Basis For Abnormal Desquamation And Permeability Barrier Dysfunction in RXLI

Peter M. Elias,*,† Debra Crumrine,* Ulrich Rassner,* Jean-Pierre Hachem,¶ Gopinathan K. Menon,* Wenyan Man,* Monica Hoi Wun Choy,* Laura Leypoldt,* Kenneth R. Feingold,† and Mary L. Williams*,#
*Department of Dermatology and #Pediatrics, University of California, San Francisco; †Dermatology and Medical Services, VAMC, San Francisco; and ¶Departments of Toxicology and Dermatology, Vrije Universiteit Brussels, Belgium

Mutations in the gene for steroid sulfatase (SSase), are responsible for recessive x-linked ichthyosis (RXLI). As a consequence of SSase deficiency, its substrate, cholesterol sulfate (CSO_4), accumulates in the epidermis. Accumulation of this amphipathic lipid in the outer epidermis provokes both a typical scaling phenotype and permeability barrier dysfunction. Research on RXLI has illuminated several, potentially overlapping pathogenic mechanisms and provided insights about the role of SSase and CSO_4 in normal differentiation, barrier maintenance, and desquamation. We now show here that SSase is concentrated in lamellar bodies (LB), and secreted into the SC interstices, along with other LB-derived lipid hydrolases. There, it degrades CSO_4, generating some cholesterol for the barrier, while the progressive decline in CSO_4 (a serine protease (SP) inhibitor) permits corneodesmosome (CD) degradation leading to normal desquamation. Two molecular pathways contribute to disease pathogenesis in RXLI: 1) excess CSO_4 produces nonlamellar phase separation in the stratum corneum (SC) interstices, explaining the barrier abnormality. 2) The increased CSO_4 in the SC interstices inhibit activity sufficiently to delay CD degradation, leading to corneocyte retention. We also show here that increased Ca^{++} in the SC interstices in RXLI could contribute to corneocyte retention, by increasing CD and interlamellar cohesion. RXLI represents one of the best understood diseases in dermatology – from the gene to the SC interstices, its etiology and pathogenesis are becoming clear, and assessment of disease mechanisms in RXLI led to new insights about the role of SSase and CSO_4 in epidermis terminal differentiation.

Key words: cholesterol sulfate/cholesterol/transepidermal water loss/serine proteases/corneodesmosomes/barrier function/ichthyosis/X-linked ichthyosis.
J Invest Dermatol 122:314–319, 2004

The Molecular Basis for Recessive X-Linked Ichthyosis

In 1978 recessive X-linked ichthyosis (RXLI) was linked to deficiency of the microsomal enzyme, steroid sulfatase (SSase) (Koppe *et al*, 1978; Webster *et al*, 1978). The SSase gene has been the subject of considerable research because of its location on the distal tip of the short arm of the X chromosome (Tiepolo *et al*, 1977; Mohandas *et al*, 1979; Tiepolo *et al*, 1980; Shapiro, 1985; Li *et al*, 1990). Patients with RXLI display gene mutations/deletions (Ballabio *et al*, 1987; Bonifas *et al*, 1987; Conary *et al*, 1987; Gillard *et al*, 1987; Shapiro *et al*, 1989), resulting in ichthyosiform skin changes with occasional extracutaneous organ system involvement, due to contiguous gene syndromes (Schmickel, 1986; Schnur and Trask, 1989).

SSase is a 62 kDa microsomal enzyme, responsible for hydrolyzing the 3β-sulfate esters from both cholesterol sulfate (CSO_4) and sulfated steroid hormones, generating their nonsulfated counterparts. As a result of enzyme deficiency in RXLI, CSO_4 accumulates in skin, plasma, and red cell membranes (up to 10- to 20-fold increase (Bergner and Shapiro, 1988; Epstein *et al*, 1981). CSO_4 is carried in blood by the low-density (β-lipoprotein) fraction, producing diagnostic electrophoretic alterations (Epstein *et al*, 1981; Ibsen *et al*, 1986). The fact that CSO_4 levels in scale are still higher than levels in blood presumably explains the prominence of skin involvement in RXLI (Bergner and Shapiro, 1988; Epstein *et al*, 1981; Williams and Elias, 1981).

Steroid sulfatase and cholesterol sulfate in normal epidermal physiology To examine the regulation of epidermal CSO_4 content by SSase activity in relation to normal cohesion and desquamation, epidermal cell populations were prepared from normal and RXLI epidermis, and assayed for both SSase activity and CSO_4 content (Elias *et al*, 1984). Whereas SSase activity is low in the basal and spinous layers, its levels peak in the SG (10–20 times

Abbreviations: CD, corneodesmosomes; CE, cornified envelope; Chol, cholesterol; CLE, corneocyte-bound lipid envelope; CSO_4, cholesterol sulfate; HMGCoA, hydroxymethyl glutaryl coenzyme A; LB, lamellar bodies; RXLI, recessive X-linked ichthyosis; SC, stratum corneum; SCCE, stratum corneum chymotryptic enzyme; SCTE, stratum corneum tryptic enzyme; SG, stratum granulosum; SPI, serine protease inhibitor; SSase, steroid sulfatase; TEWL, transepidermal water loss; TG-1, transglutaminase 1.

higher), and persist at equally high levels in the SC (Fig 1), where it is concentrated in membrane domains, primarily in the lower SC (Fig 2) In contrast, SSase activity is absent from both epidermal nucleated cell layers and the SC in RXLI (Kubilus *et al*, 1979; Elias *et al*, 1984). Normally, CSO_4 comprises about 5% of the total lipid of human stratum granulosum (SG), declining to about 1% of lipid mass in the outer SC, through ongoing hydrolysis of CSO_4 during SC transit (Fig 1). In RXLI, where SSase activity is absent, the SC contains up to 12% CSO_4 by lipid weight ($\approx$ 1% of total weight), a concentration that exceeds that in hair, nail, and ungulate hoof, where CSO_4 can account for 2–5% of total weight.

Because cholesterol sulfotransferase (sulf2Bib) activity predominates in lower nucleated cell layers, while SSase peaks in the outer epidermis, Epstein *et al* (1984) proposed an "epidermal cholesterol sulfate cycle", where cholesterol is first sulfurylated in the lower epidermis and then desulfated in outer epidermal layers, regenerating cholesterol (Fig 1). Moreover, CSO_4 levels are normally lower in the outer SC than in lower SC (Long *et al*, 1985; Ranasinghe *et al*, 1986), supporting the concept that ongoing hydrolysis of CSO_4 by SSase during SC transit may be critical for normal desquamation (Elias *et al*, 1984; Ranasinghe *et al*, 1986). The "cholesterol sulfate cycle", however, has significance that extends well beyond desquamation (see below).

Since SSase activity reportedly is not concentrated in lamellar body (LB)-enriched fractions of whole SG (Grayson *et al*, 1985), how the enzyme reaches membrane domains has remained problematic. Though a classic microsomal enzyme, ultrastructural immunocytochemical studies have also localized the enzyme to the plasma membrane, where it localizes to coated pits (Willemsen *et al*, 1988; Dibbelt *et al*, 1989). To address the question of SSase delivery to SC, we developed a new cytochemical method, utilizing both lead- and barium-capture methods, for the ultrastructural localization of SSase activity (see legend to Fig 2 for details about one of these methods). Using these techniques, we localized SSase not only within the cytosol (i.e., microsomes), but also within LB (Fig 2*B*). We further demonstrated delivery of SSase to the SC interstices following LB secretion, where its activity persists into the lower SC layers, but disappears from the outer SC (Fig 2*A*). Thus, SSase utilizes the LB secretory system to reach sites where it participates in the regulation of barrier homeostasis and desquamation like other lipid hydrolases that are involved in the extracellular processing of secreted polar lipids (Elias and Menon, 1991).

Like SSase, CSO_4 is concentrated in the SC interstices, but in contrast to other lipid precursors, it is not present in LB (Grayson *et al*, 1985). Yet, its mode of delivery to the SC interstices can be explained readily by both its lipophilicity

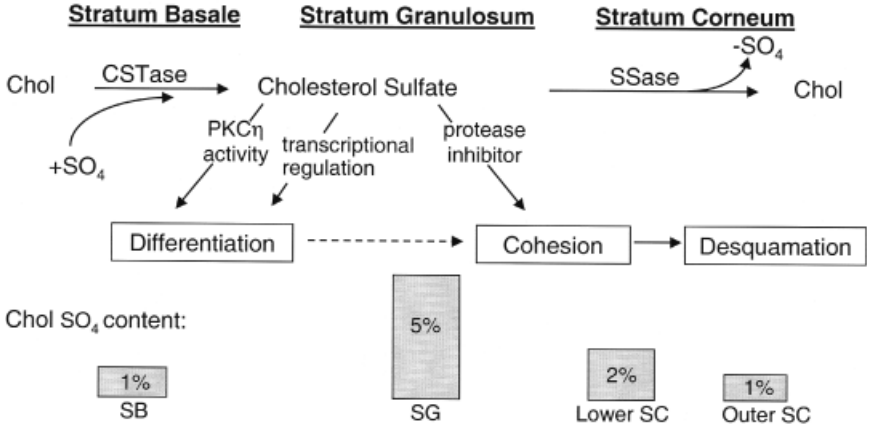

Figure 1
Cholesterol sulfate cycle in normal epidermis. Role of steroid sulfatase and cholesterol sulfate in normal epidermal physiology. Abbreviations: CSTase, cholesterol sulfotransferase; SSase, steroid sulfatase; Chol, cholesterol; PKC, protein kinase C; Chol SO_4, cholesterol sulfate; SB, stratum basale; SG, stratum granulosum; SC, stratum corneum.

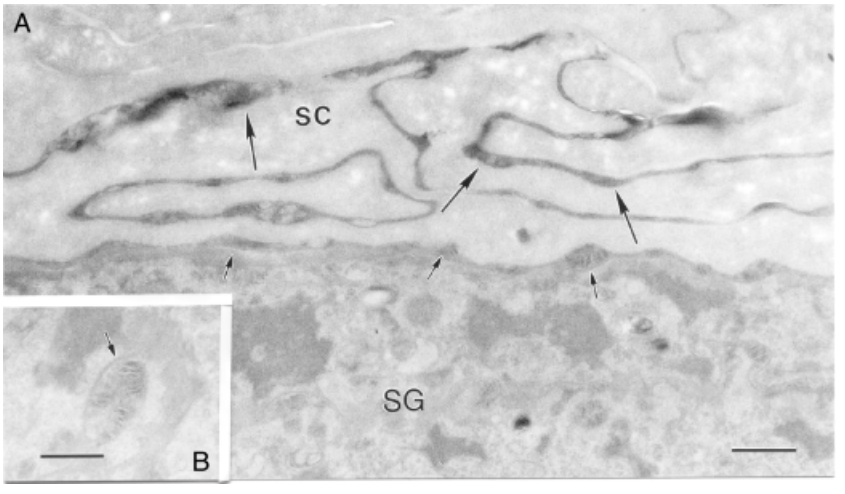

Figure 2
Steroid sulfatase activity localizes to lamellar bodies and at the stratum corneum interstices. A: Enzyme activity, shown as lead precipitate (arrows), localizes to the interstices of lower SC layers. B: SSase activity localizes to lamellar body contents and limiting membranes (not shown). Abundant activity also is seen at the stratum granulosum (SG)-stratum corneum (SC) interface. Human skin samples were aldehyde-fixed, rinsed in Hepes buffer, and microwave incubated in 5 mM 4-methylumbilliferyl sulfate and 2 mg/mL lead nitrate in Hepes buffer (Rassner *et al*, 1997). Post-fixation was in reduced osmium tetroxide with microwave irradiation for 2.5 min at 37°C. A & B, Scale bars: A: 1.0 μm; B: 0.5 μm.

and its amphilicity. CSO_4, like many oxygenated sterols (but unlike cholesterol), readily diffuses across the keratinocyte plasma membrane (Ponec and Williams, 1986). Therefore, in the absence of a lipid milieu within corneocytes, CSO_4 likely partitions preferentially to the highly hydrophobic, extracellular domains of the SC, based purely upon its solubility characteristics; i.e., it does not require delivery by a secretory organelle.

Mechanisms proposed to perturb permeability barrier homeostasis in RXLI Although patients with RXLI display only a minimal basal barrier abnormality (Lavrijsen *et al*, 1993), they demonstrate a pronounced delay in recovery kinetics following acute perturbations (Zettersten *et al*, 1998). Moreover, topically applied CSO_4 not only induces scaling (Maloney *et al*, 1984), but also a barrier abnormality (Zettersten *et al*, 1998), lending further credence to the hypothesis that excess CSO_4 plays a destabilizing role in SC barrier function. Several mechanisms have been proposed to contribute to the barrier abnormality in RXLI (Fig 3). First, CSO_4 fails to form eutectic mixtures with other SC lipids; hence, excess CSO_4 segregates into distinct nonlamellar domains in model lipid mixtures (= phase separation) (Rehfeld *et al*, 1986). Indeed, ultrastructural images of SC in RXLI show extensive, but focal nonlamellar domains, with disruption of the extracellular lamellae (Fig 3; see also Zetterston *et al*, 1998).

However, the barrier abnormality is probably due not only to excess CSO_4, but also to decreased cholesterol (the cholesterol content of the SC in RXLI is reduced by approximately 50%) (Williams and Elias, 1981), and decreased cholesterol alone can produce abnormal extracellular lamellar membranes (Mao-Qiang *et al*, 1993). Two unrelated mechanisms have been invoked to explain the decrease in cholesterol in RXLI (Fig 4): 1) reduced generation of cholesterol from CSO_4 due to the enzyme deficiency; (2) CSO_4-mediated inhibition of HMGCoA reductase, the rate-limiting enzyme of cholesterol synthesis (Williams *et al*, 1987). Thus, increased CSO_4 levels in RXLI could inhibit epidermal cholesterol synthesis, explaining the reduced levels of cholesterol in the SC of RXLI. Yet, topical cholesterol only partially improves barrier function in RXLI, and it only partially reverses the barrier abnormality in CSO_4-treated mice (Maloney *et al*, 1984), suggesting that it is increased CSO_4, rather than insufficient cholesterol, that accounts for the barrier abnormality in RXLI. Finally, CSO_4 inhibits transglutaminase 1 (TG-1)-mediated attachment of ω-hydroxyceramides to the cornified envelope (CE) *in vitro*, a step that forms the cornified-bound lipid envelope (CLE) (Nemes *et al*, 2000). Together, the CE/CLE forms a scaffold for the deposition and supramolecular organization of the SC lamellar bilayers, and in lamellar ichthyosis, decreased TG-1 activity leads to a defective CE scaffold, accounting in part for the barrier abnormality (Elias *et al*, 2002). Yet,

Figure 3
Abnormalities in barrier function in RXLI are due to lamellar-phase separation. Lamellar bilayers appear normal between numerous foci of phase separation in RXLI (A, arrows). (Sample biopsies obtained under our protocol approved by the UCSF Committee on Human Research, according to Declaration of Helsinki Guidelines). Ruthenium tetroxide postfixation. Scale bar = 0.25 μm.

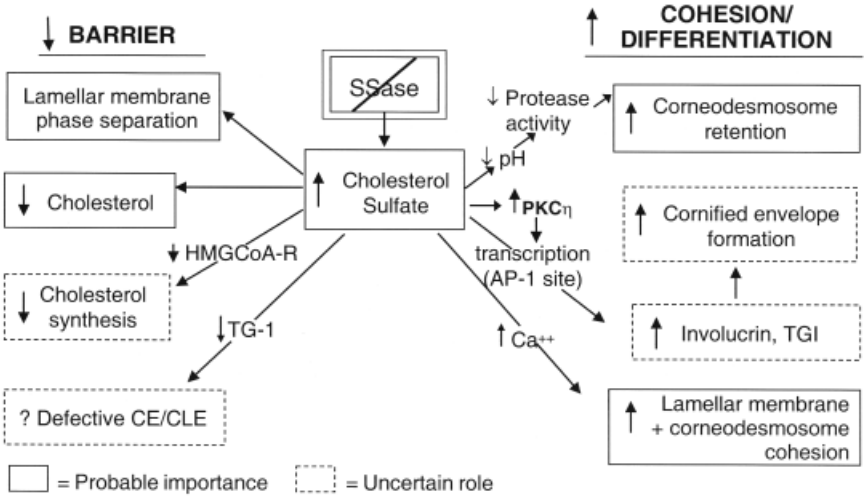

Figure 4
Pathogenic mechanisms in RXLI. Abbreviations: CE; cornified envelope; CLE, corneocyte-bound lipid envelope; TG-1, transglutaminase 1; HMGCoA-R, hydroxymethyl glutarylCoenzyme A reductase (for others see legend to Figure 1).

despite the proposition that increased CSO_4 could alter the barrier in RXLI via an effect on TG-1-mediated scaffold function (Nemes *et al*, 2000), the CE/CLE scaffold has not been demonstrated to be abnormal in RXLI.

Mechanisms proposed to cause abnormal desquamation in RXLI The hyperkeratosis in RXLI is attributed to delayed desquamation rather than to excessive hyperproliferation (Williams, 1991). Both epidermal proliferation indices and transit times are normal, consistent with an absence of significant acanthosis in RXLI (Frost and Van Scott, 1966). Thus, RXLI is classified as a classic, retention-type of ichthyosis, with delayed degradation of corneodesmosomes; i.e., these structures persist high into the SC (Figs 4, 5). Two key serine proteases, the SC chymotryptic enzyme (SCCE) and the SC tryptic enzyme (SCTE), are thought to mediate desquamation, because both can degrade corneodesmosomes *in vitro* (Brattsand *et al*, 2000). While the activity of these enzymes is restricted by the acidic pH of normal SC (SCCE and SCTE exhibit neutral pH optima), the pH of the SC in RXLI is lower than that of normal SC (Ohman and Vahlquist, 1998). Hence, we hypothesized that these serine proteases might be less operative in RXLI than in normal SC. As seen in Fig 6, serine protease activity appears to be much higher in normal than in RXLI SC (see legend for experimental details). Thus, the reduced pH of SC alone can account for the increased cohesion in RXLI.

CSO_4 could also increase SC retention through its known activity as a serine protease inhibitor (SPI): For example, CSO_4 inhibits sperm capacitation, a serine protease-catalyzed reaction (cited in Williams, 1991), and CSO_4 inhibits SC serine proteases *in vitro* (Sato *et al*, 1998). However, whether CSO_4 functions as an SPI in epidermis and its importance in relation to other known SPI of the SC (e.g., elafin, plasminogen activator inhibitor I, secretory leukocyte protease inhibitor, cystatin) is not known.

A third, unrelated mechanism which could result in increased SC cohesion posits that Ca^{++}, if present in sufficient quantities, could cross-link the highly anionic SO_4 group on adjacent lamellar bilayers (Epstein *et al*, 1984). Indeed, CSO_4-containing liposomes aggregate avidly in the presence of calcium (Abraham *et al*, 1987). Moreover, Ca^{++}, which is required for desmosome stabilization, could also stabilize attachments between opposing corneodesmosomes. To address this potential mechanism, we employed ion precipitation cytochemistry to localize Ca^{++} in RXLI *versus*. normal SC (Menon *et al*, 1985). Whereas

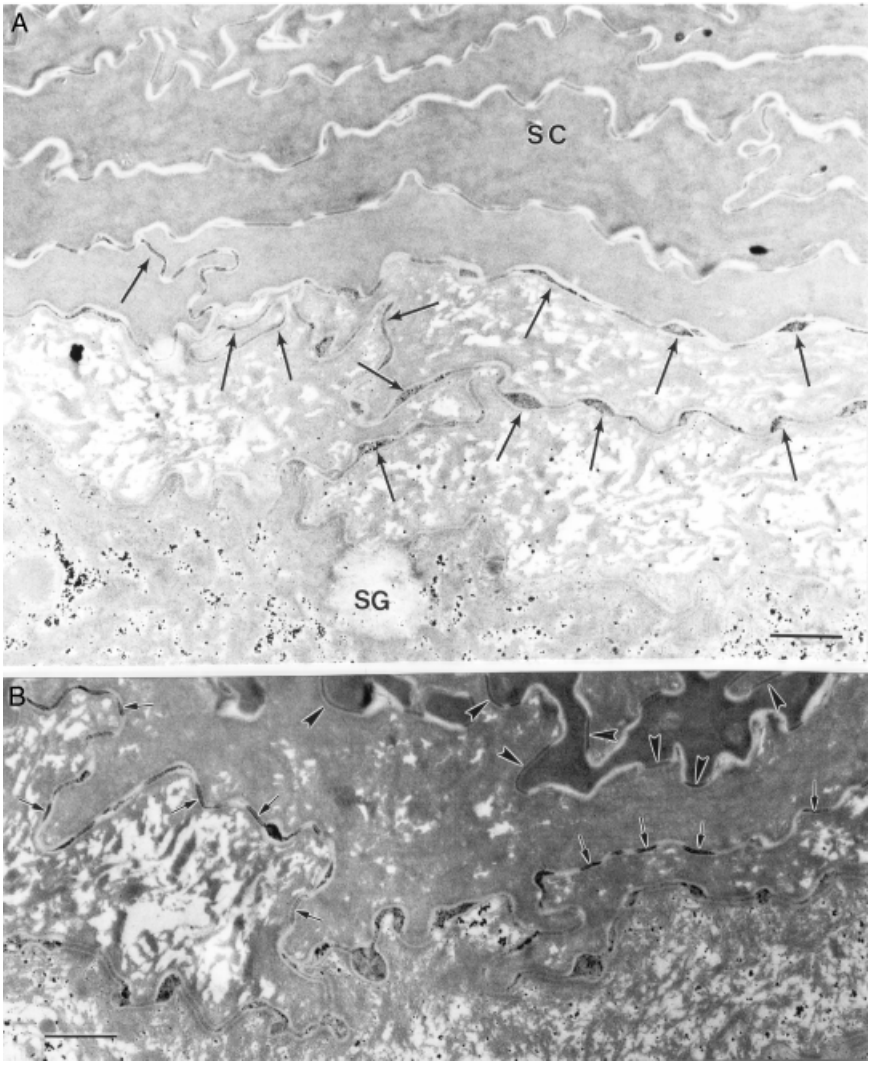

Figure 5
Calcium preferentially localizes to corneodesmosomes in extracellular spaces of lower SC in RXLI. Ca^{++} precipitates in normal epidermis do not extend above the stratum granulosum (SG); i.e., into the stratum corneum (SC) (Menon *et al*, 1985). In contrast, Ca^{++} precipitates extend above the SG–SC interface in RXLI, where they are restricted to the extracellular domains (Figure 5*A*). Corneodesmosomes are increased at all levels of SC (arrowheads). In many sites, Ca^{++} appears to specifically segregate with corneodesmosomes (arrows). A + B, osmium tetroxide postfixation. Scale bars = 1 μm.

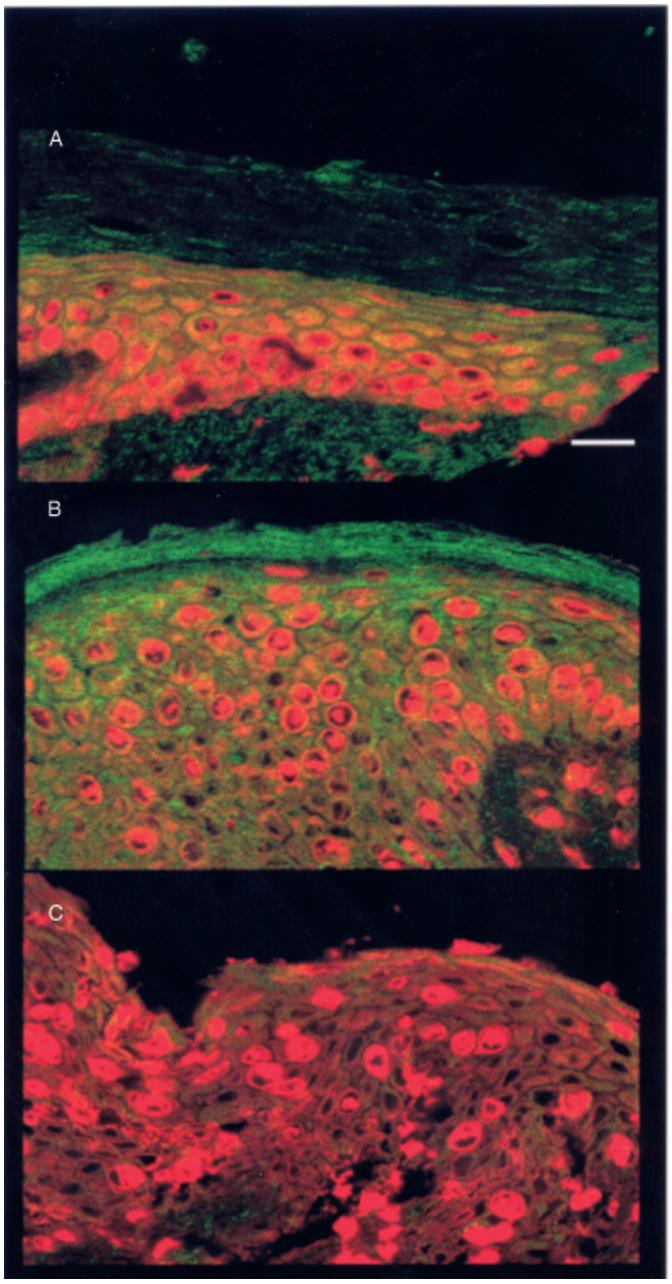

Figure 6
Serine protease activity is reduced in RXLI SC. Frozen sections (8 μm) from an X-linked ichthyosis adult (A) and from the surgical excision margins of an adult normal (B) were rinsed with a washing solution (1% Tween 20 in deionized water) and incubated at 37°C for 1 h with 250 μL of BODIPY-Fl-casein in either deionized water (2 μL/mL), 1 mM cholesterol sulfate in DMSO, or DMSO alone, as described recently (Hachem *et al*, 2003); C: normal + cholesterol sulfate. All sections then were rinsed with the 1% Tween 20 washing solution, coverslipped, and visualized under a confocal microscope (Leica TCS SP, Heidelberg, Germany) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Scale bar = 10 μM.

Ca^{++} is largely excluded from the intercellular spaces of normal SC (not shown; see Menon *et al*, 1985), the lower SC in RXLI demonstrates abundant Ca^{++} in extracellular domains (Fig 5*A,B*). Moreover, the Ca^{++} preferentially localizes along the external face of opposing corneodesmosomes (Fig 5*B*). Thus, the delayed degradation of corneodesmosomes in RXLI correlates with increased Ca^{++} in the lower SC; and therefore, this mechanism, along with Ca^{++} bridging of adjacent lamellar bilayers (Epstein *et al*, 1984), could further contribute to increased corneocyte retention in RXLI.

Regulation of differentiation by cholesterol sulfate Sulfation of cholesterol by cholesterol sulfotransferase is a step that is intimately linked to differentiation in keratinizating epithelia. For example, CSO_4 levels are several orders of magnitude higher in keratinizing than in mucosal epithelia, and induction of mucous metaplasia in keratinizing epithelia by application of exogenous retinoids induces a dramatic decline in CSO_4 levels. CSO_4 is known to stimulate epidermal differentiation by at least two related mechanisms (Fig 4). 1) It activates the η isoform of protein kinase C (Denning *et al*, 1995), which in turn stimulates the phosphorylation of differentiation-linked proteins, assessed as increased CE formation (Fig 1). 2) CSO_4 is a transcriptional regulator of TG-1 and involucrin expression, operating through an AP-1 binding site in the promoter region (Kawabe *et al*, 1998; Hanley *et al*, 2001). It is likely that these two mechanisms are linked as shown in Fig 4: PKC activation by CSO_4 could phosphorylate AP-1, leading to enhanced transcriptional regulation of TG-1 and involucrin. A key issue that remains unresolved, however, is whether the increased levels of CSO_4 in RXLI cause increased corneocyte differentiation, and whether such increased differentiation could provoke corneocyte retention. Such a mechanism could also, however, potentially explain the unique properties of hair, nail, and ungulate hooves, where CSO_4 levels are much higher than in normal SC, and even higher than in the SC of RXLI.

In summary, we have traced the evolution of our understanding of the pathogenesis of RXLI, one of the best understood of all dermatologic disease entities. Elucidation of pathogenic mechanisms in RXLI has led to new concepts about the normal regulation of epidermal differentiation, permeability barrier homeostasis, and cohesion/desquamation.

This work was supported by NIH grants AR 19098, and AR 39639, AR 39448 (PP), and the Medical Research Service, Department of Veterans Affairs. Jerelyn Magnusson and Jennifer C. Quevedo provided excellent editorial assistance.

DOI: 10.1046/j.1523-1747.2003.22258.x

Manuscript received October 16, 2003; revised December 8, 2003; accepted for publication December 8, 2003

Address correspondence to: Mary L. Williams, M.D., Department of Dermatology, Box 0316, UCSF, San Francisco, CA 94143-0316, Tel (415) 353-7884, Fax (415) 353-7850, Email: mLwilliams@orca.ucsf.edu

References

Abraham W, Wertz PW, Landmann L, Downing DT: Stratum corneum lipid liposomes: Calcium-induced transformation into lamellar sheets. J Invest Dermatol 88:212–214, 1987

Ballabio A, Parenti G, Carrizo R, *et al*: Isolation and characterization of a steroid sulfatase cDNA clone: Genomic deletions in patients with X-chromosome-linked ichthyosis. Proc Natl Acad Sci 84:4519–4523, 1987

Bergner EA, Shapiro LJ: Metabolism of 3H-dehydroepiandrosterone sulphate by subjects with steroid sulphatase deficiency. J Inherit Metab Dis 11:403–415, 1988

Bonifas JM, Morley BJ, Oakey RE, Kan YW, Epstein EH Jr: Cloning of a cDNA for steroid sulfatase: Frequent occurrence of gene deletions in patients with recessive X chromosome-linked ichthyosis. Proc Natl Acad Sci 84:9248–9251, 1987

Brattsand M, Ekholm IE, Egelrud T: Stratum corneum tryptic enzyme in normal epidermis: A missing link in the desquamation process? J Invest Dermatol 114:56–63, 2000

Conary JT, Lorkowski G, Schmidt B, *et al*: Genetic heterogeneity of steroid sulfatase deficiency revealed with cDNA for human steroid sulfatase. Biochem Biophys Res Commun 144:1010–1017, 1987

Denning MF, Dlugosz AA, Williams EK, Szellasi Z, Blumberg PM, Yuspa SH: Specific protein kinase C isozymes mediate the induction of keratinocyte differentiation markers by calcium. Cell Growth Differ 6:149–151, 1995

Dibbelt L, Herzog V, Kuss E: Human placental sterylsulfatase: immunocytochemical and biochemical localization. Biol Chem Hoppe Seyler 370:1093–1102, 1989

Elias PM, Menon GK: Structural and lipid biochemical correlates epidermal permeability barrier. Adv Lipid Res 24:1–26, 1991

Elias PM, Schmuth M, Uchida Y, *et al*: Basis for the permeability barrier abnormality in lamellar ichthyosis. Exp Dermatol 11:248–256, 2002

Elias PM, Williams ML, Maloney ME, Bonifas JA, Brown BE, Grayson S, Epstein EH Jr: Stratum corneum lipids in disorders of cornification. Steroid sulfatase and cholesterol sulfate in normal desquamation and the pathogenesis of recessive X-linked ichthyosis. J Clin Invest 74:1414–1421, 1984

Epstein EH Jr, Krauss RM, Shackleton CH: X-linked ichthyosis: increased blood cholesterol sulfate and electrophoretic mobility of low-density lipoprotein. Science 214:659–660, 1981

Epstein EH, Williams ML, Elias PM: The epidermal cholesterol sulfate cycle. J Am Acad Dermatol 10:866–868, 1984

Frost P, Van Scott EJ: Ichthyosiform dermatoses. Classification based on anatomic and biometric observations. Arch Dermatol 94:113–126, 1966

Gillard EF, Affara NA, Yates JR, Goudie DR, Lambert J, Aitken DA, Ferguson-Smith MA: Deletion of a DNA sequence in eight of nine families with X-linked ichthyosis (steroid sulphatase deficiency). Nucl Acids Res 15:3977–3985, 1987

Grayson S, Johnson-Winegar AG, Wintroub BU, Isseroff RR, Epstein EH Jr, Elias PM: Lamellar body-enriched fractions from neonatal mice: preparative techniques and partial characterization. J Invest Dermatol 85:289–294, 1985

Hachem JP, Crumrine D, Fluhr J, Brown BE, Feingold KR, Elias PM: pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. J Invest Dermatol 121:345–353, 2003

Hanley K, Wood LC, Ng DC, *et al*: Involucrin transcriptin and expression of fra-1, fra-2, and Jun D are increased by cholesterol sulfate in human keratinocytes. J Lipid Res 42:390–398, 2001

Ibsen HH, Brandrup F, Secher B: Topical cholesterol treatment of recessive X-linked ichthyosis. Lancet 2:645, 1984

Kawabe S, Ikuta T, Ohba M, Chida K, Ueda E, Yamanishi K, Kuroki T: Cholesterol sulfate activates transcription of transglutaminase 1 gene in normal human keratinocytes. J Invest Dermatol 111:1098–1102, 1998

Koppe G, Marinkovic-Ilsen A, Rijken Y, De Groot WP, Jobsis AC: X-linked icthyosis. A sulphatase deficiency. Arch Dis Child 53:803–806, 1978

Kubilus J, Tarascio AJ, Baden HP: Steroid-sulfatase deficiency in sex-linked ichthyosis. Am J Hum Genet 31:50–53, 1979

Lavrijsen AP, Oestmann E, Hermans J, Bodde HE, Vermeer BJ, Ponec M: Barrier function parameters in various keratinization disorders: Transepidermal water loss and vascular response to hexyl nicotinate. Br J Dermatol 129:547–553, 1993

Li XM, Yen P, Mohandas T, Shapiro LJ: A long range restriction map of the distal human X chromosome short arm around the steroid sulfatase locus. Nucl Acids Res 18:2783–2788, 1990

Long SA, Wertz PW, Strauss JS, Downing DT: Human stratum corneum polar lipids and desquamation. Arch Dermatol Res 277:284–287, 1985

Maloney ME, Williams ML, Epstein EH Jr, Law MY, Fritsch PO, Elias PM: Lipids in the pathogenesis of ichthyosis: Topical cholesterol sulfate-induced scaling in hairless mice. J Invest Dermatol 83:252–256, 1984

Mao-Qiang M, Feingold KR, Elias PM: Exogenous lipids influence permeability barrier recovery in acetone treated murine skin. Arch Dermatol 129:728–738, 1993

Menon GK, Grayson S, Elias PM: Ionic calcium reservoirs in mammalian epidermis ultrastructural localization by ion capture cytochemistry. J Invest Dermatol 84:508–512, 1985

Mohandas T, Shapiro LJ, Sparkes RS, Sparkes MC: Regional assignment of the steroid sulfatase-X-linked ichthyosis locus. Implications for a noninactivated region on the short arm of human X chromosome. Proc Natl Acad Sci 76:5779–5783, 1979

Nemes Z, Demeny M, Marekov LN, Fesus L, Steinert PM: Cholesterol 3-sulfate interferes with cornified envelope assembly by diverting transglutaminase 1 activity from the formation of cross-links and esters to the hydrolysis of glutamine. J Biol Chem 275:2636–2646, 2000

Ohman H, Vahlquist A: The pH gradient over the stratum corneum differs in X-linked recessive and autosomal dominant ichthyosis. A clue to the molecular origin of the "acid skin mantle". J Invest Dermatol 111:674–677, 1998

Ponec M, Williams ML: Cholesterol sulfate uptake and outflux in cultured human keratinocytes. Arch Dermatol Res 279:32–36, 1986

Ranasinghe AW, Wertz PW, Downing DT, Mackenzie IC: Lipid composition of cohesive and desquamated corneocytes from mouse ear skin. J Invest Dermatol 86:187–190, 1986

Rassner UA, Crumrine DA, Nau P, Elias PM: Microwave incubation improves lipolytic enzyme preservation for ultrastructural cytochemistry. Histochem J 29:387–392, 1997

Rehfeld SJ, Williams ML, Elias PM: Interactions of cholesterol and cholesterol sulfate with free fatty acids. Possible relevance for the pathogenesis of recessive X-linked ichthyosis. Arch Dermatol Res 278:259–263, 1986

Roberts KD, Lieberman S: In: Chemical and Biological Aspects of Steroid Conjugation. Bernstein S, Solomon S (eds). Springer-Verlag, New York: 1970, p 219–290

Sato J, Denda M, Nakanishi J, Nomura J, Koyama J: Cholesterol sulfate inhibits proteases that are involved in desquamation of stratum corneum. J Invest Dermatol 111:189–193, 1998

Schmickel RD: Contiguous gene syndromes: A component of recognizable syndromes. J Pediatr 109:231–241, 1986

Schnur RE, Trask BJ, van den Eugh G, *et al*: An Xp22 microdeletion associated with ocular albinism and ichthyosis. Approximation of breakpoints and estimation of deletion size by using cloned DNA probes and flow cytometry. Am J Hum Genet 45:706–720, 1989

Shapiro LJ: Steroid sulfatase deficiency and the genetics of the short arm of the human X chromosome. Adv Hum Genet 14:331–381, 1985

Shapiro LJ, Yen P, Pomerantz D, Martin E, Rolewic L, Mohandas T: Molecular studies of deletions at the human steroid sulfatase locus. Proc Natl Acad Sci 86:8477–8481, 1989

Tiepolo L, Zuffardi O, Fraccaro M, di Natale D, Gargantini L, Muller CR, Ropers HH: Assignment by deletion mapping of the steroid sulfatase X-linked ichthyosis locus to Xp223. Hum Genet 54:205–206, 1980

Tiepolo L, Zuffardi O, Rodewald A: Nullisomy for the distal portion of Xp in a male child with a X/Y translocation. Hum Genet 39:277–281, 1977

Webster D, France JT, Shapiro LJ, Weiss R: X-linked ichthyosis due to steroid-sulphatase deficiency. Lancet 1:70–72, 1978

Willemsen R, Kroos M, Hoogeveen AT, van Dongen JM, Parenti G, van der Loos CM, Reuser AJ: Ultrastructural localization of steroid sulphatase in cultured human fibroblasts by immunocytochemistry: A comparative study with lysosomal enzymes and the mannose 6-phosphate receptor. Histochem J 20:41–51, 1988

Williams ML, Elias PM: Stratum corneum lipids in disorders of cornification: increased cholesterol sulfate content of stratum corneum in recessive x-linked ichthyosis. J Clin Invest 68:1404–1410, 1981

Williams ML: Lipids in normal and pathological desquamation. Adv Lipid Res 24:211–262, 1991

Williams ML, Rutherford SL, Feingold KR: Effects of cholesterol sulfate on lipid metabolism in cultured human keratinocytes and fibroblasts. J Lipid Res 28:955–967, 1987

Zettersten E, Man MQ, Sato J, Denda M, Farrell A, Ghadially R, Williams ML, Feingold KR, Elias PM: Precessive x-linked ichthyosis: role of cholesterol-sulfate accumulation in the barrier abnormality. J Invest Dermatol 111: 784–790, 1998