceramides (70%) are derived from the deglycosylation of glucosylceramide (Fig. 4). However, whether the remaining ceramides (~ 30%) are delivered to the SC as free ceramide or involve an alternate metabolic precursor of ceramide, such as sphingomyelin, is not known. Furthermore, the complete lack of glucosylceramidase activity in the Gaucher mice resulted in less SC ceramide than was present in the BrCBE model (Table I) (17). The relatively normal ceramide levels, in the presence of increased glucosylceramide, could be a reflection of incomplete enzyme inhibition in the inhibitor-based model. Alternatively, these data also suggest that a compensatory normalization of SC ceramides may occur in the

Table I. Epidermal Findings in β-Glucocerebrosidase Deficiency

Finding	Topical BrCBE*	Gaucher mice	Mice heterozygous for null allele	GD type 2‡	Normal human
Clinical ichthyosis	No	Yes (mild)	No	Yes	No
Lanthanum penetration	ND	↑↑	Normal	NA	Normal
TEWL	↑	↑↑↑	Normal	NA	Normal
Altered SC bilayers	Yes	Yes	No	Yes§	No
SC GlcCer content	↑↑	↑↑	No change	NA	Low
SC Cer content	No change	↓	No change	NA	High

* Adapted from reference 17. ‡ A subset of type 2 Gaucher patients (reference 29). § Single patient investigated. ND, not determined; NA, not available, GlcCer, glucosylceramides; and Cer, ceramides.

BrCBE model through a glucosylceramide to ceramide independent pathway. Since the null allele Gaucher mice only survive 12–24 h in the dry, exutero environment, compensatory increases in ceramide levels may not yet be evident. Although acidic sphingomyelinase activity has been colocalized both to lamellar body-enriched fractions of murine epidermis and to SC extracellular domains (57), the role of sphingomyelin in providing either baseline or compensatory barrier ceramides is under investigation.

In summary, the pathogenesis of skin lesions observed with glucocerebrosidase deficiency appears to result primarily from an altered content of glucosylceramides and ceramides in the extracellular domains of the SC. The ultrastructural abnormalities induced by the persistence of glucosylceramides in the SC may be linked to the ichthyotic skin lesions that occur in certain type 2 Gaucher patients as well as in the type 2 Gaucher mouse. These studies show definitively that conversion of glucosylceramide to ceramide is required to form the intercellular membrane structures required for normal epidermal permeability barrier function.

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