See related Commentary on page ix

Stratum Corneum Defensive Functions: An Integrated View

Peter M. Elias*†
*Dermatology Service, Veterans Affairs Medical Center and †Department of Dermatology, University of California, San Francisco, California, USA

Most epidermal functions can be considered as protective, or more specifically, as defensive in nature. Yet, the term ''barrier function'' is often used synonymously with only one such defensive function, though arguably its most important, i.e., permeability barrier homeostasis. Regardless of their relative importance, these protective cutaneous functions largely reside in the stratum corneum (SC). In this review, I first explore the ways in which the multiple defensive functions of the SC are linked and interrelated, either by their shared localization or by common biochemical processes; how they are co-regulated in response to specific stressors; and how alterations in one defensive function impact other protective functions. Then, the structural and biochemical basis for these defensive functions is reviewed, including metabolic responses and signaling mechanisms of barrier homeostasis. Finally, the clinical consequences and therapeutic implications of this integrated perspective are provided.

Key words: barrier function/corneodesmosomes/cytokines/desquamation/hydration/lamellar bodies/pH/psychological stress/stratum corneum/lamellar body
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Defensive Functions of the Stratum Corneum (SC)

Defensive functions of SC primarily localize to either the cellular or extracellular compartment Virtually all epidermal functions (with the exception of vitamin D production) can be considered protective, and most of these critical protective functions further localize to the SC (Elias and Feingold, 2003). The structural organization of the SC into a two-compartment system largely dictates the localization of specific defensive functions to either the corneocyte or extracellular matrix (Table I). Lamellar bodies (LB), which secrete a variety of proteins and lipids into the extracellular spaces, further mandate that an array of functions reside in the SC interstices (Fig 1). In addition to the provision of lipids for the barrier, LB deliver: (1) *lipid processing enzymes* that process lipid precursors into their respective products (Freinkel and Traczyk, 1983; Grayson *et al*, 1985); (2) *proteases* that orchestrate desquamation (e.g., serine proteases (SP)) (Sondell *et al*, 1994; Braissant *et al*, 1996; Braissant and Wahli, 1998); (3) *anti-proteases*, such as elafin and cystatin M/E; (4) *corneodesmosin* (CDSN), a novel protein of the outer epidermis that coats the external face of corneodesmosomes (CD) (Haftek *et al*, 1996), rendering them resistant to premature proteolysis (Lundstrom *et al*, 1994); and (5) *antimicrobial peptides*, e.g., human β-defensin 2 (hBD2) (Oren *et al*, 2003).

How are protective functions linked? Although it is convenient to consider each defensive function as a discrete process, many individual functions co-localize, and/or they are linked biochemically or by common regulatory mechanisms.

Co-localization of permeability and antimicrobial barriers In addition to presenting a daunting physical barrier to pathogenic microbes that attempt to penetrate from the environment, the epidermis also generates a spectrum of antimicrobial lipids, peptides, toll-like receptors, and chemokines that together comprise cutaneous innate immunity (Gallo and Nizet, 2003; Ganz, 2003). The ability of the skin to both restrict water loss from the body, and the ingress of microbial pathogens through the SC, results from its organization into a two-component system of lipid-depleted corneocytes embedded in a lipid-enriched, extracellular matrix (Table I). It is the: (i) absolute quantities, (ii) hydrophobic character, (iii) lipid distribution, and (iv) supramolecular organization of its constituent lipids into a series of lamellar bilayers (Elias and Menon, 1991) that together account for the permeability barrier. Three of these SC lipids exhibit robust antibacterial activity *in vitro* (i.e.,

Abbreviations: AD, atopic dermatitis; aSMase, acidic sphingomyelinase; CD, corneodesmosome; CDSN, corneodesmosin; CE, cornified envelope; CLE, corneocyte-bound lipid envelope; DSC, desmocollin; DSG, desmoglein; EHK, epidermolytic hyperkeratosis; FFA, free fatty acids; FLIM, fluorescence life-time imaging; β-GlcCer'ase, β-glucocerebrosidase; hBD, human beta-defensin; ; IL-1, interleukin-1; LB, lamellar body; LI, lamellar ichthyosis; LXR, liver X receptor; NHE, sodium-proton exchanger; NHR, nuclear hormone receptors; ω-OH-Cer, ω-hydroxy ceramides; PL, phospholipids; PPAR, peroxisome proliferator activator receptor; SC, stratum corneum; SCCE, stratum corneum chymotryptic enzyme; SCCP, stratum corneum cysteine protease; SCTE, stratum corneum tryptic enzyme; SG, stratum granulosum; SP, serine proteases; $sPLA_2$, secretory phospholipase A_2; SPT, serine palmitoyl transferase; SREBP, sterol regulatory element binding proteins; TG, transglutaminase; TNF, tumor necrosis factor; UCA, urocanic acid; UV, ultraviolet light

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Table I. Protective functions of mammalian stratum corneum

Function	Localization
Permeability barrier[a]	Extracellular
Cohesion (integrity) → desquamation[a]	Extracellular
Antimicrobial barrier (innate immunity)[a]	Extracellular
Mechanical (impact and shear resistance)	Corneocyte
Toxic chemical/antigen exclusion	Extracellular
Selective chemical absorption	Extracellular
Hydration	Corneocyte
UV barrier	Corneocyte
Initiation of inflammation (cytokine activation)[a]	Corneocyte
Psychosensory interface	Unknown
Thermal barrier	Unknown

[a]Regulated or thought to be activated by (SC) pH.

free fatty acids (FFA), glucosylceramides, and the Cer hydrolytic product, sphingosine (Miller *et al*, 1988; Bibel *et al*, 1992).

Mammalian epidermis also expresses several types of antimicrobial peptides: the α-defensins, hNP1 and 2, the β-defensins, hBD1–4, and a cathelicidin, hCAP (Gallo and Nizet, 2003; Ganz, 2003), the S100 protein, psoriasin (Glaser *et al*, 2005), and a novel antimicrobial RNase, RNase 7 (Harder and Schröder, 2002). Defensins comprise small, cationic, and cysteine-enriched members of a highly conserved gene family, consisting of α and β subtypes, which differ slightly in their disulfide-bond pairing, genomic organization, and tissue distribution (Ganz, 2003). α-defensins are present in neutrophils and the small intestine (Paneth cells), but only at low levels in the skin. All four β-defensins are expressed in keratinocytes, and exhibit potent antimicrobial activity against a variety of Gram-negative and Gram-positive bacteria, yeast, and viruses. But the activity of individual defensins against specific microbial targets differs greatly, e.g., hBD3, but not hBD2, is active against *Staphylococcus aureus* (Dunsche *et al*, 2002; Ganz, 2003).

hBD2 and 3 further localize to the outer epidermis and both are activated by primary cytokines, such as interleukin (IL-1)α and tumor necrosis factor (TNF)α (Huh *et al*, 2002; Liu *et al*, 2002; Oren *et al*, 2003). The co-localization of the permeability and antimicrobial barriers is demonstrated vividly by the translocation of hBD2 from the endoplasmic reticulum to epidermal LB following IL-α stimulation (Oren *et al*, 2003), with subsequent sequestration of hBD2 within SC membrane domains (Huh *et al*, 2002). Through its localization in the SC interstices, hBD2 is positioned to intercept pathogenic microbes as they attempt to penetrate between corneocytes.

The human epidermis also expresses the cathelicidin, hCAP-18 (Zaiou and Gallo, 2002; Gallo and Nizet, 2003). Cathelicidins are a class of small, cationic peptides with a highly conserved, NH-terminal cathelin segment that possesses cysteine protease inhibitory activity, and a C-terminal segment (LL-37) (Zaiou and Gallo, 2002). The latter possesses a broad spectrum of antimicrobial activity including against viruses (Howell *et al*, 2004), but not antistaphylococcal activity, and it, too, is delivered by LB secretion (Braff *et al*, 2004). The biologic importance of

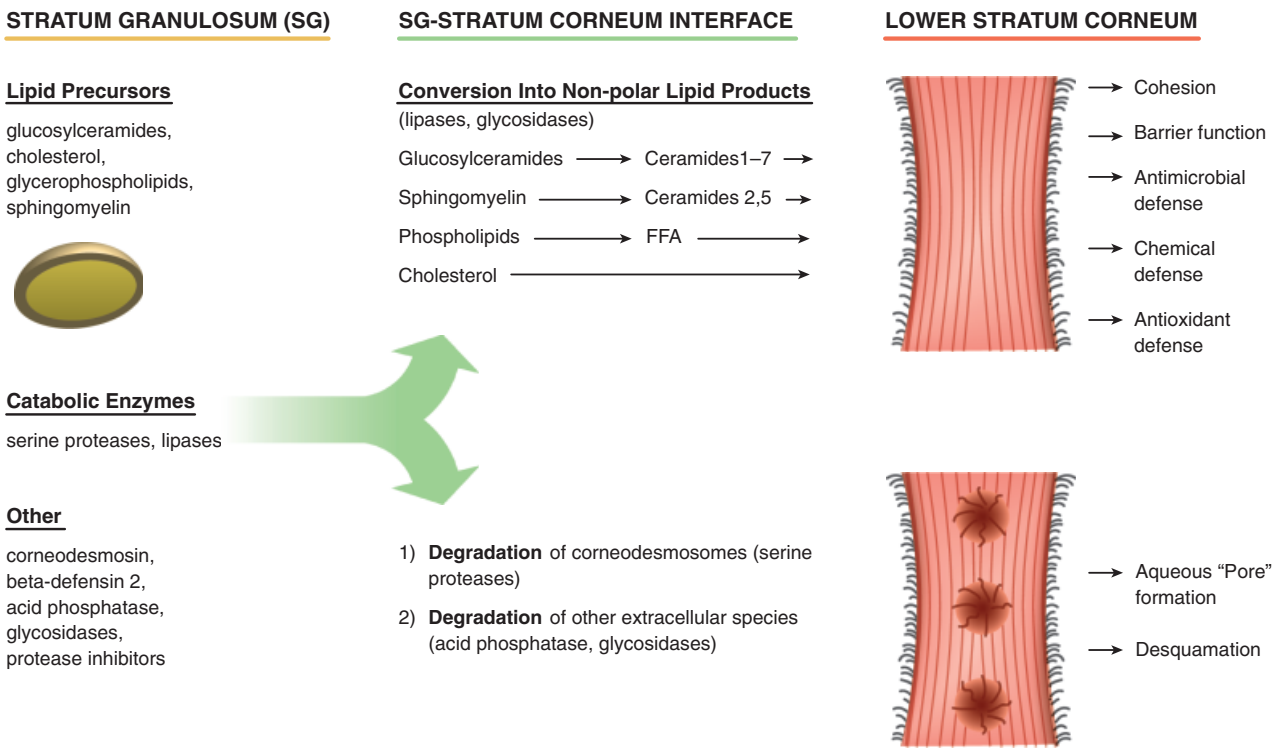

Figure 1
Lamellar body secretion dictates localization of multiple functions to extracellular compartment.

cathelicidins in antimicrobial defense is shown by the increased incidence of skin infections in knockout mice (Nizet *et al*, 2001). Moreover, whereas hBD2 and hCAP-18 levels upregulate in psoriasis, which rarely becomes secondarily infected, these peptides fail to upregulate in lesional skin of active atopic dermatitis (AD) (Ong *et al*, 2002), which correlates with a high cutaneous carriage of *S. aureus* in AD. Psoriasis and RNase 7 appear to primarily protect against fecal-derived gram-negative bacteria (Harder and Schröder, 2002). Because of their overlapping spectra of activity against different pathogens, the various antimicrobial peptide families (hBD and hCAP-18) act synergistically in antimicrobial defense (Gallo and Nizet, 2003; Zaiou *et al*, 2003). Thus, antimicrobial peptides co-localize both biochemically and topographically to the SC interstices.

Hydration, ultraviolet (UV) filtration, and immunosuppression are linked through the histidase pathway In addition to resistance of the skin to *mechanical* or blunt *injury*, corneocytes ("bricks") generate filaggrin-derived peptides and their deiminated products, which, along with sebaceous gland-derived glycerol (Fluhr *et al*, 2003), regulate not only SC *hydration* but also several other downstream functions (Fig 2). Early in cornification, filaggrin, the predominant, histidine-enriched, basic protein in F-type keratohyalin granules, disperses around keratin filaments within the stratum compactum (Dale *et al*, 1997). At ambient humidities (<85% RH), i.e., above the stratum compactum, filaggrin is largely hydrolyzed by a still-uncharacterized, cytosolic protease into free amino acids, including histidine, glutamine (glutamic acid), and arginine (Scott and Harding, 1986; Harding *et al*, 2000). These amino acids, and their distal, deinminated products (urocanic acid, pyrrolidone carboxylic acid, and ornithine/citrulline/aspartic acid, respectively) comprise much of the osmotically active material that regulates SC hydration (Harding *et al*, 2000). Histidine is deiminated enzymatically to its acidic, polar, plurifunctional metabolite, trans-urocanic acid (tUCA), by the enzyme, histidine ammonia lyase (histidase) (Scott, 1981). As it acts as an *endogenous sunscreen*, tUCA is photoisomerized by UV-B to cUCA (De Simone *et al*, 2001), a potent *immunosuppressive* molecule, implicated in the pathogenesis of UV-induced skin cancers (Noonan and De Fabo, 1992; Finlay-Jones and Hart, 1998). Thus, products of the histidase pathway regulate two key defensive functions as well as a pathophysiologic function of the SC.

Multiple defensive functions can be altered by a single stressor

Relationship of psychological stress to barrier function and SC integrity/cohesion Some key SC functions can be coregulated in response to single common stressors. A pertinent example is *psychological stress*, which exerts negative effects not only on *permeability barrier* homeostasis (Denda *et al*, 2000; Altemus *et al*, 2001; Garg *et al*, 2001) but also on SC *integrity* and *cohesion*. Stress impacts these functions by a common mechanism, i.e., an increase in endogenous glucocorticoids (Denda *et al*, 2000), and glucocorticoids, in turn, alter barrier function and SC integrity/cohesion by a common mechanism, i.e., suppression of epidermal lipid synthesis and LB production (Kao *et al*, 2003). Accordingly, not only the stress-induced barrier abnormality, but also the abnormality in SC integrity can be reversed (overridden) by a mixture of physiologic lipids, containing all three key SC species (i.e., ceramides, FFA, and cholesterol) (Kao *et al*, 2003).

Impact of pH on multiple defensive functions A second example of a common stressor that modulates multiple functions is SC pH. In fact, pH orchestrates at least three important SC defensive functions (Table I). Using a flat surface electrode, the pH of mammalian SC typically ranges from 4.5 to 5.0 in the outer SC, approaching neutrality in the lower SC (Ohman and Vahlquist, 1994). Recent studies, utilizing fluorescence-lifetime imaging (FLIM) combined with mutliphoton microscopy, show that SC pH is heterogeneously distributed, and that the pH gradient is non-linear (Behne *et al*, 2002, 2003). Most importantly, FLIM demonstrated that membrane domains even at the level of the stratum granulosum (SG)–SC interface are selectively acidified (Behne *et al*, 2002).

Although at least two endogenous, biochemical pathways regulate SC pH, the full array of functions that are impacted by each mechanism is still largely unclear. A third mechanism is the histidine-to-UCA pathway, which can account quantitatively for SC acidification (Krien and

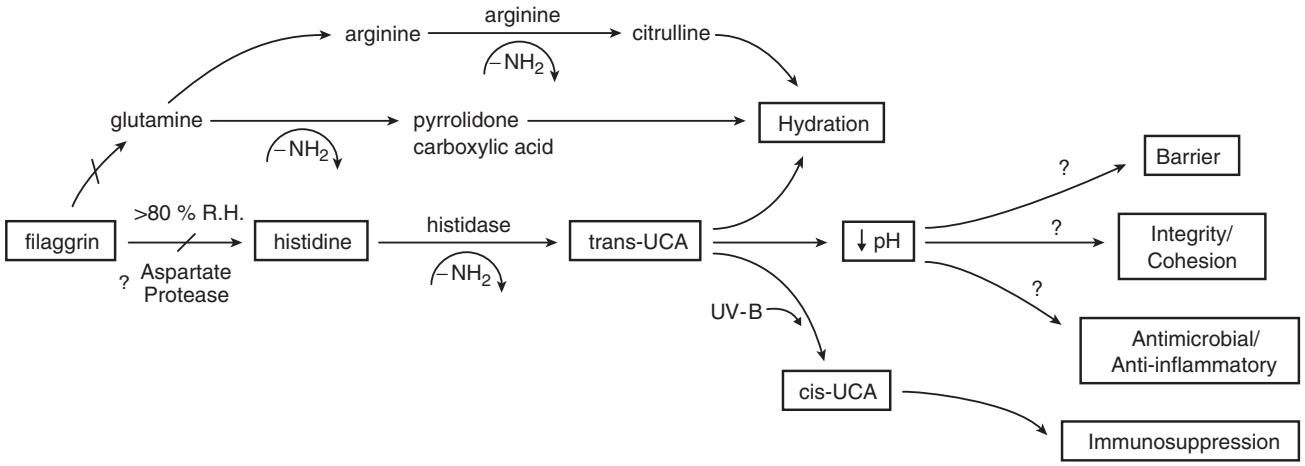

Figure 2
Functions potentially impacted by filaggrin metabolism in the stratum corneum.

Kermici, 2000), but its importance is unclear, because: (1) This mechanism would be inoperative in the fully hydrated inner SC (Scott and Harding, 1986; Bouwstra *et al*, 2003). (2) Because tUCA is extremely polar, it might not influence pH within the relatively hydrophobic, extracellular membrane domains. (3) UCA levels peak immediately after birth, following initial exposure of neonatal SC to a dry environment (Scott and Harding, 1986; Fluhr *et al*, 2004), but acidification of human SC develops much more slowly, i.e., over several weeks to months (Giusti *et al*, 2001). (4) Patients and mice with histidinemia (histidase mutations) exhibit no known abnormalities in skin morphology, or function (Fluhr *et al*, 2004). Thus, other acidifying mechanisms probably predominate as SC acidifying mechanisms.

Phospholipid (PL)-to-FFA hydrolysis by an as yet incompletely characterized extracellular secretory phospholipases ($sPLA_2$) clearly influences SC pH (Fluhr *et al*, 2001). $sPLA_2$ represent a growing family of non-polar lipids of homologous enzymes of low molecular mass (13–18 kDa) that catalyze the hydrolysis of glycerophospholipids at the sn-2 position, generating FFA and lysophospholipids (Redoules *et al*, 1999; Maury *et al*, 2000; Schadow *et al*, 2001). Whereas the epidermis expresses several $sPLA_2$ isoforms, i.e., groups I, II, and group X $sPLA_2$ (Maury *et al*, 2000; Schadow *et al*, 2001), only the group I isoform is known to persist into the SC (Redoules *et al*, 1999; Maury *et al*, 2000; Mazereeuw-Hautier *et al*, 2000). Like its PL substrates, $sPLA_2$ activity localizes to LB, which deliver the enzyme to the SC interstices (Grayson *et al*, 1985; Elias *et al*, 1988). $sPLA_2$-mediated hydrolysis of PL generates not only FFA that are required as structural components of the SC extracellular lamellar bilayers (Mao-Qiang *et al*, 1995b; Mao-Qiang *et al*, 1996), but also protons that impact at least two SC defensive functions (Fluhr *et al*, 2001). When $sPLA_2$ activity is blocked, SC pH increases, producing abnormalities in both barrier function and SC integrity/cohesion (Fluhr *et al*, 2001). Although altered SC integrity/cohesion is linked to premature dissolution of CD, paralleled by reduced desmoglein 1 (DSG1), the alterations in barrier homeostasis are linked not only to depletion of FFA, but also to downstream deactivation of β-GlcCer'ase and acidic sphingomyelinase (aSMase) activities (Mauro *et al*, 1998; Fluhr *et al*, 2001; Hachem *et al*, in press). Thus, acidification blockade has downstream consequences for lipid-processing enzymes with acidic pH optima.

Non-energy requiring, integral membrane transporters that extrude protons in exchange for sodium–proton exchanger (NHE) comprise at least six 80–90 kDa gene products, with 35%–60% homology (Orlowski and Grinstein, 1997). Whereas NHE2–5 are present in extracutaneous tissues, NHE1 is expressed ubiquitously, including in keratinocytes/epidermis (van Hooijdonk *et al*, 1997; Behne *et al*, 2001; Sarangarajan *et al*, 2001), where it localizes to the outer nucleated cell layers of the epidermis (Behne *et al*, 2001). Both k.o. and NHE1 inhibitor-treated mice reveal an increase in SC pH (≈ 1/3 pH unit), and delayed barrier recovery after acute abrogations (Behne *et al*, 2001). Because NHE1 k.o. mice reveal a selective increase in pH in membrane domains in the lower SC, and NHE1 is inserted into the apical plasma membrane of SG cells, it appears to selectively acidify extracellular domains just above the SG–SC interface (Behne *et al*, 2002, 2003).

The prevalent hypothesis about the principal role for SC pH is its putative importance for *antimicrobial defense* (Aly *et al*, 1978). The microflora of the skin comprise: (a) transient, (b) temporary-resident, and (c) permanent-resident species (Leyden *et al*, 1979), including coagulase-negative staphylococci, which prevail on dry surfaces, whereas Propionibacteriae predominate on sebaceous gland-enriched skin sites. These normal flora grow best at a more acidic pH, whereas pathogenic bacteria, such as *S. aureus*, grow best at a neutral pH (Korting *et al*, 1990). The importance of pH for antimicrobial function is further suggested by neonatal, eczematous, and atopic skin, which display a neutral pH (Sparuvigna *et al*, 1999; Visscher *et al*, 2000; Giusti *et al*, 2001), coupled with an enhanced risk of infection (Beare *et al*, 1958; Yosipovitch *et al*, 1993; Fluhr *et al*, 2004). As compelling as these data are, the role of the acidic pH in antimicrobial defense has not been demonstrated directly.

The importance of SC pH for *permeability barrier homeostasis* is suggested both by the worsening of barrier function when intact skin is exposed to an alkaline pH (Thune *et al*, 1988), and by the delay in barrier recovery that occurs when perturbed skin is exposed to a neutral pH (Mauro *et al*, 1998; Hachem *et al*, 2003). pH influences barrier function directly through its effects on membrane bilayer organization (Bouwsta *et al*, 2000), and/or secondarily through its regulation of extracellular lipid processing (Fig 1). Although β-GlcCer'ase and aSMase exhibit acidic pH optima, $sPLA_2$ and steroid sulfatase (SSase) display neutral-to-alkaline pH optima, suggesting that sequential changes in SC pH within microenvironments of the SC interstices orchestrate lipid processing (Fig 1).

Integrity/cohesion, which is inversely related to rates of corneocyte shedding (*desquamation*), represents a second SC function that is pH dependent. Although a large family of SP are present in SC, two SP, the epidermis-specific *SC chymotryptic* (SCCE) and *SC tryptic* (SCTE) *SP*, (kalleikreins 5 and 7) appear to be critical mediators of this function. Because SCCE and SCTE exhibit neutral-to-alkaline pH optima (Brattsand and Egelrud, 1999; Hanson *et al*, 2002), they would exhibit higher activities when SC pH increases, as in inflammatory dermatoses. But SP could sustain low rates of normal desquamation even in normal SC, because these enzymes exhibit residual activity against physiologic substances, even at an acidic pH (Caubert *et al*, 2004), where aspartate and cysteine proteases, with acidic pH optima, could also become operative (Fig 3). In contrast, it is likely that SCCE/SCTE activities dominate at all levels of

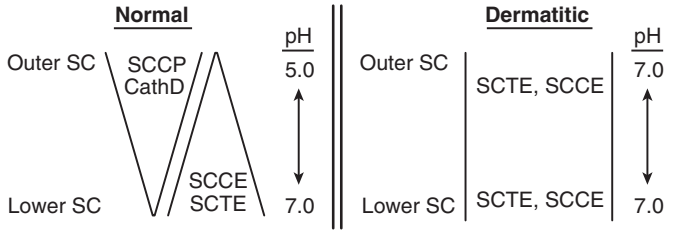

Figure 3
Proposed pH-dependent role of different desquamatory proteases in normal and dermatitic skin. SCCE and SCTE, see text; Cath D, cathepsin D; SCCP, SC cysteine protease.

SC in pathological skin, which is characterized by optimal conditions for SP activation (i.e., increased pH, hydration, and Ca^{2+} levels).

Perturbations of one defensive function can alter other key functions

Humidity (SC hydration) influences permeability barrier. The interactivity of certain defensive functions is further demonstrated by the changes in *permeability barrier function* that follow changes in *external humidity*. Whereas sustained exposure to a physiologically relevant humid environment (85% RH) downregulates barrier competence, prolonged exposure of normal skin to low ambient humidities (below 20% RH) enhances barrier homeostasis (Denda *et al*, 1998) (Fig 6). Thus, hydration stimulates appropriate alterations in barrier function. Yet, more extreme switches in hydration, i.e., from skin previously exposed to a humid to a dry environment, paradoxically produce a profound, though temporary, deficit in barrier function (Sato *et al*, 2002) (Fig 4). Apparently humid-adapted granular cells cannot upregulate their metabolic and secretory machinery. The relevance of this work for humans, who often travel between such extremes in humidity, is unclear. Fortunately, the barrier defect is temporary, as defective cells are quickly replaced by new, functionally competent arrivals to the SG (Sato *et al*, 2002).

Key mechanical structures regulate permeability barrier Another pertinent example of one function impacting a second is shown by the role of the cornified envelope (CE) not only as a *mechanical barrier*, but also as a scaffold that organizes secreted extracellular lipids into the continuous lamellar membrane structures, which mediate *permeability barrier* function. Defective lamellar structures and increased transcutaneous water loss occur in both transglutaminase 1 (TG1)-deficient lamellar ichthyosis (LI) (Lavrijsen *et al*, 1995; Elias *et al*, 2002c) and loricrin keratoderma (Vohwinkel's disease) (Schmuth *et al*, 2004b), when an attenuated or fragile CE is associated with abnormalities in adjacent lamellar membranes, in turn provoking altered barrier function.

Permeability barrier disruption initiates inflammation Current fashion views the skin as a proinflammatory tissue (e.g., distinctive T cell abnormalities occur in common dermatoses, such as psoriasis, contact dermatitis, and AD). Yet, these diseases are often initiated and sustained by external perturbations (e.g. Koebner phenomenon in psoriasis). It appears increasingly likely that cutaneous *immune phenomena* can be triggered by both the release of a preformed pool of primary cytokines, IL-1α, IL-1β, and TNFα, from the corneocyte and granular cell cytosol, as well as increased production of these cytokines, in response to minimal *external* (barrier) *perturbations* (Elias and Feingold, 1999, 2003). Following their release, these cytokines signal divergent, downstream pathways that initiate both homeostatic (repair-related) and pro-inflammatory processes, restoring barrier competence while at the same time initiating a signal cascade that stimulates cutaneous inflammation by downstream recruitment/entrapment of inflammatory cells. According to this "outside–inside" perspective, many cutaneous inflammatory phenomena, including disease-specific T cell responses, are recruited primarily as incidental participants in a defensive sequence aimed at normalizing SC function. Thus, barrier function and inflammatory signaling are linked, defensive functions of the epidermis.

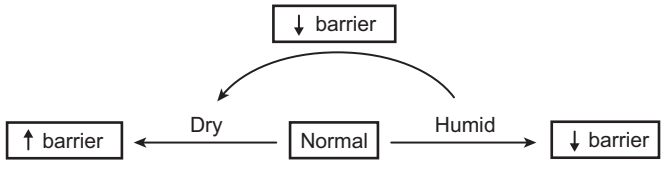

Figure 4
Changes in external humidity alter permeability barrier function.

Structural and Biochemical Basis for the SC Barrier

Epidermal differentiation leads to the formation of the SC, a heterogeneous tissue composed of lipid-depleted corneocytes embedded in a lipid-enriched extracellular matrix, which subserves the barrier. These lipids derive from a highly active, lipid-synthetic factory, operative in all of the nucleated cell layers of the epidermis (Feingold, 1991), which generates a unique lipid and hydrolase-enriched, secretory organelle, the epidermal LB (Odland and Holbrook, 1981; Landmann, 1988). LB are $0.3-0.4 \times 0.25$ mm structures, which comprise about 10% of the cytosol of the SG (Elias *et al*, 1977a, 1998a). Following secretion of their contents at the SG–SC interface, LB contents are processed from a polar lipid mixture into a hydrophobic mixture of ceramides, "FFA", and cholesterol, organized into the lamellar membranes that form the hydrophobic matrix within which corneocytes are embedded (Elias and Menon, 1991).

The corneocyte Although corneocyte proteins have been studied intensively as markers of epidermal differentiation (Eckert *et al*, 1997), their role in the permeability barrier is less clear. Yet, these anucleated cells are well known to perform other critical epidermal functions (Table I). The corneocytes influence the *permeability barrier* through their function as "spacers", i.e., they force water and xenobiotes to traverse a tortuous, extracellular hydrophobic pathway (Potts and Francoeur, 1990), and by serving as a scaffold for lamellar membrane organization.

Proteins of the CE The CE, and its external, ceramide-enriched, cornified-bound lipid envelope (CLE) together provide a stable, mechanically and chemically resistant scaffold for the deposition and organization of the extracellular matrix (Hohl, 1990). The CE, a uniform, 15 nm thick, peripheral envelope that encloses the corneocyte cytosol (Hohl, 1990; Ishida-Yamamoto and Iizuka, 1998), consists of several highly cross-linked, cytosolic proteins, including involucrin, loricrin, elafin, desmoplakin, envoplakin, cytostatins, and pancornulins/cornifins (small proline-rich proteins) (Hohl *et al*, 1991; Steinert and Marekov, 1997).

Involucrin, a 68 kDa rod-shaped molecule with a series of highly conserved 10 amino acid repeats, containing 3 gluta-

mine residues each as potential cross-linking sites (Steinert and Marekov, 1997), accounts for 5%–15% of the CE expressed in the late spinous and SG layer, and it appears to be the first envelope precursor that is cross-linked by TG1, and therefore localizes to the outer SG. Loricrin is a cysteine (7%), serine (22%), and glycine (55%) enriched, 38 kDa, highly insoluble peptide, comprising one component of keratohyalin granules (Hohl *et al*, 1991), and accounts for up to 80% of CE mass. Loricrin is cross-linked into the CE late in differentiation, i.e., immediately after LB secretion (Bickenbach *et al*, 1995). A mutation resulting in elongation of the C-terminal domain of one loricrin allele occurs in some kindreds with Vohwinkle's keratoderma (Maestrini *et al*, 1996). These patients display not only digital constrictions (pseudo-ainhum) and a dense, honeycomb keratoderma, but also a mild, generalized ichthyosis, and a permeability barrier abnormality, as in LI (Schmuth *et al*, 2004b).

Formation of the CE is catalyzed largely by the epidermal-specific, calcium-dependent enzyme, *TG1*, a 92 kDa protein, which forms γ-glutamyl-E-lysine isopeptide bonds between constituent CE proteins (Hennings *et al*, 1981; Eckert *et al*, 1997; Kalinin *et al*, 2002). Although TG1 mRNA is expressed in lower layers, the enzyme becomes active after membrane anchorage to the outermost granular cell (Chakravarty and Rice, 1989), coinciding with the calcium (Ca) gradient peaks in the outer epidermis (Menon *et al*, 1985a, b), and with activation by an aspartate protease, cathepsin D (Egberts *et al*, 2004). At least two other TG isoforms, TG2 (82 kDa) and TG3 (77 kDa), contribute variably to CE cross-linking (Kalinin *et al*, 2002). Yet, because the CE from corneocytes of patients with LI is invariably defective, residual TG2 and 3 activities cannot compensate completely for TG1 deficiency (Hohl *et al*, 1993; Elias *et al*, 2002c). Whereas autosomal recessive forms of primary ichthyosis comprise a heterogeneous group, patients with the severe, classic LI phenotype typically display TG1 deficiency (Huber *et al*, 1995), whereas patients with less severe phenotypes reflect a variety of genotypes, including TG1 deficiency (Choate *et al*, 1996b, 1998). LI patients display a severe permeability barrier abnormality (Lavrijsen *et al*, 1995; Choate *et al*, 1996a, b; Elias *et al*, 2002c), but despite the attenuated CE in TG1-deficient LI, increase in permeability occurs through the SC interstices (Elias and Ghadially, 2002). Although the extracellular membranes in LI display minor abnormalities in interlamellar spacing (Ghadially *et al*, 1992b; Lavrijsen *et al*, 1995), the basis for increased intercellular permeability is best explained by the fragmentation of extracellular lamellae that occurs in these patients (Elias and Ghadially, 2002).

Finally, CE-associated proteins, while being necessary for the steady-state maintenance of normal barrier homeostasis, are transiently downregulated following acute barrier perturbations, apparently as a form of metabolic conservation (Elias *et al*, 2002b) (Fig 4), but subsequently upregulated during later stages of barrier recovery (Ekanayake-Mudiyanselage *et al*, 1998).

Structural proteins of the corneocyte cytosol. Keratins are the most abundant structural proteins of the epidermis and its appendages, contributing to the mechanical properties of these epithelia (Fuchs and Weber, 1994). Keratins are of two types, type I or acidic (K9–20) and type II or basic (K1–8), which are co-expressed in pairs, and all keratins display a similar secondary structure, with a central, rod domain comprising four α helices, and distinctive, non-helical, head and tail sequences (Kalinin *et al*, 2002). Whereas K5 and 14 are expressed in the basal layer, K1, 2e, and 10 are expressed in suprabasal layers, eventually accounting for 80% of the mass of the corneocyte (Fuchs, 1994; Fuchs and Weber, 1994; Eckert *et al*, 1997). Deletions of as few as 10 amino acids from the rod domain of either K1, 2e, or 10 result in keratin clumping, i.e., the defective allelic protein binds to its partner in a dominant-negative fashion, forming defective intermediate filaments, resulting in several forms of epidermolytic hyperkeratosis (EHK) (Rothnagel *et al*, 1992; Corden and McLean, 1996), which shift from predominantly blistering in neonates to predominantly hyperkeratosis after exposure to the xeric, post-natal environment (Williams and Elias, 1993). The basis for the permeability barrier abnormality in EHK results from the cytoskeleton abnormality: rather than simply producing corneocyte fragility, the abnormal keratin pairs interfere with LB secretion, resulting in decreased amounts of extracellular lipids (Schmuth *et al*, 2001b).

Type II keratin filaments and their partners attach covalently to the CE via a single lysine residue in a highly conserved region of the VI subdomain of KI (Kimonis *et al*, 1994). Accordingly, mutations in the VI domain, affecting a highly conserved lysine residue, interfere with the insertion of type II keratins to desmoplakin in the CE (Kimonis *et al*, 1994). These patients display a diffuse, non-epidermolytic palmo-plantar keratoderma (NSPPK) with acral keratotic plaques (Candi *et al*, 1998). Detachment and retraction of keratin bundles from CD in the SC result in both microvesiculation and deformation of the keratinocyte periphery (Candi *et al*, 1998) (i.e., corneocytes do not flatten properly).

Profilaggrin is a large, histidine-rich, highly cationic phosphoprotein, consisting of 37 kDa filaggrin repeats, connected by peptide segments enriched in hydrophobic amino acids (Fleckman *et al*, 1985). Profilaggrin is concentrated within keratohyalin granules, where it may sequester loricrin, which also localizes to keratohyalin. During terminal differentiation, profilaggrin is both dephosphorylated and proteolytically processed by a Ca^{2+}-dependent protein convertase, furin, at the N-terminus to yield filaggrin (Resing *et al*, 1993), which ionically binds to KI/I0, inducing the formation of macrofibrils in the corneocyte cytosol (Dale *et al*, 1997). Immunolocalization studies suggest that processed filaggrin peptides initially associate via their N-terminal domain with the CE in the lower SC, but filaggrin later detaches, allowing proteolysis into hygroscopically active amino acids and their deiminated products.

Extracellular lipids of the SC The processing of secreted LB contents leads to the progressive generation of a mixture of relatively non-polar lipids, which is enriched in ceramides, cholesterol, and FFA, present in an approximately equimolar ratio (Schurer and Elias, 1991). Lesser, but variable amounts of cholesterol esters, triglycerides, and diglycerides, which may, in part, be of sebaceous gland origin, also persist. The FFA comprise a mixture of essential (EFA) and non-essential FFA (NEFA) (Lampe *et al*, 1983), and

both are required separately as critical structural ingredients of the barrier (Schurer and Elias, 1991). NEFA derive largely, if not entirely, from the hydrolysis of PL, which are co-secreted with their respective sPLA at the SG–SC interface (Elias *et al*, 1988; Mao-Qiang *et al*, 1995b; Mao-Qiang *et al*, 1996), which also results in acidification of the SC interstices (Fluhr *et al*, 2001).

The "mortar" lipids also contain abundant cholesterol, which is secreted unchanged from LB; lesser quantities of cholesterol derive from the hydrolysis of cholesterol sulfate to cholesterol by the enzyme, steroid sulfatase (Elias *et al*, 2004). Although a variety of studies have shown that cholesterol is critical for permeability barrier function (e.g., Feingold *et al*, 1990), cholesterol derived from cholesterol sulfate is not required (Zettersten *et al*, 1998).

A third, key lipid constituent of the mortar is a family of nine ceramide species (Fig 1), which vary according to their (Wertz *et al*, 1984): (1) sphingosine *versus* phytosphingosine (extra-OH group) base; (2) α- *versus* non-α-hydroxylated *N*-acyl fatty acids, which typically are $>$C30 in length; and (3) the presence of an additional, ω-esterified linoleic acid residue. Whereas all nine ceramides are generated from their glucosyl/ceramide precursors, only ceramides 2 and 5 derive from sphingomyelin (Uchida *et al*, 2000). Ceramides 1, 4, and 7 are the principal repositories for the essential fatty acid (EFA), linoleic acid, a critical structural ingredient in the barrier (Elias and Brown, 1978; Elias *et al*, 1980). These epidermis-unique molecules appear to link adjacent bilayers through their highly elongated *N*-acyl chains, with ω-esterified linoleic acid (Bouwstra *et al*, 1998). The esterified linoleate moiety is important for barrier function, because in EFA deficiency, the primary biochemical defect is a substitution of NEFA for linoleic acid, a biochemical alteration that results in a pronounced permeability barrier defect (Elias *et al*, 1980).

CLE. A 10 nm, tightly apposed, electron-lucent, plasma membrane-like structure, now termed the CLE, replaces the plasma membrane on the external aspect of mammalian corneocytes (Swartzendruber *et al*, 1987; Marekov and Steinert, 1998). After aggressive solvent treatment (e.g., chloroform:methanol extraction), the CLE appears to comprise a single, outer electron-dense leaflet, separated from the CE by an electron-lucent space (Swartzendruber *et al*, 1987). But following treatment with the polar solvent, pyridine, the CLE instead can be seen to comprise a trilaminar structure, intimately related to the CE (Elias *et al*, 1977a, 2000, 2002c). Involucrin, a major constituent of outer portion of the CE, is cross-linked prior to cornification, forming a scaffold for CLE formation (Steinert and Marekov, 1997). The CLE comprises ω-hydroxyceramides (Cer), with very long-chain, *N*-acyl fatty acids, covalently bound to the CE (Swartzendruber *et al*, 1987; Marekov and Steinert, 1998). These ω-hydroxy derive from insertion of the β-glucosyl ω-hydroxyceramide-enriched limiting membrane of the LB into the apical plasma membrane of the outermost granular (SG) cells (Wertz *et al*, 1989; Behne *et al*, 2000). Bound glucosyl-β-hydroxy ceramides, however, are quickly deglucosylated (Doering *et al*, 1999). The CLE could serve: (1) as a "molecular rivet" in SC cohesion (Wertz *et al*, 1989); and (2) as a scaffold for extracellular lamellar membrane organization (Elias and Ghadially, 2002; Schmuth *et al*, 2004a–c). Yet, despite its extreme hydrophobicity, its ability to restrict water movement is unclear, because solvent extraction of SC leaves the CLE largely intact, whereas the SC becomes porous to transcutaneous water loss (Elias *et al*, 2000). Yet, the CLE could still restrict water movement to extracellular domains, while limiting both water uptake into the corneocyte, and egress of water-soluble, hygroscopic amino acids out of the corneocyte cytosol (Elias *et al*, 2000).

Metabolic Regulation of Permeablity Barrier Homeostasis

Although the SC has many functions, its ability is to serve as a protective barrier that prevents excess loss of fluids and electrolytes, allows life in a terrestrial environment (Scheuplein and Blank, 1971). As noted above, the permeability barrier is mediated by the organization of the extracellular lipids of the SC into a series of parallel membrane structures, and its distinctive composition (Elias and Menon, 1991).

Dynamics of barrier recovery. The "cutaneous stress test" comprises any type of acute barrier disruption (organic solvent, detergent, tape stripping) that depletes the SC of its complement of lipids (Feingold, 1991; Elias and Feingold, 1992). Although the total time required for barrier recovery varies according to age and species, there is always an initial, rapid recovery phase, followed by a prolonged recovery phase that requires about 35 h for completion in rodents (Grubauer *et al*, 1989). In young humans, the acute recovery phase is somewhat longer (about 12 h), and the later recovery phase is prolonged to 72 h, but in aged humans, the recovery phase can last over 1 wk (Ghadially *et al*, 1995). Restoration of barrier function is accompanied by reaccumulation of lipids within the SC interstices, visible with Nile red fluorescence, and by the reappearance of lamellar membranes, as early as 2 h after acute disruption (Grubauer *et al*, 1987, 1989). Because artificial restoration of the barrier with a vapor-impermeable, but not vapor-permeable membrane, inhibits barrier recovery and all attendant metabolic processes (Grubauer *et al*, 1989), the entire metabolic response is aimed specifically at normalization of barrier function (Feingold, 1991; Elias and Feingold, 1992).

The same test can also be deployed as a physiologic challenge (i.e., stress test) that can discern abnormal function, even when basal parameters are normal. Thus, the cutaneous stress test is analogous to other clinical maneuvers used to identify pathology (e.g., cardiac treadmill exam, end respiratory volume, overnight water deprivation, etc.). Indeed, the cutaneous stress test revealed deficient barrier function in both aged (Ghadially *et al*, 1995) and neonatal skin (Fluhr *et al*, 2004), despite deceptively normal function under basal conditions. Moreover, psychologic stress results in a delay in barrier recovery (Denda *et al*, 2000; Altemus *et al*, 2001; Garg *et al*, 2001), and it amplifies differences in barrier function among still other "normal" groups, such as individuals with type 1 vs. type 4–5 pigmentation, and in testosterone-replete males (Elias and Feingold, 2003). Although clinicians have not yet exploited

the stress test as a clinical tool, these results explain the propensity for inflammatory dermatoses to be more severe in males than in females, and for fair-skinned individuals to have "sensitive" (? atopic) skin.

LB secretion leading to lamellar membrane formation The first step in the repair response following barrier disruption is rapid secretion (within minutes) of performed LB contents from cells of the outer SG, which leaves the cytosol of these cells largely devoid of LB (Menon *et al*, 1992; Elias *et al*, 1998a). Newly formed LB then begin to reappear in SG cells by 30–60 min, and by 3–6 h the number (density) of LB in SG cells exceeds normal. Because of accelerated secretion and organellogenesis between 30 min and 6 h, the quantities of secreted LB contents increase at the SG–SC interface, and by 2 h new, lipid-enriched lamellar bilayers begin to appear in the lower SC (Grubauer *et al*, 1989; Menon *et al*, 1992). Thus, the exocytosis of LB provides a pathway by which the epidermis delivers lipids and their respective lipid-processing enzymes simultaneously to the extracellular spaces of the SC (Fig 1).

Structural adaptations of the outermost granular cell that facilitate secretion. Although LB secretion remains largely restricted to the outermost SG cell (Menon *et al*, 1992; Elias *et al*, 1998a), certain structural features of the SG cell facilitate delivery of LB-derived lipid and enzyme contents to the SG–SC interface, designating the outermost SG cell as the "secretory granulocyte" (Elias *et al*, 1998a): (1) a highly disbursed, tubulo-reticular trans-Golgi network that extends throughout the apical cytosol (see also Madison *et al*, 1998); (2) deep invaginations of the SG–SC interface that form an extensive honeycomb/latticework, and are continuous with the intercellular domains; and (3) arrays of contiguous LB that undergo compound exocytosis (organelle-to-organelle, rather than solely organelle-to-plasma membrane fusion) following acute perturbations. Together, these specialized structures explain the ability of the outermost SG cell to function as a secretory cell, despite being both heavily keratinized and in possession of a partially developed CE (Elias *et al*, 1998a). Although others have interpreted images of the investigations quite differently; i.e., that the invaginations reflect a non-energy-requiring unfurling (continuous phase from LB to the lamellar membranes) of lipids (Norlen, 2001a, b), this hypothesis contradicts the large body of evidence that LB assembly and secretion are highly regulated processes that can be blocked by inhibitors or by exposure to low temperatures that slow energy-requiring functions.

Regulation of lipid synthesis by barrier requirements. The rapid formation of LB following acute barrier disruption requires increased availability of the major lipid components of LB, i.e., cholesterol, glucosylceramides, and PL (Feingold, 1991; Elias and Feingold, 1992). Although the epidermis is a very active site of lipid synthesis under basal conditions (Feingold *et al*, 1983), barrier disruption stimulates a further increase in the synthesis of cholesterol, ceramides, and FA (a major component of both PL and ceramides) (Feingold *et al*, 1983; Menon *et al*, 1985a, b; Proksch *et al*, 1990; Holleran *et al*, 1991a) (Fig 6). The increase in cholesterol synthesis is associated with an increase in the activity, protein levels, and mRNA levels of HMG CoA reductase (Proksch *et al*, 1990; Harris *et al*, 1997), and other key enzymes in the cholesterol synthetic pathway; i.e., HMG CoA synthase, farnesyl diphosphate synthase, and squalene synthase (Fig 6) (Harris *et al*, 1997). Whereas the increase in FA synthesis occurs because of an increase in the activity and mRNA levels of both of the key enzymes of FA synthesis, acetyl CoA carboxylase, and FA synthase (Ottey *et al*, 1995), the increase in ceramide synthesis is because of an increase in the activity and mRNA levels of serine palmitoyl transferase (SPT) (Holleran *et al*, 1991a) (Fig 6), the enzyme which catalyzes the first committed step in ceramide synthesis. In contrast, glucosylceramide synthase, the enzyme which synthesizes glucosylceramides, does not increase following barrier disruption (Chujor *et al*, 1998). But glucosylceramide synthesis could still be dependent upon the availability of FA for both sphingoid base formation and N-acylation. Thus, a specific, coordinate increase in the synthesis of the key lipid constituents of LB provides the pool of lipids, required for the formation of new LB.

Barrier homeostasis requires synthesis of each of the three key lipids Using specific inhibitors of key synthetic enzymes, cholesterol, FA, ceramide, and glucosylceramide synthesis were shown to be individually required for barrier formation (Feingold *et al*, 1990; Holleran *et al*, 1991b; Mao-Qiang *et al*, 1993a; Chujor *et al*, 1998). For example, blockade of cholesterol synthesis with topical inhibitors of HMG CoA reductase (e.g., statins) slows barrier recovery and selectively delays the return of cholesterol to the SC (Feingold *et al*, 1990, 1991). These effects are because of a specific block in cholesterol synthesis, because co-applications of either mevalonate or cholesterol (distal products) normalize barrier recovery. Likewise, topical 5-(tetradecyloxy)-2-furancarboxylic acid (TOFA), an inhibitor of acetyl CoA carboxylase, one of the two key enzymes of FA synthesis, inhibits epidermal FA synthesis and delays barrier repair (Man *et al*, 1993; Mao-Qiang *et al*, 1993a). Yet, TOFA-induced inhibition of barrier repair again can be overcome by topical co-applications of enzyme product (i.e., FFA), again demonstrating the specificity of the inhibitor. Moreover, β-chloroalanine, a selective inhibitor of SPT, also delays barrier recovery after acute barrier disruption (Holleran *et al*, 1991b), and again, inhibition of barrier recovery can be overcome by co-applications of exogenous ceramides. Finally, PDMP, a selective inhibitor of glucosylceramide synthase (GC synthase), also inhibits barrier recovery after acute disruption (Chujor *et al*, 1998). Inhibition of each of these enzymes produces a similar result: decreased LB formation, contents, and secretion, as well as a paucity of extracellular lamellar bilayers (Feingold, 1991; Elias and Feingold, 1992). These studies clearly demonstrate that epidermal cholesterol, FA, ceramide, and glucosylceramide synthesis are required individually for LB formation and barrier homeostasis.

The key SC lipids are required in an equimolar distribution. Whereas the above-described studies clearly demonstrate the individual requirement for each of the three key lipids (cholesterol, FA, and ceramides) for the permeability

barrier, these lipids must be supplied together in a proper proportion for normal barrier recovery in rodents and humans (Man *et al*, 1993, 1996). Topical applications of any one or two of the three key lipids to acutely perturbed skin actually delays barrier recovery, whereas application of the three key lipids in an equimolar mixture normalizes recovery rates (Man *et al*, 1993, 1996). Both incomplete and complete mixtures of the three key lipids rapidly traverse the SC, internalize within the granular cell layer, bypassing the endoplasmic reticulum and proximal Golgi apparatus, targeting distal sites (i.e., the trans-Golgi network), where LB are formed (Mao-Qiang *et al*, 1995a). Within LB, the exogenous and endogenous lipids mix, producing normal or abnormal LB contents and derived lamellar membrane structures, depending on the molar distribution of the applied lipids. Further acceleration of barrier recovery can be achieved in both rodents and humans by increasing the proportion of any of the three key lipids to a 3:1:1 ratio (Man *et al*, 1996; Zettersten *et al*, 1997). Thus, physiologic mixtures of topical lipids influence barrier function, not by occluding of the SC, but rather by contributing to the lipid pool within SG cells, thereby regulating the contents of newly generated LB. In contrast, non-physiologic lipids, like petrolatum, function like vapor-permeable membranes at the surface of the SC (Ghadially *et al*, 1992a; Mao-Qiang *et al*, 1995a).

The contrasting features of non-physiologic and physiologic lipids dictate the clinical settings where each is useful. Bolstering epidermal barrier status should decrease susceptibility to the large group of skin diseases that are triggered, sustained, or exacerbated by external perturbations, such as AD, contact dermatitis, and psoriasis (Elias *et al*, 1999). All of these diseases are characterized by a barrier abnormality, and the extent of the barrier abnormality parallels clinical severity (Ghadially *et al*, 1996b; Proksch *et al*, 2003; Sugarman *et al*, 2003), hence the recent emergence of "barrier repair" strategies to decrease the susceptibility to these disorders. These approaches can be classified into three subcategories (Elias and Feingold, 2001): (1) optimized mixtures of the three *physiologic lipids* (ceramides, cholesterol, and FFA) in appropriate molar ratios to correct the targeted disease; (2) one or *more non-physiologic* lipids (e.g. petrolatum, lanolin); and (3) *dressings*, either *vapor permeable*, which allow metabolic (repair) processes to continue in the underlying epidermis, or *vapor impermeable*, which shut down metabolic responses in the underlying epidermis. Thus, we can now choose an appropriate barrier strategy for a specific clinical indication, based upon knowledge of disease pathogenesis (Elias and Feingold, 2001; Williams and Elias, 2003) (Table II). For example, AD is characterized by a global decrease in SC lipids with a steep reduction in ceramides (Imokawa *et al*, 1991; Proksch *et al*, 2003), attributable to increased sphingomyelin/glucosylceramide deacylase activity in affected epidermis (Hara *et al*, 2000); hence, the apparent utility of a ceramide-dominant mixture of physiologic lipids in AD (Chamlin *et al*, 2002). In contrast, aged and photoaged epidermis exhibits a global reduction in SC lipids (Ghadially *et al*, 1995), with a further decrease in cholesterol synthesis (Ghadially *et al*, 1996a), hence, the success of a cholesterol-dominant mixture of physiological lipids in this setting (Zettersten *et al*, 1997).

Signals of Barrier Homeostasis

Transcriptional regulation of corneocyte protein expression by nuclear hormone receptor (NHR) ligands. Members of both the Class I and Class II family of NHR influence epidermal barrier formation, function, and development (Table II). At least three ligands (glucocorticoids, estrogens, and androgens) of the Class I family (receptors for the steroid hormones) regulate permeability barrier development in fetal skin, as well as barrier homeostasis in adult skin. In fetal skin both exogenous glucocorticoids and estrogens, either administered *in utero* or added directly to fetal skin explants in organ culture, accelerate the development of a mature permeability barrier, whereas in contrast, administration of androgens retards barrier ontogenesis (Hanley *et al*, 1996a, b, 1998). Although the impact of Class I ligands on barrier function in post-natal skin is less well understood, normal to supra-normal levels of androgens provoke an analogous decline in permeability barrier homeostasis in adult murine and human skin (Kao *et al*, 2001). Yet, an increase in endogenous glucocorticoids, induced by either psychological stress (Denda *et al*, 2000), or systemic administration of exogenous steroids (Kao *et al*, 2003), alters permeability barrier homeostasis and SC integrity/cohesion.

Table II. Cutaneous effects of class II NHR ligands

Receptor	Ligands/activators	Fetal barrier development	Adult barrier homeostasis	Anti-inflammatory
Classic				
RAR	All-trans-retinoic acid	None	Worse	Improves
T3R	Triodothyronine (T3)	Accelerates	?	?
D3R	1,25$(OH)_2$ vitamin D3	None	Worse	Improves
Liposensor				
PPARα	Leukotriene B4, fatty acids, fibrates	Accelerate	Accelerate	Improve
PPARγ	Prostaglandin J_2, troglitazone	None	None	Improve
PPARδ	Free fatty acids	None	Accelerate	Improve
LXR	Oxygenated sterols	Accelerate	Accelerate	Improve

NHR, nuclear hormone receptor; PPAR, peroxisome proliferator activator receptor; LXR, liver X receptor.

Why certain observations in fetal and post-natal skin (e.g., effects of glucocorticoids) diverge is not yet known.

The Class II family of NHR includes receptors for both well-known ligands, such as thyroid hormone (T3R), retinoic acid, and 1,25 $(OH)_2$ vitamin D3 (D3R), and a number of receptors ("liposensors"), whose activators/ligands are endogenous lipids. The roles of these receptors, which include the peroxisome proliferator activator receptor (PPAR) and liver X receptor (LXR) in epidermal function, are currently being characterized (Table II). The activators for most of these receptors, e.g., PPARα, PPARα/β, and LXRα/β, include lipid synthetic intermediates or metabolites, such as certain FFA, leukotrienes, prostanoids, and oxygenated sterols (Duplus and Forest, 2002; Fitzgerald *et al*, 2002). Because it is likely that increased quantities of sterol and fatty acid metabolites are generated both during epidermal development (Hurt *et al*, 1995), and as co-products of the increase in lipid synthesis that follows barrier disruption (Feingold, 1991), processes leading to formation/restoration of the lipid matrix could also concurrently promote corneocyte protein production (Fig 5). Recent studies have shown that these liposensor activators accelerate fetal skin development and induce keratinocyte differentiation (Hanley *et al*, 1994, 1997; Komuves *et al*, 1998). By regulating both the transcription of key corneocyte proteins; e.g., involucrin, loricrin, and TG1 (Hanley *et al*, 2000a, b), and stimulating epidermal lipid synthesis (Rivier *et al*, 2000), these liposensors potentially influence both the "brick" and "mortar" compartments of the barrier (Fig 5). Because these agents also reverse the barrier abnormalities, epidermal hyperplasia, and cutaneous inflammation in various animal models (Komuves *et al*, 2000; Sheu *et al*, 2002; Fowler *et al*, 2003; Ruhl *et al*, 2003), they hold substantial promise as therapeutic agents in dermatology (Ellis *et al*, 2000).

Transcriptional regulation of epidermal lipid synthesis

Cholesterol and FA synthesis. Seminal studies by the Brown and Goldstein laboratory have demonstrated that both cholesterol and FA synthesis are feedback regulated by a group of transcription factors, the sterol regulatory element binding proteins (SREBP) (Brown and Goldstein, 1998). Two SREBP genes (SREBP-1 and SREBP-2) encode three proteins: SREBP-1A, SREBP-1C, and SREBP-2 (Smith *et al*, 1988; Vallett *et al*, 1996). These tripartite proteins, which are $\approx$ 1150 amino acids in length, with a $\approx$ 480 amino acid NH2-terminal segment facing the cytosol, are members of the basic–helix–loop–helix–leucine zipper family of transcription factors. This NH2-terminal segment is followed by a membrane attachment fragment of $\approx$ 80 amino acids, containing two membrane-spanning sequences separated by a $\approx$ 31 amino acid hydrophilic loop tat projects into the lumen of the ER and nuclear envelope, and a COOH-terminal segment of $\approx$ 590 amino acids that projects into the cytosol. A novel sensing mechanism is activated by sterol depletion (Loewen and Levine, 2002; Rawson, 2003). A two-step proteolytic process occurs that results in the release of the NH2-terminal segments, allowing them to enter the nucleus, where they bind as homodimers to cis-elements in the promoters of multiple SREBP-responsive genes stimulating various enzymes of cholesterol and FA synthesis (Vallett *et al*, 1996; Brown and Goldstein, 1998). In contrast, when excess cholesterol accumulates, proteolytic cleavage is inhibited and transcription of target genes declines (Loewen and Levine, 2002; Rawson, 2003). The two-step cleavage process leading to the release of active SREBP begins at site 1, between the Leu and Ser of the RSVLS sequence, in the middle of the hydrophilic loop (Vallett *et al*, 1996). The site 1 protease (S1P) is a SP that splits the lumenal loop of SREBP into two halves that remain attached by their membrane attachment domains. Because the S1P localizes to the Golgi, cleavage of SREBP is initiated there. Transport of SREBP from the endoplasmic reticulum to the Golgi is facilitated by SREBP cleavage-activating protein (SCAP), an elongated protein whose COOH terminus complexes with the COOH-terminus of SREBP (Rawson, 2003). The final release of the active NH2 fragment of SREBP requires a second proteolytic step at Site 2, a Leu–Cys bond at the border of the cytosolic and membrane portion of the NH2 segment. Although the Site 2 protease (S2P) is not regulated directly by changes in cholesterol levels, it can only act after prior Site 1 cleavage has occurred. Together, this feedback system insures that cells obtain an uninterrupted supply of optimal levels of cholesterol and fatty acids, whereas also preventing their accumulation.

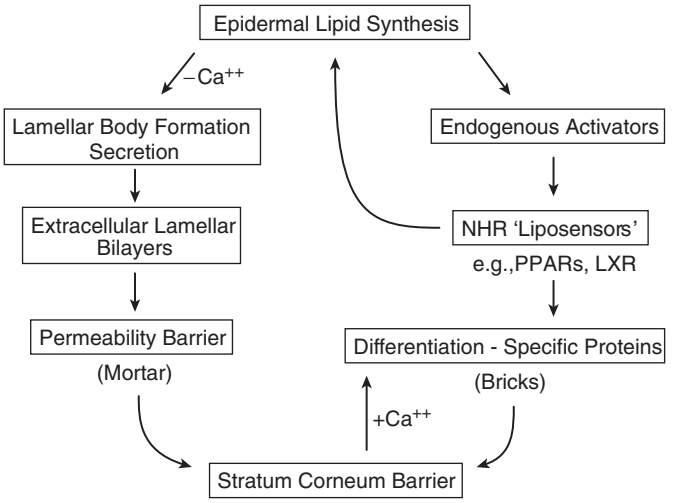

Figure 5
Co-ordinate regulation of epidermal differentiation and lamellar body secretion by calcium and nuclear hormone receptors.

SREBP-1 and SREBP-2 activate the same family of genes, but in different proportions (Smith *et al*, 1988; Vallett *et al*, 1996; Brown and Goldstein, 1998; Narce and Poisson, 2002). In general, SREBP-2 is a more important regulator of cholesterol synthesis, e.g., cholesterol-depleted cells preferentially upregulate SREBP-2, and SREBP-1 knockout animals upregulate SREBP-2 and normalize cholesterol synthesis in response to decreased cellular sterols. SREBP-1a is as effective as SREBP-2 as a regulator of HMGCoA synthase and HMGCoA reductase (i.e., cholesterol synthesis), but it has a greater effect on FA synthesis than does SREBP-2, whereas SREBP-1c primarily regulates FA synthesis. Although both SREBP-1a and SREBP-1c are present (Harris *et al*, 1998), SREBP-2 is the predominant species present in both murine epidermis and in cultured human

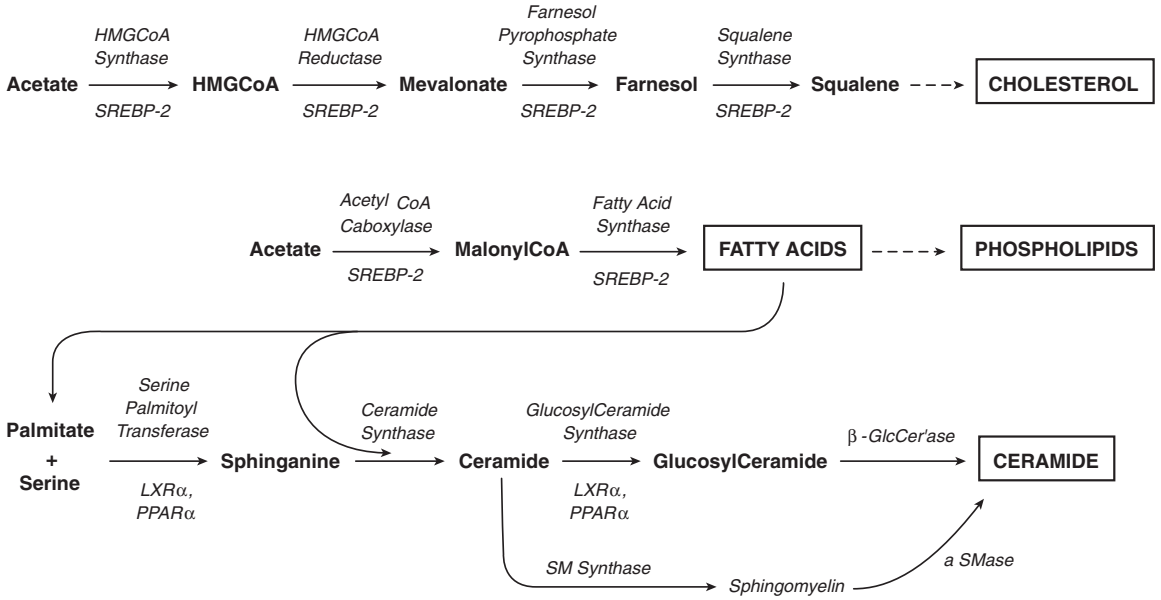

Figure 6
Metabolic pathways leading to synthesis of cholesterol, fatty acids, and ceramides.

keratinocytes. Moreover, SREBP-2 appears to coordinately regulate cholesterol and fatty acid synthesis in keratinocytes. But its role in the transcriptional regulation of the lipid synthetic response to barrier disruption is not yet clear.

Ceramide synthesis. SPT catalyzes the initial and first committed step in ceramide synthesis (Fig 6). Regulation of ceramide synthesis occurs predominantly by alterations in the activity of SPT, and/or the availability of palmitic acid, a substrate for SPT (Holleran *et al*, 1991a). Thus, alterations in FA synthesis, potentially regulated by SREBP, could indirectly affect ceramide production (Harris *et al*, 1998; Narce and Poisson, 2002). Yet, the expression of this enzyme is not regulated directly by SREBP, as SPT mRNA levels increase, rather than decrease, with oxysterol blockade of SREBP activation (Harris *et al*, 1998). In contrast, SPT expression increases in response to inflammatory stimuli, such as UV light, endotoxin, and cytokines (TNF and IL-1) (Farrell *et al*, 1998). To what extent and how liposensor activators regulate ceramide/glucosylceramides production is a subject of intense current study (Narce and Poisson, 2002).

Calcium (Ca) signaling of barrier homeostasis. Based upon phenomena such as the recovery response that follows acute barrier disruption, and the epidermal response to altered external humidities, signaling mechanisms must be operative in the epidermis. The epidermis displays a distinctive Ca gradient (Menon *et al*, 1985b), which is regulated passively by barrier integrity (Elias *et al*, 2002a), with the highest levels of Ca in the outer SG, tapering to very low levels in both the lower epidermis and SC. Indeed, changes in Ca that occur in response to an altered barrier, rather than barrier disruption *per se*, regulate barrier recovery (Lee *et al*, 1992; Menon *et al*, 1994b). In the presence of high Ca and $K+$, barrier recovery is inhibited, but recovery normalizes with co-applications of inhibitors of voltage-sensitive Ca channels (e.g., nifedipine, verapamil) (Lee *et al*, 1992). Ion-induced regulation of barrier recovery targets LB secretion, which normally occurs at low rates, sufficient to meet basal barrier requirements (Menon *et al*, 1994a). LB secretion rates accelerate when Ca levels surrounding SG cells decline in parallel with barrier disruption (Menon *et al*, 1994b) (Fig 5). Conversely, the high levels of Ca levels in the outer nucleated layers of the epidermis directly regulate epidermal differentiation, after with downregulation of protein synthesis, after acute barrier disruption (Elias *et al*, 2002b), followed by increased expression of the same proteins at later time points as the Ca gradient returns (Ekanayake-Mudiyanselage *et al*, 1998). Thus, Ca coordinately regulates a critical subset of homeostatic responses in the epidermis; i.e., those related to LB secretion and epidermal differentiation.

Cytokines and growth factors. Both release of cytokines from a preformed pool (Wood *et al*, 1996) and production of several cytokines and growth factors increase with either acute or prolonged barrier disruption (Wood *et al*, 1992, 1994b, 1997; Nickoloff and Naidu, 1994), and in chronic inflammatory skin diseases, which generally display high levels of primary cytokines, chemokines and other inflammatory markers (Kupper, 1990). Although the role of injury-provoked cytokine generation in epidermal pathophysiology seems increasingly evident, the importance of individual cytokines as regulators of metabolic processes that lead to barrier recovery can be controversial, because occlusion, which normalizes barrier function artificially, fails to block the upregulation of cytokine production (Wood *et al*, 1994a). Likewise, occlusion has little or no effect on the expression levels of the receptors for these cytokines (Wood *et al*, 1994b). Yet, occlusion does, in fact, downregulate expression levels of primary cytokines in chronically perturbed skin (e.g., EFA deficiency) (Wood *et al*, 1994a), and it also blocks

upregulation of certain growth factors, e.g., amphiregulin and nerve growth factor, whose expression increases greatly after acute barrier disruption (Liou *et al*, 1997). Finally, transgenic mice with double knockouts of both the functional IL-1 and TNF receptors display a delay in barrier recovery (Kreder *et al*, 1999); and finally, injections of these cytokines accelerate barrier recovery (Jensen *et al*, 1999; Ye *et al*, 2002). Primary cytokines could regulate barrier homeostasis by two mechanisms: (1) cytokines stimulate keratinocytes lipid synthesis (Ye *et al*, 2002); and (2) they stimulate keratinocyte proliferation (Kupper, 1990; Feliciani *et al*, 1996).

Repeated disruption of the permeability barrier by either tape stripping or acetone treatment leads to cutaneous inflammation, epidermal hyperplasia, and impaired differentiation (Denda *et al*, 1996). Because such disturbances in barrier function can provoke important cutaneous abnormalities, it is possible that alterations in barrier function contribute to the pathogenesis of inflammatory dermatoses, particularly in disorders, such as psoriasis and AD, which are associated with barrier abnormalities (Elias and Feingold, 2001). Although specific T cell abnormalities are undeniably important in the *etiology* of psoriasis, AD (Kupper, 1990), and allergic contact dermatitis, the studies described above support a possible epidermal *pathogenesis* for these disorders. Together, these studies show that the SC is an exquisite biosensor, which responds to barrier disruption and/or injury with the release and activation of a variety of signaling molecules, which are putative regulators of the homeostatic repair response in the underlying epidermis. These cytokines can, however, also signal a downstream cascade that eventually leads to inflammation.

Regulation of Desquamation

SC integrity/cohesion. If not the most important defensive function, perhaps the most distinctive characteristic of the SC is its ability to desquamate invisibly from the skin surface. Cohesion between adjacent corneocytes is facilitated by the "tongue-and-groove" arrangement of adjacent cells (Christophers and Kligman, 1964; Menton and Eisen, 1971), by CD (=specialized desmosomes of the SC), which rivet together adjacent cells (Serre *et al*, 1991; Harding *et al*, 2000), and by extracellular lipids (Chapman *et al*, 1991). SC "integrity" is a measure of resistance of adjacent corneocytes, to dissociation quantified as a rate of increase in transepidermal water loss with succesive tape strippings (Ghadially *et al*, 1995; Horikoshi *et al*, 1999; Fluhr *et al*, 2001). A related index, SC "cohesion", is the amount of protein removed per stripping (Fluhr *et al*, 2001). Although desquamation allows the distal, invisible shedding of corneocytes, dyshesion is largely completed within the lower SC by proteolytic degradation of CD (Simon *et al*, 2001). Morphological evidence for loss of CD (Chapman *et al*, 1991; Menon and Elias, 1997) is paralleled by the progressive disappearance of its constituent proteins, desmocollin 1 (DSC1), DSG1, and CDSN) (Lundstrom *et al*, 1994; Suzuki *et al*, 1996; Harding *et al*, 2000). DSG and DSC are desmosomal cadherins, encoded by separate genes that mediate cell-to-cell adhesion. Whereas epidermal desmosomes contain four isoforms of DSG and DSC, CD contain only the DSG1 and DSC1 isoforms (Harding *et al*, 2000). CDSN is a recently described, 36–48 kDa differentiation product of the epidermis, which is secreted by LB (Serre *et al*, 1991), accounting for its localization on the extracellular surfaces of CD (Simon *et al*, 1997), where it shields DSG1 and DSC1 from premature proteolysis (Serre *et al*, 1991; Lundstrom *et al*, 1994), and mediates homophilic binding to counterparts on adjacent corneocytes (Jonca *et al*, 2002). The cross-linking of additional CD constituents (e.g., envoplakin, periplakin) into the CE further stabilizes these structures, and in addition, dictates that CD degradation must occur *in situ* (Harding *et al*, 2000).

Proteolytic processes leading to desquamation. The progressive changes that occur in CD and their constituent peptides point to a critical role for extracellular proteases in the orchestration of corneocyte shedding (Suzuki *et al*, 1996; Horikoshi *et al*, 1999; Harding *et al*, 2000). At least ten types of SP activity have been identified in SC, with a convincing link to desquamation for two SP, SCCE, and SCTE (kallikrein 7 and 5, respectively) (Hansson *et al*, 1994; Ekholm *et al*, 2000). SCCE is predominantly extracellular (Sondell *et al*, 1994), and SCTE activity also is present in membrane domains (Hachem *et al*, 2003), but its subcellular distribution is not known. Based upon *in vitro* inhibitor studies, both SCCE and SCTE appear to be major regulators of desquamation (Suzuki *et al*, 1993; Horikoshi *et al*, 1999; Ekholm *et al*, 2000). The mRNA for human SCCE is expressed in the outer epidermis (Sondell *et al*, 1994), where it is synthesized as a 25 kDa inactive precursor, bearing seven amino acid propeptide residue, delivered to the SC interstices by LB secretion (Hansson *et al*, 1994). SCCE can be cleaved to its active form by SCTE, a 28–33 kDa epidermis-specific, plurifunctional SP (Brattsand *et al*, 2000). SCTE not only activates SCCE but it also degrades CD directly and activates itself (from a 37 kDa precursor) (Ekholm *et al*, 2000). SCCE activation is strictly controlled under basal conditions by both the requirement for precursor activation by SCTE (Hansson *et al*, 1994), and the presence of endogenous SP inhibitors plus certain inhibitory SC lipids (e.g., cholesterol sulfate and FFA are inhibitors of SP) (Suzuki *et al*, 1996; Elias *et al*, 2004), and the physiologic state of SC under basal condition (i.e., low pH, low Ca^{2+}, and low hydration; Watkinson *et al*, 2001; Bouwstra *et al*, 2003). Conversely, barrier disruption not only removes inhibitory lipids but it also allows increased Ca^{2+} and water movement into the SC interstices (Lee *et al*, 1992; Menon *et al*, 1994a), along with an increase in SC pH, conditions that favor SCCE-mediated desquamation. Although both cysteine and aspartate protease activities, with acidic pH optima, have been found in the SC (Suzuki *et al*, 1993, 1996; Horikoshi *et al*, 1998; Watkinson *et al*, 2001; Bernard *et al*, 2003), assignment of specific roles for these proteases in desquamation is complicated both by uncertainties about their localization, and by over-reliance on *in vitro* models to characterize their putative activities.

Protease inhibitors in the SC (Table III) Proteolytic events leading to desquamation are restricted by co-secreted and

Table III. Protease–anti-protease reactions in stratum corneum

	Preferred substrate	Anti-proteases
Serine proteases		
SCTE	DSG1, pro-SCCE	LEKTI 1
SCCE	DSC1, CDSN	SKALP, SLPI, LEKTI 1
Cysteine proteases		
SCCP	DSC1, CDSN	Cystatin M/E, Cystatin α, SLPI
Aspartate proteases		
Cathepsin G	DSC1, CDSN	SLPI

SC, stratum corneum; SCTE, SC tryptic; SCCE, SC chymotryptic; SCCP, SC cysteine protease; DSG1, desmoglein 1; DSC1, desmocollin 1; CDSN, corneodesmosin; SLPI, secretory leukocyte protease inhibitor; SKALP, skin-derived antileukocyte proteinase.

co-localized protease inhibitors in the SC. The SP inhibitors (SPI) include: secretory leukocyte protease inhibitor (SLPI), a non-glycosylated, cysteine-enriched, 12 kDa protein, which potently inhibits SP (Thompson and Ohlsson, 1986; Wiedow *et al*, 1990, 1993). SLPI consists of two cysteine core domains in a tandemly repeating motif, of which one domain is a trappin gene product (Thompson and Ohlsson, 1986). Trappins comprise a family of low-molecular-weight proteinase inhibitors, which include another SPI found in SC, skin-derived antileukocyte proteinase (SKALP) or elafin (Molhuizen *et al*, 1993; Alkemade *et al*, 1994). SKALP is a smaller, 6 kDa peptide that shares a single linear epitope of six amino acids with SLPI (Molhuizen *et al*, 1993). Because both SLPI and SKALP contain TGase substrate domains, they are extensively cross-linked to loricrin residues in the CE (Thompson and Ohlsson, 1986; Molhuizen *et al*, 1993; Alkemade *et al*, 1994). Likewise, plasminogen activator inhibitor type 2 (PAI-2) represents an additional SPI that is cross-linked into the CE (Risse *et al*, 1998, 2000).

The inhibitory spectra of SLPI and SKALP are both overlapping and complementary. Because SLPI exhibits stronger inhibitory activity against SCCE than does SKALP, it is thought to be the principal regulator of SCCE *in vivo*, but SKALP is 100-fold more effective as a trypsin inhibitor than is SLPI (Molhuizen *et al*, 1993; Alkemade *et al*, 1994). Although expressed minimally in normal epidermis, both SLPI and SKALP expression become highly expressed in suprabasal cells in psoriasis, during wound healing, and following epidermal injury (Wiedow *et al*, 1990; Alkemade *et al*, 1994).

The SC has recently been shown to contain another important SPI, i.e., LEKTI 1 (Komatsu *et al*, 2002). The genetic abnormality in Netherton's syndrome (NS) comprises mutations in the SPINK 5 gene, which encodes the 15-member LEKTI 1 class of SPI (Chavanas *et al*, 2000; Bitoun *et al*, 2002). LEKTI generally are expressed in squamous epithelia and thymus (Komatsu *et al*, 2002), but neither their natural substrates nor their localization in epidermis are known. NS is a severe autosomal recessive disorder, characterized by congenital ichthyosis, severe atopic manifestations, and a propensity for hypernatremic dehydration because of the severity of the putative barrier defect (Moskowitz *et al*, 2004). Whereas inflammation and secondary infections are common features of NS (Traupe, 1989), much more prominent features are the loss (thinning) of SC and the extraordinary barrier abnormality (Moskowitz *et al*, 2004), which could result from reduced SC integrity because of unchecked proteolysis (Komatsu *et al*, 2002). The presumed basis for the barrier defect is severe abnormalities in lamellar membrane structure (Fartasch *et al*, 1999), which could result from the premature destruction of the lipid-processing enzymes needed to generate the lamellar bilayers from secreted precursors (Fartasch *et al*, 1999). Finally, the SC contains two cysteine protease inhibitors, cystatin α and cystatin M/E, which again are constituents of the CE (Takahashi *et al*, 1996; Zeeuwen *et al*, 2001). Their potential physiologic role is shown by the Harlequin mouse, where mutations in cystatin M/E result in ichthyosis and hair abnormalities (Zeeuwen *et al*, 2003).

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Address correspondence to: Peter M. Elias, MD, Dermatology Service (190), VA Medical Center, 4150 Clement Street, San Francisco, California 94121, USA (reprints are not available). Email: eliaspm@itsa.ucsf.edu

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