

# Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens

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**Background:** Mutations in the human filaggrin gene (*FLG*) are associated with atopic dermatitis (AD) and are presumed to provoke a barrier abnormality. Yet additional acquired stressors might be necessary because the same mutations can result in a noninflammatory disorder, ichthyosis vulgaris. **Objective:** We examined here whether *FLG* deficiency alone suffices to produce a barrier abnormality, the basis for the putative abnormality, and its proinflammatory consequences. **Methods:** By using the flaky-tail mouse, which lacks processed murine filaggrin because of a frameshift mutation in the gene encoding profilaggrin that mimics some mutations in human AD, we assessed whether *FLG* deficiency provokes a barrier abnormality, further localized the defect, identified its subcellular basis, and assessed thresholds to irritant- and hapten-induced dermatitis.

**Results:** Flaky-tail mice exhibit low-grade inflammation with increased bidirectional, paracellular permeability of water-soluble xenobiotics caused by impaired lamellar body secretion and altered stratum corneum extracellular membranes. This barrier abnormality correlates with reduced inflammatory thresholds to both topical irritants and haptens. Moreover, when exposed repeatedly to topical haptens at doses that produce no inflammation in wild-type mice, flaky-tail mice experience a severe AD-like dermatosis with a further deterioration in barrier function and features of a T<sub>H</sub>2

immunophenotype (increased C<sub>RT</sub>H levels plus inflammation, increased serum IgE levels, and reduced antimicrobial peptide [mBD3] expression).

**Conclusions:** *FLG* deficiency alone provokes a paracellular barrier abnormality in mice that reduces inflammatory thresholds to topical irritants/haptens, likely accounting for enhanced antigen penetration in *FLG*-associated AD. (J Allergy Clin Immunol 2009;124:496-506.)

**Key words:** Atopic dermatitis, barrier function, contact dermatitis, filaggrin, flaky-tail mouse, lamellar body

Atopic dermatitis (AD) is a chronic, relapsing, inflammatory skin condition that displays an associated abnormality in permeability barrier function.<sup>1-4</sup> Until recently, AD was viewed as a primary immunologic disorder in which sustained allergen exposure in susceptible hosts leads to T<sub>H</sub>2-dominant inflammation, which then secondarily provokes defects in barrier function (the inside-outside paradigm).<sup>5</sup> However, recent genetic studies have shown a strong association between AD and several loss-of-function mutations in the gene encoding the stratum corneum structural protein filaggrin (*FLG*).<sup>6,7</sup> Moreover, there is a dose-response relationship between *FLG* deficiency and disease severity, such that patients with double-allele or compound heterozygote mutations in *FLG* display more severe and earlier-onset AD, as well as an increased propensity for AD to persist into adulthood.<sup>8-11</sup> This rapidly growing body of work has led to a paradigm shift in conceptions of AD pathogenesis, with increasing weight being placed on the role of a primary barrier abnormality that then precipitates downstream immunologic abnormalities (the outside-inside paradigm).<sup>1,12</sup> According to this concept, T<sub>H</sub>2-dominant inflammation results from sustained epicutaneous access of haptens through a defective skin barrier.<sup>13,14</sup> T<sub>H</sub>2 cytokines then downregulate expression of T<sub>H</sub>1 cytokines,<sup>5</sup> epidermal antimicrobial peptide expression,<sup>15,16</sup> and epidermal structural proteins,<sup>1,2</sup> completing an outside-inside-(back to) outside paradigm for AD.<sup>2,17</sup>

*FLG* is an intracellular protein with important roles in stratum corneum hydration<sup>18</sup> and as a structural component of the corneocyte cytosol.<sup>19</sup> However, normal permeability barrier function is not regulated by the corneocyte but rather by the lipid-enriched extracellular matrix of the stratum corneum.<sup>20,21</sup> Indeed, in all inflammatory dermatoses studied to date, including those associated with inherited disorders of corneocyte proteins, the barrier abnormality has been linked to accelerated paracellular water loss.<sup>22,23</sup> Thus the newfound link between *FLG* and AD leaves unanswered whether *FLG* deficiency alone provokes a barrier

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Supported by National Institutes of Health grants AR019098, AG028492, AI059311 and the Medical Research Service, Department of Veterans Affairs.

Disclosure of potential conflict of interest: J. P. Sundberg has received research support from the National Alopecia Areata Foundation, Proctor & Gamble, the Ellison Medical Foundation, and the National Institutes of Health. T. M. Mauro has received research support from the National Institutes of Health. M. Schmuth has received and honorarium for lecturing from Astellas Pharma and has received research support from the Austrian Science Fund and the National Institutes of Health. K. R. Feingold has received research support from Merck. The rest of the authors have declared that they have no conflict of interest.

Received for publication April 13, 2009; revised June 24, 2009; accepted for publication June 26, 2009.

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0091-6749/\$36.00

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doi:10.1016/j.jaci.2009.06.046

#### Abbreviations used

|                |                                                                            |
|----------------|----------------------------------------------------------------------------|
| AD:            | Atopic dermatitis                                                          |
| CRTH2:         | Chemoattractant receptor-homologous molecule expressed on T <sub>H</sub> 2 |
| <i>FLG</i> :   | Human filaggrin gene                                                       |
| <i>flg</i> :   | Murine filaggrin gene                                                      |
| <i>ft/ft</i> : | Flaky tail                                                                 |
| mBD3:          | Mouse $\beta$ -defensin 3                                                  |
| Ox:            | Oxazolone                                                                  |
| TEWL:          | Transepidermal water loss                                                  |
| TPA:           | 12-O-tetradecanoylphorbol-13-acetate                                       |

abnormality in the epidermis and, if so, the identity of the subcellular basis for such an abnormality. Although it has been widely hypothesized that an *FLG*-associated barrier defect facilitates hapten ingress,<sup>12,13</sup> this relationship was only recently demonstrated.<sup>24</sup> Yet the mechanism whereby *FLG* deficiency compromises barrier function, thereby provoking inflammation, remains unknown. Pertinently, *FLG* mutations that occur in patients with AD also occur in the inherited, noninflammatory condition ichthyosis vulgaris,<sup>25,26</sup> raising questions about whether *FLG* deficiency alone suffices to provoke inflammation or whether additional acquired stressors are required to elicit AD.<sup>1,2</sup>

The above questions are difficult to address within the context of human AD, in which multiple genetic and environmental factors intertwine to provoke disease pathogenesis. Thus we used a pre-existing model, the flaky-tail (*ft/ft*) mouse, which exhibits very reduced murine filaggrin gene (*flg*) expression.<sup>27</sup> Flaky-tail mice possess a homozygous frameshift mutation in profilaggrin that prevents the processing of profilaggrin into *flg*,<sup>24</sup> such that *ft/ft* mice simulate double-allele, loss-of-function mutations in certain human patients with AD that also result in reduced levels of processed filaggrin protein.<sup>6,28</sup> The *ft/ft* mutation was shown previously to yield a phenotype of diffuse mild flaking analogous to that seen in human ichthyosis vulgaris.<sup>27</sup> Using the *ft/ft* model, we demonstrate that *ft/ft* mice display (1) abnormal barrier function and low-grade inflammation at baseline, confirming the recent observations of Fallon et al,<sup>24</sup> and (2) enhanced bidirectional, paracellular penetration of water-soluble tracers. We demonstrate further that (1) increased penetration through the stratum corneum can be attributed to structural defects in the lamellar body secretory system, (2) *flg* deficiency alone reduces inflammatory thresholds to both irritants and allergens, and (3) *ft/ft* mice have a hapten-induced, AD-like dermatosis at lower challenge doses than do wild-type mice, mirroring recent work in *ft/ft* mice exposed to a complete antigen.<sup>24</sup> Thus *flg* deficiency yields a paracellular barrier abnormality that likely also favors elicitation of AD by allowing enhanced/sustained hapten access.

## METHODS

### Materials and animals

Studies were conducted in *maft/maft/J (ft/ft)* mice (new designation: a/a *ft/ft*; *ma ft/JSun JR#9078*) and age- and sex-matched *C57Bl/6J<sup>+/+</sup>* mice (The Jackson Laboratory, Bar Harbor, Me) housed under pathogen-free conditions. Flaky-tail mice are compound mutants at 2 loci, both matted (*ma/ma*) and *ft/ft*. Matted confers a stable, lifelong, and readily identifiable phenotype of subtle hair coarseness that was introduced by Jackson Laboratories to facilitate identification of mutants without reliance on genotyping.<sup>29</sup> Young *ft/ft* and wild-

type mice were between 6 and 12 weeks of age, whereas old mice were 50 to 52 weeks of age. Ethanol, acetone, and lanthanum nitrate were purchased from Fisher Scientific (Fairlane, NJ). Oxazolone (Ox) and calcium green were purchased from Sigma Chemical Co (St Louis, Mo). Affinity-purified rabbit anti-mouse antibodies to both profilaggrin and *flg* were purchased from BabCo (Richmond, Calif). Rabbit anti-mouse antibody against the prostaglandin D<sub>2</sub> receptor chemoattractant receptor-homologous molecule expressed on T<sub>H</sub>2 (CRTH2)/DP2, which is expressed solely on T<sub>H</sub>2-bearing lymphocytes and mast cells, was purchased from Cayman Chemical (Ann Arbor, Mich). Rabbit anti-mouse antibody against CD3 was purchased from BD Biosciences (San Jose, Calif).

## Experimental protocols and functional studies

All animal procedures were approved by the Animal Studies Subcommittee (Institutional Animal Care and Use Committee) of the San Francisco Veterans Administration Medical Center and performed in accordance with their guidelines. Basal stratum corneum hydration and surface pH were measured (CM 825/PH 900; Courage & Khazaka, Köln, Germany) on shaved back skin of *ft/ft* and wild-type mice ( $n = 4-6$  in all experimental groups, except where noted in figure legends). Transepidermal water loss (TEWL) measurements were taken under basal conditions with an electrolytic water analyzer (Meeco, Warrington, Pa), as well as 2 and 4 hours after a tape stripping-induced, 3-fold increase in TEWL. Barrier recovery was calculated as percentage barrier recovery from 0 levels immediately after acute disruption.<sup>30-37</sup>

Irritant and acute allergic contact dermatitis were induced in *ft/ft* and wild-type mice by using 12-O-tetradecanoylphorbol-13-acetate (TPA) and Ox, respectively, as described previously.<sup>38</sup> TPA (either 0.3% or 0.1% in acetone) was applied once to the inner and outer surface of the ear. Challenge doses of Ox (either 0.02% or 0.5% in acetone) were applied once after initial Ox sensitization (2% Ox). In all cases ear thickness was measured 2 hours after treatment.

Flaky-tail and wild-type mice were sensitized with 2% Ox, as above, to induce an AD-like dermatosis in mice. One week later, shaved areas on the flanks of *ft/ft* and wild-type mice were treated every other day topically with 60  $\mu$ L of 0.5% or 0.02% Ox for an additional 3 weeks (10 challenges). Other groups of *ft/ft* and wild-type mice were treated with ethanol alone as the vehicle control group ( $n = 10-12$  in each group). At the end of treatments, basal TEWL and stratum corneum hydration were measured again.

## Immunohistochemistry

Changes in overall morphology were visualized after hematoxylin and eosin staining of 5- $\mu$ m paraffin-embedded tissue samples. Both the number of epidermal nucleated cell layers and the density of the inflammatory infiltrate were measured in 20 to 30 randomly chosen fields from *ft/ft* and wild-type mice before and after Ox challenge doses, as above. Immunohistochemical assessment of changes in profilaggrin/filaggrin, CRTH2, and CD3 levels were performed, as described previously.<sup>39</sup> Briefly, 5- $\mu$ m paraffin sections were incubated with the primary antibodies overnight at 4°C. After washing, sections were incubated with the secondary antibody for 30 minutes. Staining was detected with the ABC-peroxidase kit from Vector Laboratories (Burlingame, Calif). Frozen sections were examined with a Zeiss fluorescence confocal microscope (Jena, Germany), and digital images were captured with AxioVision software (Carl Zeiss Vision, Munich, Germany). A similar sequence was used to assess 2 antimicrobial peptides in 5- $\mu$ m frozen sections (primary antibodies to mouse  $\beta$ -defensin 3 (mBD3) from Alpha Diagnostics; mouse cathelicidin antimicrobial peptide antibody was a gift from Dr Richard Gallo, University of California San Diego).

## Ultrastructural lipase detection

Ultrastructural cytochemistry of lipase activity has been used as a content marker for epidermal lamellar bodies, allowing assessment of the efficacy of organelle secretion.<sup>40</sup> Briefly, samples were incubated in 5% Tween 85 (1 mL) in 0.2 mol/L HEPES buffer (2.5 mL), 2.5% sodium taurocholate (2 mL), and 10% calcium chloride (1 mL) in 25 mL of distilled water (pH 7.4). Microwave

incubations were carried out twice for 30 seconds at 2450 MHz, and the water bath was changed between the pulses to maintain temperatures at less than 40°C.<sup>41</sup> After incubation in the same medium for an additional 30 minutes at 37°C, all samples were incubated further in 0.1 mol/L cacodylate buffer containing 0.2% Tween 85 with or without 200  $\mu$ M of the lipase inhibitor tetrahydrolipstatin, as previously described.<sup>42</sup>

### Tracer penetration

Freshly obtained explants of back skin from untreated *fl/fl* and wild-type mice were exposed dermis side downward (30 minutes to 2 hours) to the low-molecular-weight tracer colloidal lanthanum nitrate (4%) in Karnovsky solution to assess the outward permeation of water-soluble markers. To assess permeation from the outside, we placed 3 wild-type control and 3 *fl/fl* mice in Petri dishes after achievement of general anesthesia, with one side of each mouse immersed in 4% colloidal lanthanum in a 37°C incubator for 2 hours. Lanthanum nitrate was prepared in 4% wt/vol sucrose in 0.05 mol/L Tris buffer, pH 7.5. After immersion, biopsy samples were taken and processed as below. In parallel studies a 20  $\mu$ M/L solution of calcium green 5 N (Invitrogen, Carlsbad, Calif) in nanopure water was applied on the stratum corneum side of *fl/fl* and wild-type mice for 10 and 75 minutes. Ten-millimeter biopsy specimens were mounted immediately on microscope coverslips, washed in nanopure water, and imaged in a Zeiss 510 meta NLO (Zeiss Germany) at the Live Cell Analysis Core Facility at the University of California San Francisco. An optically pumped (Coherent, Inc, Santa Clara, Calif) mode-locked Mira 900 Ti:Saph laser (Coherent) was used as the 2-photon excitation source at a wavelength of 800 nm. A 500- to 550-nm band pass filter was placed in the detection path to minimize sample autofluorescence. Under these experimental conditions, we estimated the contribution of autofluorescence to the total signal to be less than 5%. Z-stacks of 235X235X20  $\mu$ m<sup>3</sup> images were acquired, beginning with the outer surface of the stratum corneum at z increments of 1  $\mu$ m by using a 40 $\times$  oil immersion objective with a 1.3 numerical aperture (Zeiss). Matlab software (The Math Works, Natick, Mass) was used to quantitate image intensity at each z-stack and to calculate mean penetration depths.

### Electron microscopy

Aldehyde-fixed biopsy specimens were postfixed with either 0.25% ruthenium tetroxide or 1% aqueous osmium tetroxide containing 1.5% potassium ferrocyanide, as described previously.<sup>43</sup> Ultrathin sections were examined with an electron microscope (Zeiss 10A; Carl Zeiss, Thornwood, NY) operated at 60 kV. Images were captured with Digital Micrograph 3.10.0 software from Gatan, Inc (Pleasanton, Calif).

### Serum IgE

Blood samples were collected from *fl/fl* and wild-type mice under basal conditions and after prior Ox sensitization, followed by repeated treatments with either Ox or vehicle. Serum IgE concentrations were determined with a mouse IgE ELISA quantitation kit from Bethyl Laboratories (Montgomery, Tex), according to instructions provided by the manufacturer.

In these and all other studies, statistical analyses were performed with the Student *t* test to compare differences between 2 groups and with a further ANOVA analysis when 3 or more groups were compared.

## RESULTS

### Filaggrin deficiency results in abnormal barrier function

Prior studies have shown that *fl/fl* mice display markedly reduced F-type keratohyalin granules, with an absence of *flg*,<sup>24</sup> caused by a recently identified frameshift mutation in the gene encoding profilaggrin.<sup>24</sup> By using a consensus antibody that recognizes both profilaggrin and *flg*, we found intense positive immunostaining restricted to the stratum granulosum (granular

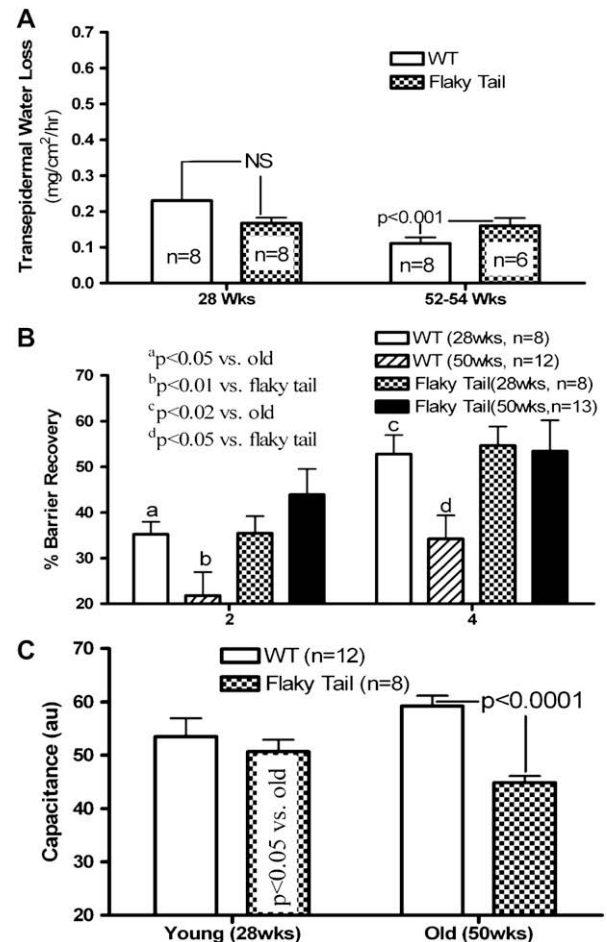


**FIG 1.** Abnormal barrier function and stratum corneum hydration in *fl/fl* mice. **A**, Basal TEWL levels were assessed with an electrolytic analyzer as milligrams per square centimeter per hour and expressed as means  $\pm$  SEMs. **B**, Barrier disruption was achieved with repeated tape stripping until TEWL levels were 3 $\times$  normal or greater. Then percentage recovery from an initial 0 value was measured 2 and 4 hours after stripping and expressed as means  $\pm$  SEMs. **C**, Stratum corneum hydration was measured as electrical capacitance on shaved back skin from *fl/fl* and wild-type mice and shown as changes in arbitrary units  $\pm$  SEMs ( $n = 8-13$ , as indicated). WT, Wild-type; NS, not significant.

layer), with no residual staining of *flg* in the stratum corneum in *fl/fl* mice (see Fig E1, green staining, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). In contrast, wild-type epidermis displayed immunostaining in the granular layer, as well as diffuse staining of the stratum corneum. Thus the mutation in the gene encoding profilaggrin in *fl/fl* mice results in reduced *flg* expression in the stratum corneum, which is analogous to the situation seen in some human subjects with *FLG*-associated AD.<sup>6,28</sup>

We next assessed epidermal functional parameters in *fl/fl* and wild-type mice using biophysical instrumentation. Permeability barrier homeostasis, as assessed as the kinetics of TEWL levels after acute disruption, is normal<sup>44</sup> but basal barrier function decreases with aging in wild-type aged (52-54 weeks) mice, as previously described (Fig 1, A).<sup>44</sup> Although within the range of normal values ( $\leq 2.5$  mg/cm<sup>2</sup>/h), basal TEWL levels increase significantly (by approximately 20% to 30%) in 52- to 54-week-old *fl/fl* mice compared with those seen in age-matched wild-type

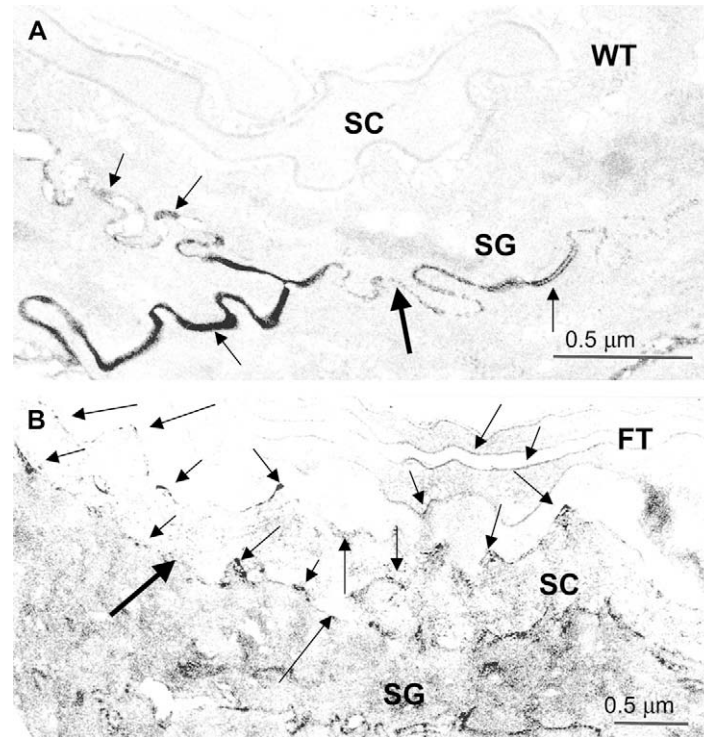


**FIG 2.** Abnormal paracellular permeability barrier to a subcutaneous, water-soluble tracer in *fl/fl* mice. Explants of back skin from sex-matched, 12-week-old, wild-type (WT) and *fl/fl* (FT) mice ( $n = 3$  each) were immersed dermis side downward on a solution containing 4% colloidal lanthanum nitrate (pH 7.5) for 30 minutes to 2 hours, followed by fixation in Karnovsky solution (see the Methods section). Colloidal lanthanum travels outward through the extracellular spaces but does not reach the stratum corneum (SC) in WT skin (A, arrows). In contrast, in FT skin lanthanum tracer extends into the lower SC, primarily through the extracellular spaces (B, arrows), suggesting impaired inside-to-outside barrier function. SG, stratum granulosum. Fig 2, A and B, Osmium tetroxide postfixation. Mag bars = 0.5  $\mu$ m.

control animals (Fig 1, A). As occurs in human AD,<sup>30</sup> the kinetics of barrier recovery after acute barrier abrogation accelerate in old *fl/fl* versus wild-type mice (Fig 1, B). Such accelerated barrier recovery also occurs in other chronic inflammatory dermatoses and is attributed to ongoing signaling of repair mechanisms, which are induced by barrier abrogation.<sup>31,32</sup> Finally, stratum corneum hydration, assessed as changes in electrical capacitance, does not differ in young *fl/fl* versus wild-type mice (Fig 1, C), but stratum corneum hydration is significantly decreased in young *fl/fl* mice, with a further decrease in older *fl/fl* mice (Fig 1, C), again mirroring known changes in human AD.<sup>33,45</sup>

Because basal TEWL levels lie within the normal range in *fl/fl* mice, we next assessed barrier function in these mice under basal conditions using an alternate approach (ie, ultrastructural visualization of tracer perfusion). The high resolution of this method also allowed us to determine simultaneously whether the barrier abnormality can be attributed to a defective corneocyte or to enhanced paracellular transport. In wild-type mice the water-soluble, low-molecular-weight, electron-dense tracer lanthanum nitrate does not breach the stratum granulosum–stratum corneum interface (Fig 2, A). In contrast, the tracer not only breaches this layer but also extends several layers outward into the stratum corneum in *fl/fl* mice (Fig 2, B). Notably, despite the intracellular localization of *flg*, the tracer remains largely confined to the stratum corneum extracellular spaces in *fl/fl* mice (Fig 2, B, arrows). Together, these results demonstrate that *flg* deficiency leads to

alterations in basal barrier function through a defect in the stratum corneum extracellular matrix.

### Enhanced uptake of epicutaneously applied, water-soluble tracers through the extracellular route

Normal stratum corneum does not allow entry of water-soluble xenobiotics.<sup>46</sup> Therefore we next determined whether epicutaneously applied, water-soluble tracers enter the stratum corneum of *flg*-deficient mice and, if so, through which route. Lanthanum nitrate tracer was applied topically to the flanks of *fl/fl* and wild-type mice for 30 minutes to 2 hours after achievement of general anesthesia. As shown previously,<sup>46</sup> lanthanum fails to breach the stratum corneum of wild-type mice (Fig 3, A). In contrast, the tracer permeates 3 to 5 layers deep into the stratum corneum of *fl/fl* mice by 2 hours (Fig 3, B and C). Notably, the tracer localizes solely within the extracellular spaces, indicating again that penetration occurs through the paracellular route (Fig 3, C, arrows). In parallel studies we visualized the permeation of the low-molecular-weight, water-soluble fluorophore calcium green using dual-photon confocal microscopy. Differences in the extent of penetration can be seen as early as 10 minutes after application of tracer to *fl/fl* skin, with further penetration evident by 75 minutes in *fl/fl* mice (Fig 3, D and E). In contrast, very little of the fluorophore can be detected beneath the outer stratum corneum of wild-type mice at 75 minutes. Together, these results

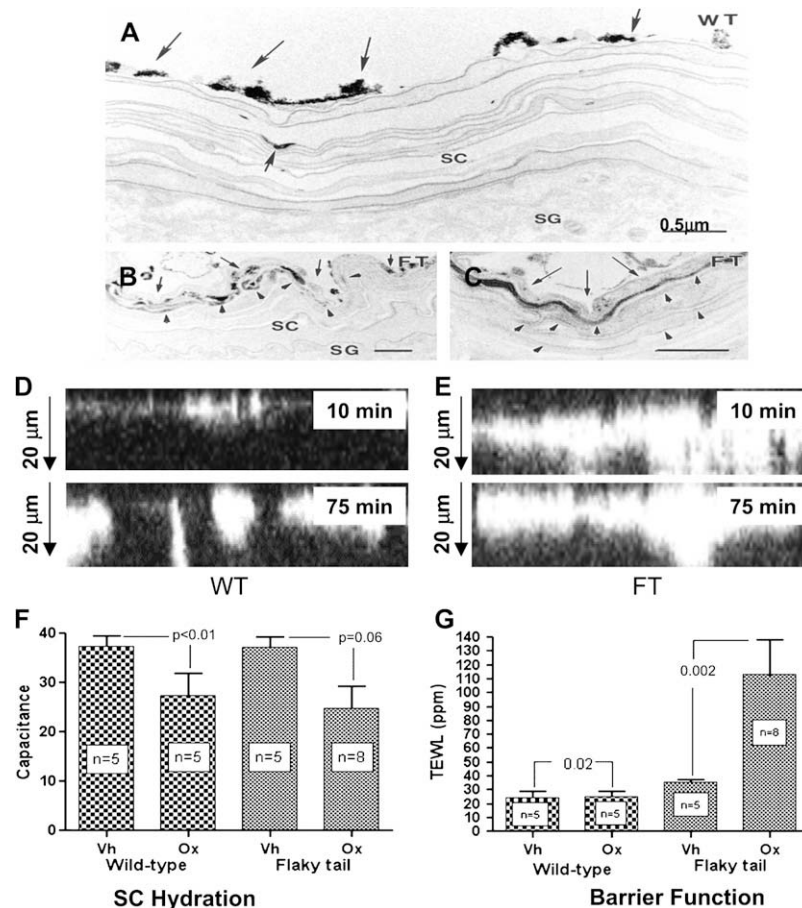


**FIG 3.** Epicutaneous tracer penetrates *ft/ft* (FT) stratum corneum (SC) through the extracellular spaces. **A-C**, Flanks of FT and wild-type (WT) mice ( $n = 3$  each) were immersed in 4% lanthanum nitrate solution for 30 minutes to 2 hours, followed by aldehyde fixation. After 2 hours, little or no tracer entered the SC of the WT mice (Fig 3, A, single small arrow). In contrast, abundant tracer reaches 4 or more layers into the SC in FT mice (Fig 3, B, arrowheads), which on higher magnification (Fig 3, C) can be seen to localize to extracellular domains (arrowheads). **D** and **E**, Calcium green was applied to freshly obtained explants from FT and WT mice ( $n = 3$  each), and penetration was assessed by means of dual-photon confocal microscopy along the z axis after 10 and 75 minutes. Note much deeper outside-to-inside penetration of calcium green in FT mice at both time points. FT mice, repeatedly challenged with low-dose Ox, display a more severe barrier abnormality (**G**) but no further decrease in SC hydration in comparison with WT mice (**F**). SG, Stratum granulosum; Vh, vehicle. Fig 3, A-C, Osmium tetroxide postfixation. Mag bar = 0.5 μm.

demonstrate that the stratum corneum of *flg*-deficient mice is more permeable to water-soluble tracers and that penetration of an electron-dense, water-soluble tracer occurs through the same paracellular pathway that is used by water itself when exiting the skin.

### Structural basis for altered permeability in *ft/ft* mice

We next assessed the structural basis for the extracellular barrier abnormality in *ft/ft* mice. By using standard transmission electron microscopy, *ft/ft* mice display not only the previously reported paucity of F-type keratohyalin granules in the stratum granulosum<sup>19</sup> but also a previously undescribed abnormality in lamellar body secretion (Fig 4). Lamellar body formation appears normal (ie, neither lamellar body density nor organelle contents differ in *ft/ft* versus wild-type mice). Yet substantial numbers of lamellar bodies appear to be retained within terminally differentiating keratinocytes in *ft/ft* mice (Fig 4, A, vs

Fig 4, B). These unsecreted organelles then become entombed within the cytosol of nascent corneocytes instead of being secreted *in toto* into the extracellular matrix, as occurs in wild-type mice (Fig 4, C, vs Fig 4, D). Partial entombment (failed secretion) of lamellar bodies could also be demonstrated by means of an alternate method: identification of the fate of a lamellar body content marker (acid lipase) by using ultrastructural cytochemistry.<sup>32,40</sup> In wild-type epidermis enzyme activity localizes both to lamellar body contents (see Fig E2, A, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)) and within the stratum corneum solely to the extracellular domains (see Fig E2, B). Flaky-tail mice reveal a similar localization of enzyme activity to lamellar bodies but evidence of reduced secretion (ie, lower amounts of initially secreted enzyme at the stratum granulosum–stratum corneum interface, as well as sparse [reduced] enzyme product in the stratum corneum interstices; see Fig E2, C, arrows). In contrast to wild-type mice, the cytosol of many *ft/ft* corneocytes contains abundant enzyme activity, with enzyme activity localizing near retained lamellar arrays (see Fig E2, D),

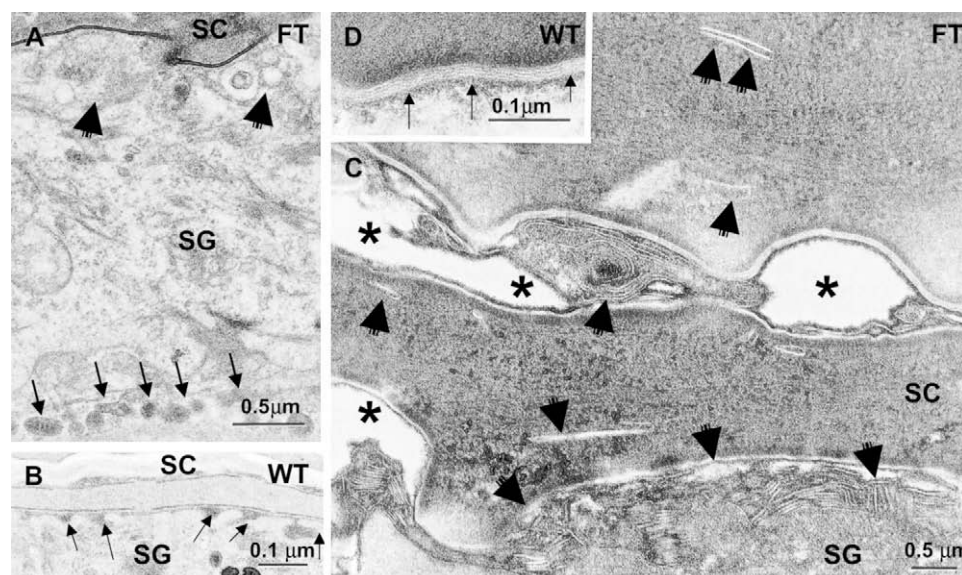


**FIG 4.** Abnormalities in the lamellar body secretory system in young *ft/ft* (FT) mice. Partial failure of lamellar body exocytosis is evident in FT epidermis (A and C). Note lamellar bodies lined up in peripheral cytosol (Fig 4, A, multiple thin arrows), decreased secreted material at the stratum granulosum (SG)–stratum corneum (SC) interface (Fig 4, A and C, short fat arrows), decreased numbers of lamellar bilayers (Fig 4, C), delayed maturation of lamellar bilayers (Fig 4, C), and entombed lamellar body contents within the corneocyte cytosol (Fig 4, C, short thin arrows). B and D, Normal lamellar body secretion (Fig 4, B, arrows) and extracellular lamellar bilayers in wild-type (WT) epidermis (Fig 4, D, thin arrows). Fig 4, A and B, Osmium tetroxide postfixation; Fig 4, C and D, ruthenium tetroxide postfixation. Mag bars = Fig 4, A and C, 0.5  $\mu$ m; Fig 4, B and D, 0.1  $\mu$ m.

providing further evidence for entombment of lamellar body contents.

We next assessed the consequences of impaired lamellar body secretion and enhanced paracellular transport in *ft/ft* mice. As a result of impaired lamellar body secretion, the quantities of extracellular lamellar bilayers correspondingly decrease in the stratum corneum of *ft/ft* mice (Fig 4, C, vs Fig 4, D). Moreover, the extracellular processing of newly secreted lamellar body contents into mature lamellar bilayers appears to be impaired or delayed in *ft/ft* mice (Fig 4, C, vs Fig 4, D). Together, these results suggest that either profilaggrin accumulation or *flg* deficiency impedes lamellar body secretion, leading to an abnormal stratum corneum extracellular matrix in *ft/ft* mice.

### Filaggrin deficiency alters thresholds for irritant and acute allergic contact dermatitis

We next assessed whether the barrier abnormality in *ft/ft* mutant mice results in an increased propensity to cutaneous inflammation. Flaky-tail skin displays low-grade inflammation under basal conditions, as indicated by the presence of increased numbers of inflammatory cells under basal conditions (Fig 5, B, E, and F; histologic images that correspond to these quantitative data are shown in Figs E3, C, vs Fig E3, A, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Moreover, most but not all *ft/ft* mice display increased serum IgE levels, even under basal conditions (Fig 5, G). Together, these results suggest that *ft/ft* mice display low-grade inflammation, even under basal conditions.

The phorbol ester TPA elicits irritant contact dermatitis when applied topically to mouse ears, with the severity of the inflammatory response correlating with an increase in ear thickness 2

hours after topical applications.<sup>34,47</sup> TPA was first applied at a standard concentration of 0.3% to the ears of *ft/ft* and wild-type mice. Although inflammation appeared greater in *ft/ft* mice, the differences in ear thickness in *ft/ft* versus wild-type mice do not achieve statistical significance (Fig 6, A). However, at a lower dose of TPA (0.1%) that provokes only marginal inflammation in wild-type mice, *ft/ft* mice display a marked inflammatory response with a significant increase in ear thickness (Fig 5, A;  $P < .001$ ; parallel histologic differences are shown in Fig E4 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Together, these results demonstrate that *ft/ft* mice display an enhanced propensity to irritant contact dermatitis.

We next assessed whether *ft/ft* mice display an altered threshold to the development of acute allergic contact dermatitis. For these studies, we used a single challenge dose (2%) of the universal hapten oxazolone (Ox), followed 1 week later by topical application to the ear of either a single challenge dose at a concentration (0.5%) that produces a prominent dermatitis after 24 to 48 hours<sup>47,48</sup> or a much lower hapten concentration (0.02%) that does not produce visible inflammation in wild-type mice. As with the topical irritant (compare with Fig 5, A), dermatitis appears more exaggerated in *ft/ft* mice after challenges with the standard (0.5%) hapten dose, but again, the results do not achieve statistical significance (Fig 5, B). At the lower challenge dose of Ox (0.02%), dermatitis does not develop in wild-type mice, whereas in contrast, substantial inflammation is evident in similarly challenged *ft/ft* mice (Fig 5, B;  $P < .0001$  vs wild-type mice; parallel histologic differences are shown in Fig E4). These results show that *ft/ft* mice display a reduced threshold for the development of hapten-induced acute allergic contact dermatitis.

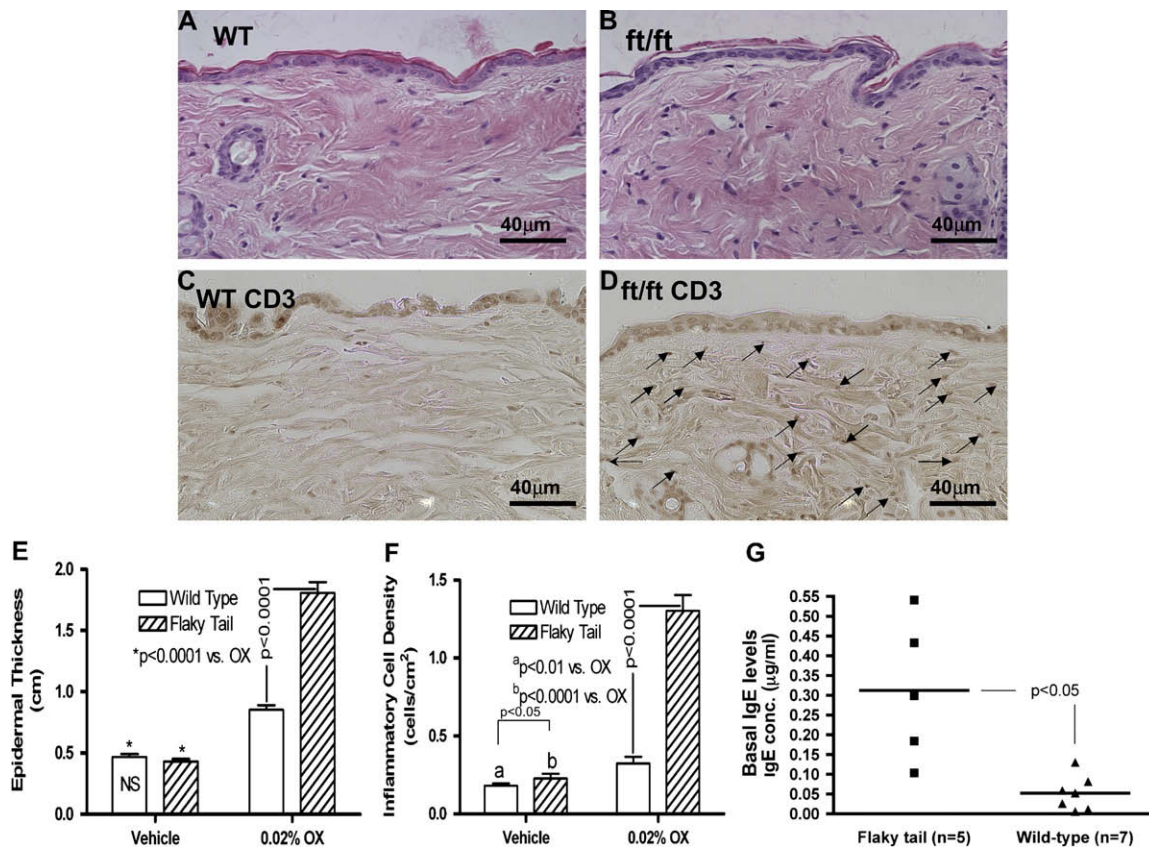


**FIG 5.** Reduced threshold for development of AD-like inflammation in response to repeated Ox challenges in *ft/ft* mice. Flaky-tail and wild-type (WT) mice were sensitized to Ox and then repeatedly challenged with a subthreshold concentration of Ox (0.02%) every other day to a shaved area of back skin for a total of 10 challenges. Under basal conditions, *ft/ft* mice display low-grade inflammation (F; see also Fig E3, C, vs Fig E3, A). Flaky-tail skin displayed much more prominent erythema, scaling, and excoriations than WT mouse skin (see text), a change that is associated with a modest inflammatory infiltrate (B vs A, and see Fig E3, C), that was enriched in CD3<sup>+</sup> dermal lymphocytes (D vs C). Quantitative changes in epidermal hyperplasia and inflammation are shown in E and Fig 5, F, G. Serum IgE levels are significantly increased in most *ft/ft* mice, even under basal conditions. Mag bars = 40 μm. NS, Not significant.

### Filaggrin deficiency predisposes to the development of an AD-like dermatosis in mice

We previously showed that Ox sensitization, followed by repeated (10×) hapten challenges over 20 to 21 days, produces a dermatosis with certain structural, biochemical, and immunologic features of human AD.<sup>49</sup> In the next set of studies, we used reduced challenge doses of Ox (10 challenges with 0.02% vs the standard 0.5% concentration) in *ft/ft* and wild-type mice. Whereas wild-type mice display minimal signs of inflammation, *ft/ft* mice reveal erythema, hyperkeratosis, epidermal hyperplasia, and histologically prominent inflammation at the lower hapten concentration (Fig 5, E and F, and see Fig E3). Moreover, although virtually no CD3<sup>+</sup> lymphocytes are detected in wild-type murine skin, a moderately high density of CD3<sup>+</sup> cells can be seen in *ft/ft* mice repeatedly challenged with low-dose Ox (Fig 6, D). Flaky-tail mice also display numerous prostaglandin D<sub>2</sub> receptor (CRTH2)-positive cells, which is consistent with a T<sub>H</sub>2-dominant immunophenotype, as well as much higher serum IgE levels than do comparably treated wild-type mice (Fig 7, A and B). Finally, *ft/ft* mice repeatedly challenged with low-dose Ox display reduced

immunostaining for mBD3 (murine homologue of hBD2; see Fig E5, D, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)), but staining for cathelicidin antimicrobial peptide (cathelicidin antimicrobial peptide = murine homologue of LL37) instead appears to increase (see Fig E5, C). Together, these results indicate that *flg* deficiency predisposes to the development of a hapten-stimulated AD-like dermatosis in mice.

### More severe barrier abnormality in *ft/ft* mice with AD

In healthy mice repeated challenges with 0.5% Ox result in a progressively more severe barrier abnormality,<sup>49</sup> which is consistent with a proposed outside-inside-outside pathogenic cycle in AD.<sup>1,2</sup> We next assessed whether repeated subthreshold (0.02%) doses of Ox would provoke functional abnormalities in *ft/ft* mice that occur only at higher doses in wild-type mice. Although stratum corneum hydration does not decrease in such Ox-challenged mice (Fig 3, F), *ft/ft* mice experience a severe permeability barrier abnormality at subthreshold doses of hapten

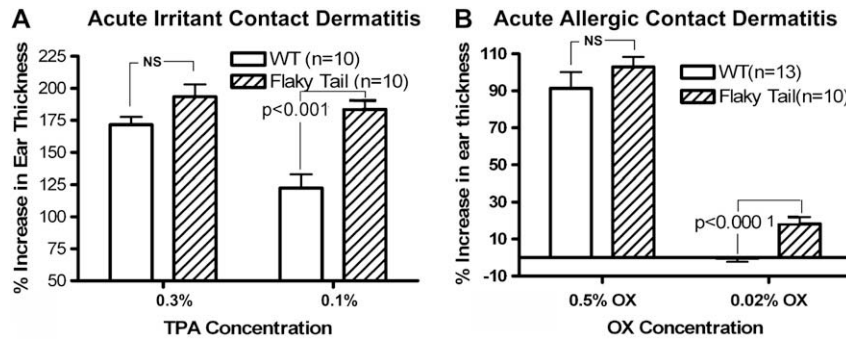


**FIG 6.** Altered sensitivity to irritant/hapten-induced allergic contact dermatitis in *ft/ft* mice. In young wild-type mice TPA induces irritant contact dermatitis, whereas Ox induces allergic contact dermatitis after a single, full-strength challenge dose.<sup>38</sup> Although higher concentrations produced comparable changes in young wild-type (*WT*) and *ft/ft* mice, lower doses of either TPA (**A**) or Ox (**B**) induce inflammation only in *ft/ft* mice. Changes in ear thickness correlate with severity of inflammation (see Fig E4). NS, Not significant.

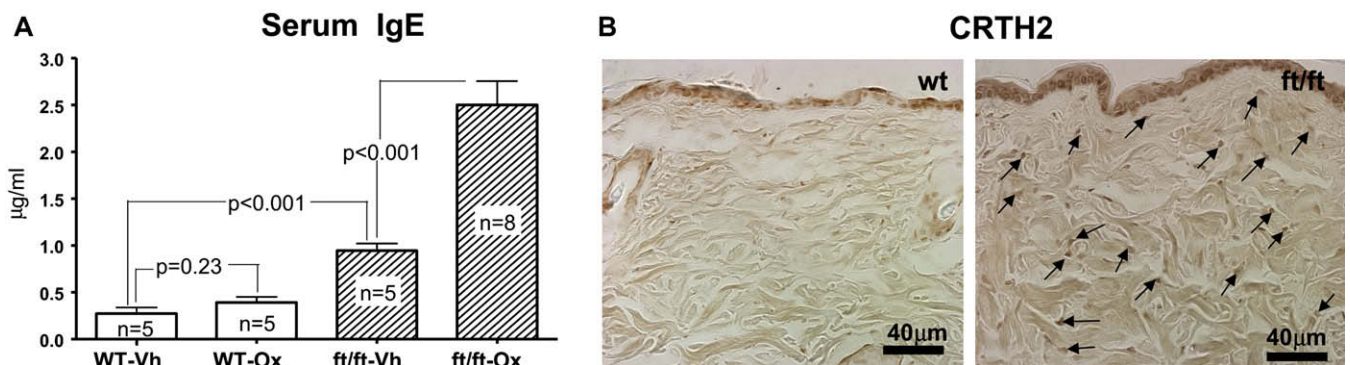


**FIG 7.** Flaky-tail mice demonstrate increased  $T_H2$  responses to repeated low-dose Ox challenges. Serum was collected from young *ft/ft* and wild-type (*WT*) mice before and after Ox sensitization and either 10 low-dose Ox (0.02%) or vehicle treatments, followed by assessment of IgE levels by means of ELISA. Although no difference from baseline was seen between vehicle- and Ox-treated *WT* mice, a large increase was observed in *ft/ft* mice (**B**). Likewise, low-dose Ox-challenged *ft/ft* mice demonstrate increased staining for CRTH2-bearing cells (**A**, arrows) in Ox-treated *ft/ft* mice. Mag bars = 40 µm.

(Fig 3, G), as evidenced by an acceleration of TEWL levels to levels produced by full-challenge hapten doses in wild-type or healthy mice.<sup>49</sup> These results show that development of an AD-like dermatosis in *flg*-deficient mice is paralleled by a further exacerbation of the permeability barrier abnormality.

## DISCUSSION

Human AD is increasingly viewed as a primary barrier disorder based on recent data from multiple populations that have demonstrated an association with mutations in *FLG*, the gene encoding the stratum corneum structural protein filaggrin. Yet although these molecular genetic studies have shifted views of AD pathogenesis toward a new outside-to-inside pathogenic paradigm,<sup>1,2</sup> they leave unanswered mechanistic questions of how *FLG* deficiency leads not only to a barrier abnormality but also to an inflammatory phenotype in AD. Because such questions are difficult to address in the complex multifactorial environment of human disease, we addressed these issues in an animal model of *flg* deficiency, the *ft/ft* mouse. Recently, these mice were shown to carry a frameshift mutation with one base pair deletion

(5303delA) in exon 3 of *flg*<sup>24</sup> that mimics 2 common distal mutations in human AD (ie, R2447X in repeat 7 and 1033del4 in repeat 10).<sup>6,28</sup>

In our examination of *ft/ft* mice, we first confirmed that *flg* deficiency alone suffices to induce a barrier abnormality and low-grade inflammation under basal conditions, as shown recently for ichthyosis vulgaris.<sup>50</sup> We also provided subcellular pathogenic insights showing that despite its intracellular localization, *flg* deficiency results in enhanced paracellular permeability to both intradermally and epicutaneously applied water-soluble tracers, which do not penetrate wild-type stratum corneum. Movement of these low-molecular-weight, water-soluble tracers through the stratum corneum interstices likely reflects the pathway for hapten ingress in AD because similarly sized water-soluble antigens, such as nickel, more frequently induce allergic contact dermatitis in human subjects with AD.<sup>51</sup> However, because our *ft/ft* mice were not on a homogenous C57BL/6 background but also express a hair phenotype (matted), we cannot eliminate the possibility that some of our observations could have been influenced by the concurrent matted mutation. Nevertheless, the recent work by Fallon et al<sup>24</sup> shows enhanced

antigen ingress in mice with the same *flg* mutation but no maturation in their background. Thus *flg* deficiency alone likely explains the barrier abnormality that we demonstrate here in *fl/fl* mice.

Localizing the barrier defect in *FLG* deficiency to the extracellular matrix is an important first step in understanding AD pathogenesis. Yet a key question remains unanswered: How can a defect in an intracellular protein, such as filaggrin, yield a more porous extracellular pathway? *FLG* has 2 putative functions: (1) mediation of stratum corneum hydration through humidity-dependent proteolysis of *FLG* into its constituent amino acids and their deaminated products (eg, histidine → transurocanic acid)<sup>18,52</sup> and (2) keratin intermediate filament compaction within the corneocyte cytosol.<sup>19,53</sup> Examination of either of these roles does not immediately suggest a mechanism by which *FLG* deficiency could lead to increased extracellular permeability. Potential clues are provided by other inherited defects of intracellular corneocyte proteins that also provoke increased paracellular permeability but through divergent mechanisms.<sup>54</sup> In one example in K1/10-deficient epidermolytic hyperkeratosis, dominant-negative pairing of the mutant protein disrupts the cytoskeleton, impairing lamellar body secretion.<sup>32</sup> In a second alternate mechanism, both loricrin keratoderma and transglutaminase 1-deficient lamellar ichthyosis yield an abnormal corneocyte scaffold that disrupts the supramolecular organization of the extracellular lamellar bilayers.<sup>36,37</sup> A third mechanism, mutations in the V1 subdomain of K1 that produce palmar plantar keratoderma, provokes detachment of keratin filaments from the cornified envelope.<sup>55</sup> This defect could link an abnormally collapsed corneocyte to an extracellular defect, yet frozen sections of *flg*-deficient stratum corneum display corneocytes of normal shape and dimensions (Elias, unpublished observations). Our ultrastructural studies on *fl/fl* mice show a partial failure of lamellar body secretion, as shown previously for nongenotyped human AD.<sup>56</sup> Likewise, our preliminary studies in genotyped ichthyosis vulgaris wild-type patients with AD show a similar blockade in lamellar body secretion.<sup>50</sup> Thus analogous to K1/10-deficient epidermolytic hyperkeratosis,<sup>32</sup> unprocessed profilaggrin might impede lamellar body secretion (see Fig E2).<sup>50</sup> This mechanism likely also pertains to human AD associated with distal *FLG* mutations, in which residual profilaggrin expression is seen but processed *FLG* is reduced.<sup>28</sup> Yet it is not clear how this mechanism might apply to those cases of AD caused by more proximal *FLG* mutations, which instead decrease both profilaggrin and filaggrin. A fourth mechanism, which could apply to both categories of *FLG* mutations, could be linked to decreased generation of acidic metabolites of *FLG* (see Fig E6 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).<sup>1,2</sup> Deficiency in these polycarboxylic acids could result in a net increase in the pH of the stratum corneum, which in turn could activate neutral pH-dependent kallikreins, with a variety of negative downstream consequences for the barrier.<sup>1,2</sup> Such a pH-dependent mechanism could also lead to deactivation of 2 key ceramide-generating enzymes: acidic sphingomyelinase and  $\beta$ -glucocerebrosidase (see Fig E6).<sup>57</sup> This mechanism might not, however, be operative in *fl/fl* mice because the surface pH of these mice remains normal, perhaps because of compensatory upregulation of alternate, endogenous acidifying mechanisms,<sup>58</sup> such as solute carrier family 9 (sodium-hydrogen exchanger 1 [slc9a]; old symbol, NHE1),<sup>59</sup> secretory phospholipase A<sub>2</sub>,<sup>60</sup> or both. Nonetheless, surface pH measurements do not always reflect pH alterations within specific

microdomains of the stratum corneum.<sup>59</sup> We did not specifically examine such potential compensatory mechanisms in this study.

Although our studies demonstrate the increased movement of topically applied, water-soluble compounds through the stratum corneum in *fl/fl* mice, the movement of water from inside to outside through the stratum corneum (TEWL) is not markedly altered in young *fl/fl* mice, becoming only slightly increased in older *fl/fl* mice in comparison with that seen in older wild-type mice. The development of a barrier abnormality with aging of *fl/fl* mice could reflect the added stress from a steeper water gradient across the stratum corneum of older animals, reflecting the observed decrease in stratum corneum hydration (Fig 1, C). Alternatively or additionally, it could reflect age-related differences in permeability barrier homeostasis.<sup>44,61</sup> Although the explanation for the discordance in TEWL rates versus xenobiotic penetration is not clear, it could reflect differences in the thresholds for the barriers to water loss versus xenobiotic ingress/egress, respectively. Pertinently, to increase TEWL levels by means of tape stripping, multiple layers of the stratum corneum must be removed.<sup>62</sup> Thus there appears to be substantial redundancy, with only a relatively small amount of stratum corneum required to maintain a normal TEWL. Conversely, the structural basis for inhibiting water movement from inside to outside (ie, TEWL) could differ from the structural basis for inhibiting the egress or ingress of somewhat larger water-soluble compounds. Because there are several possible mechanisms that could explain how *FLG* deficiency leads to a marked abnormality in xenobiotic permeation without altering water loss, additional studies will be required to distinguish among these alternatives.

Having established that *FLG* deficiency results in an extracellular barrier defect, we next addressed a question of key functional significance; namely, does this barrier defect suffice to alter thresholds to the development of inflammation from epicutaneously applied irritants and haptens? Although *FLG* mutations are associated with the inflammatory disease AD, the relationship between *FLG* deficiency and inflammation is obscured by the association of the same *FLG* mutations with the supposedly non-inflammatory disorder of cornification ichthyosis vulgaris. Additional stressors to the barrier could be required to provoke human AD<sup>1,2</sup> because human skin displays an inherently more competent barrier (see below).<sup>63-65</sup> Nevertheless, the presumption that ichthyosis vulgaris is non-inflammatory should now be re-examined in light of our observations and those of Fallon et al,<sup>24</sup> which show that *flg* deficiency alone results in epidermal hyperplasia, low-grade inflammation, and increased IgE levels in otherwise normal mice.

To ascertain whether *FLG* deficiency alone leads to increased susceptibility to inflammatory dermatoses, we used previously described methods for eliciting irritant-induced and acute allergic contact dermatitis,<sup>38,47</sup> as well as an AD-like mouse model,<sup>49</sup> in *fl/fl* versus wild-type mice. Our results show that *FLG* deficiency alone decreases thresholds to inflammation after epicutaneous applications of either irritants or haptens. Most importantly, we demonstrate development of an AD-like phenotype after applications of reduced concentrations of haptens, which do not produce T<sub>H</sub>2-dominant inflammation in wild-type mice. The AD-like phenotype included increased CRTH2<sup>+</sup> cells, decreased immunostaining for mBD3 (the murine homologue of hBD2), and increased serum IgE levels. Similarly, antigen-challenged mice with the same *flg* mutation as *fl/fl* mice also display multiple features of T<sub>H</sub>2 inflammation.<sup>24</sup> Interestingly, these *flg*-deficient

mice reportedly did not experience significantly higher IgE levels,<sup>24</sup> likely reflecting a less severe phenotype caused by differences in bioavailability of our hapten versus the complete antigen used by Fallon et al.<sup>24</sup> Finally, the apparent reduction in mBD3 expression could reflect downstream downregulation by T<sub>H</sub>2 cytokines.<sup>16</sup>

Notably, inflammation can be induced in *flg*-deficient mice without a requirement for additional acquired stressors, such as reduced ambient humidity, high pH surfactants, and/or increased psychologic stress, conditions that are known to both further compromise barrier function and to precipitate AD in human subjects.<sup>1-3</sup> This leaves unanswered, however, the question of why many patients with ichthyosis vulgaris, including some with double-allele *FLG* mutations, do not display a concomitant AD phenotype.<sup>25,26</sup> Two alternative or perhaps coincident explanations could explain this apparent paradox. First, it is possible that all patients with *FLG* mutations have the potential to have AD based on their inherited barrier defect alone, but repeated epicutaneous deposition of allergens sufficient to provoke T<sub>H</sub>2-dominant inflammation might not occur in some patients with ichthyosis vulgaris. Second, in mice, unlike in human subjects, the development of inflammation might not require the superimposition of additional acquired stressors, because as noted above, murine skin displays reduced barrier competence in comparison with human skin.<sup>63-65</sup> This inherent difference could also explain why hapten-induced, acute allergic contact dermatitis converts to an AD-like phenotype after repeated challenges in mice, a transition that is not known to occur in human subjects.

In summary, we show here that *FLG* deficiency, as occurs in many cases of human AD, suffices to provoke a barrier abnormality, which in turn allows enhanced permeation of water-soluble tracers through the paracellular route. The paracellular defect can be attributed to abnormal extracellular lamellar bilayers resulting from compromised lamellar body secretion. These structural abnormalities allow enhanced irritant/hapten ingress, resulting in reduced inflammatory thresholds and providing a mechanistic link between *FLG* deficiency and development of AD. Finally, these studies substantiate and explain the new outside-to-inside paradigm of AD pathogenesis.

We thank Ms Joan Wakefield for superb editorial assistance.

#### Key messages

- *FLG* deficiency alone provokes a permeability barrier abnormality (no further acquired stressors are necessary).
- The barrier abnormality localizes to the extracellular spaces of the stratum corneum, where it facilitates bidirectional, paracellular percutaneous transport of water-soluble xenobiotics.
- The paracellular abnormality can be ascribed to impaired secretion of epidermal lamellar bodies.
- The barrier abnormality correlates with reduced inflammatory thresholds to topical irritants and haptens.
- Repeated low-dose hapten applications that fail to elicit inflammation in wild-type mice provoke inflammation with certain T<sub>H</sub>2 features in *flg*-deficient mice, worsening the barrier abnormality, thereby completing an outside-inside-(back to) outside pathogenic cycle in murine AD.

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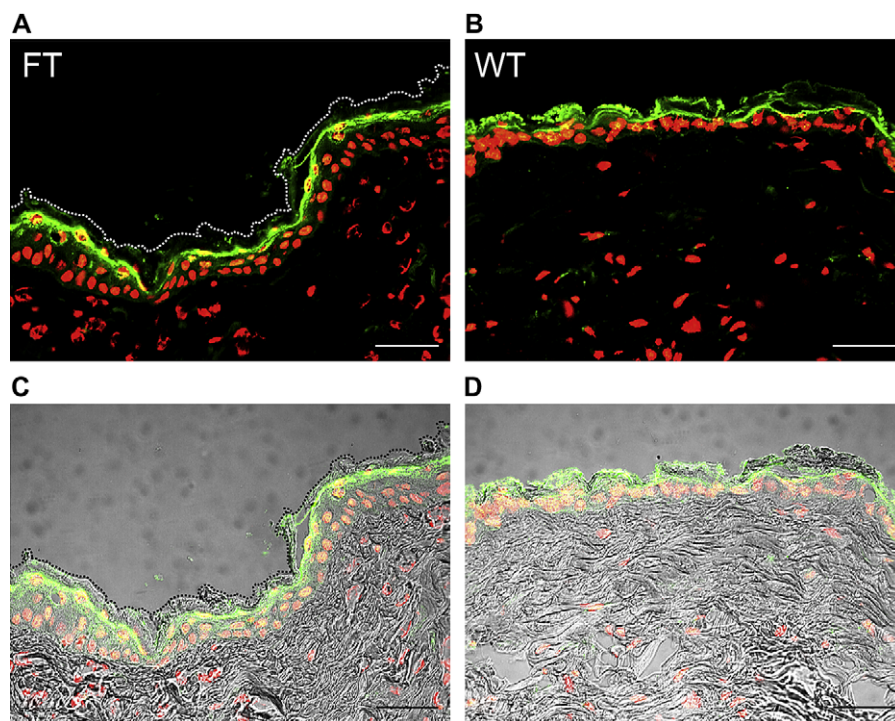


**FIG E1.** Immunostaining for filaggrin is restricted to the granular layer in *ft/ft* (FT) mice. Frozen sections of FT and wild-type (WT) skin were immunostained with a consensus antibody that recognizes both profilaggrin and filaggrin ( $n = 3$  each). **A**, Immunostaining for profilaggrin (green) is restricted to the stratum granulosum in FT mice (dotted line indicates the outer surface of the stratum corneum). **B**, WT skin displays immunostaining for profilaggrin and filaggrin that extends from the stratum granulosum into the stratum corneum. **C** and **D**, phase microscopy of merged images of Fig E1, **A** and **B**, respectively, also show lack of *flg* in the stratum corneum. Propidium iodide was used to counterstain nuclei (red). Mag bars = 40  $\mu$ m.

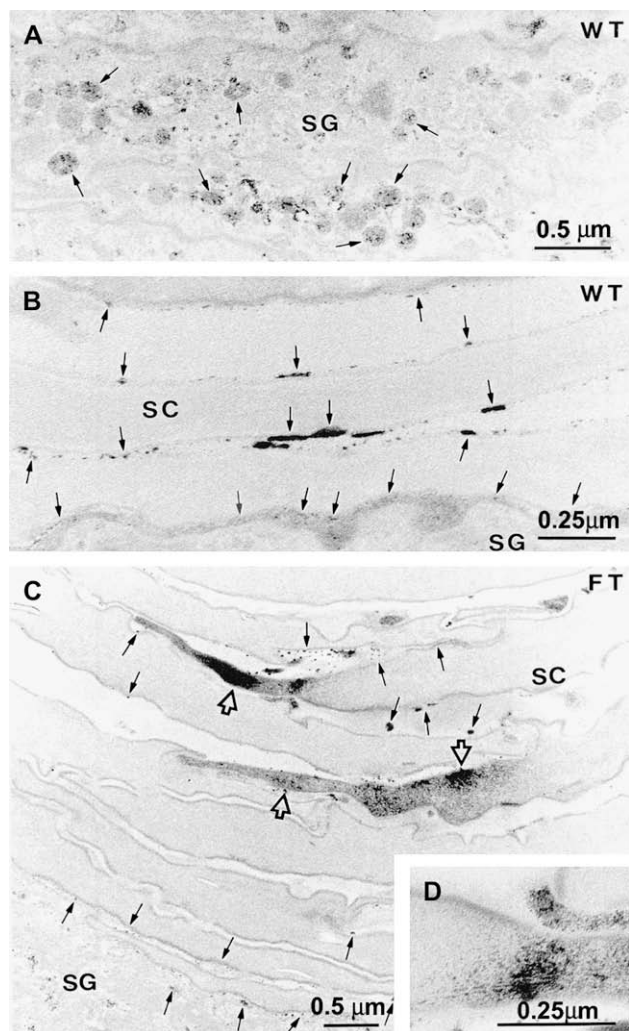


**FIG E2.** Cytochemical demonstration of impaired lamellar body secretion in *ft/ft* (FT) mice. By using an ultrastructural cytochemical marker (ie, acidic lipase), enzyme activity is restricted to lamellar bodies (**A**, arrows) in the stratum granulosum (SG) and to the stratum corneum (SC) interstices (**B**, arrows) in wild-type (WT) mice. In contrast, some enzyme activity is retained within the corneocyte cytosol in FT mice, where it aggregates around retained lamellar membranes (**C** and **D**, arrows,  $n = 3$  each). **Fig E2, A-D**, Osmium tetroxide postfixation. **Mag bars:** Fig E2, A and C = 0.5  $\mu\text{m}$ ; Fig E2, B and D = 0.25  $\mu\text{m}$ .

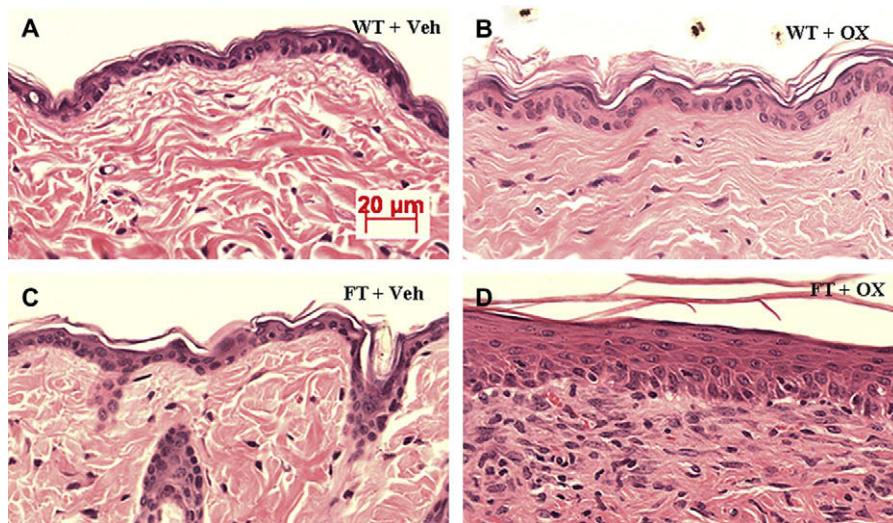


**FIG E3.** Flaky-tail (*FT*) mice display low-grade inflammation at baseline and more severe inflammation after Ox challenges. Wild-type (*WT*) and *FT* mice ( $n = 4$  each) were treated repeatedly with low concentrations of Ox (0.02%). Although *WT* mice show few signs of inflammation (**B** vs **A**), *FT* mice display low-grade inflammation at baseline (**C**) and severe inflammation after repeated Ox challenges (**D**). Quantitative data are in shown in Fig 5, E-G. Fig E3, A-D, Hematoxylin and eosin stain. *Mag bars* = 40 µm. *Veh*, Vehicle.

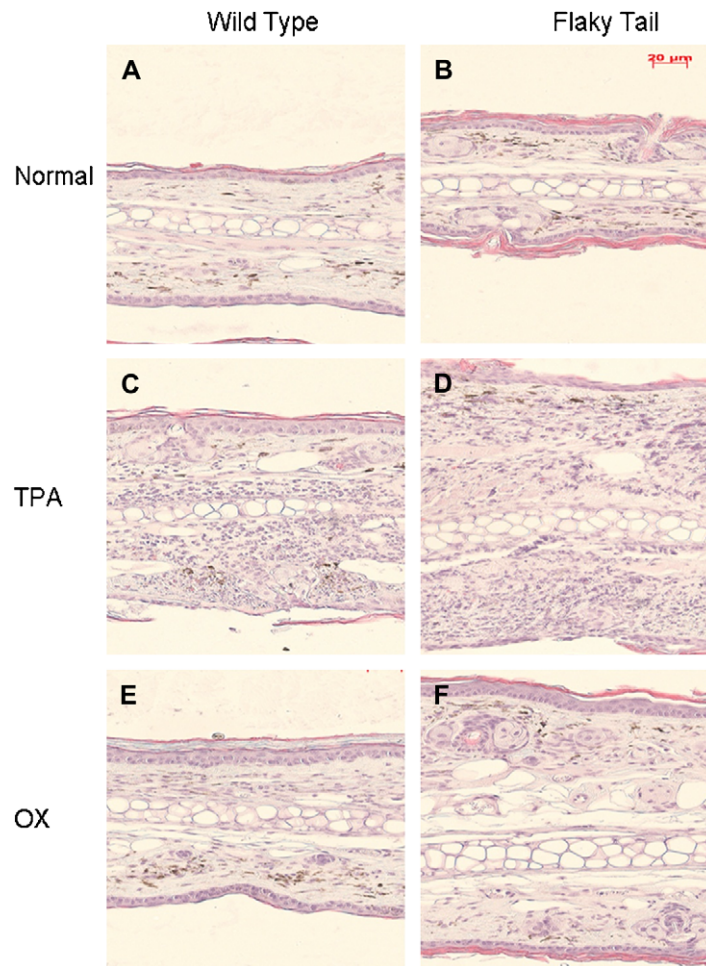


**FIG E4.** Increased sensitivity to both irritant and allergic contact dermatitis in *ft/ft* mice and wild-type mice (baseline [normal] morphology shown in **A** and **B**). In young wild-type mice 0.1% TPA induces a minimal inflammation, as demonstrated by a minimum increase in ear thickness and inflammatory cell infiltration (**C**), whereas ear thickness and inflammatory cell infiltration markedly increase in *ft/ft* mice after 0.1% TPA treatment (**D**). Likewise, 0.02% Ox challenges induce only a minimum increase in ear thickness and inflammation in WT mice (**E**). In contrast, 0.02% Ox challenges cause more severe inflammatory cell infiltration and a significant increase in ear thickness in *ft/ft* mice (**F**). All samples were obtained 2 hours after TPA applications. **Fig E4, A-E**, Hematoxylin and eosin stain. **Mag bars** = 20 μm.

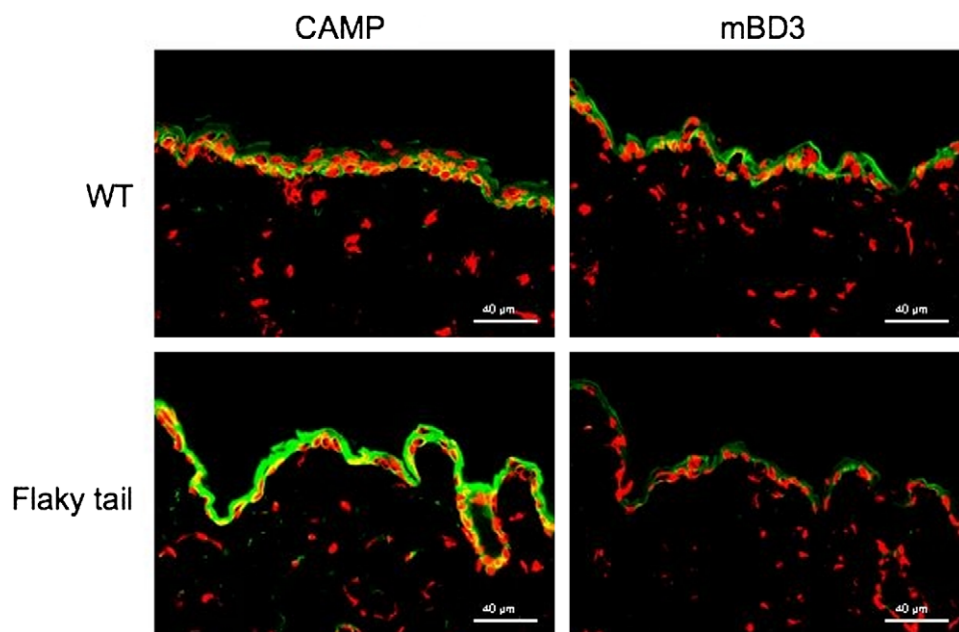


**FIG E5.** Reduced mBD3 expression in *ft/ft* mice repeatedly challenged with low-dose Ox. Young *ft/ft* and wild-type (*WT*) mice were challenged with low-dose Ox (0.02%) or vehicle  $\times$  10 doses over 20 days. mBD3 immunostaining (*green*) appears to be reduced in *ft/ft* mice (**B** vs **A**), but cathelicidin antimicrobial peptide (*CAMP*) levels instead appear to increase (**D** vs **C**). Propidium iodide was used to counterstain nuclei (*red*). Mag bars = 40  $\mu$ m.

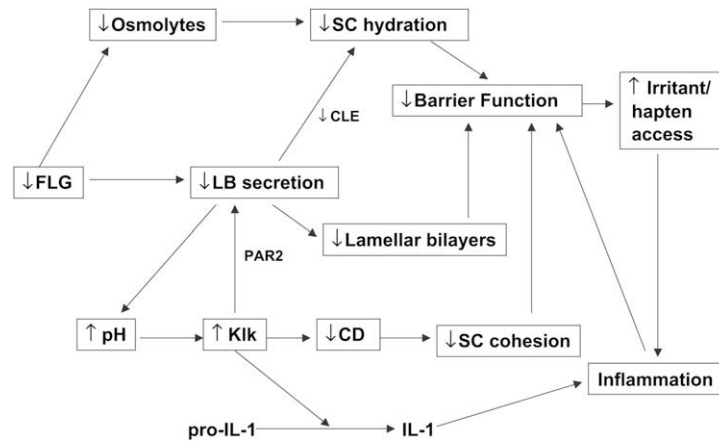


**FIG E6.** Mechanisms whereby *FLG* deficiency could provoke barrier abnormality and inflammation. Decreased filaggrin levels lead to decrease levels of osmolytes, which in turn increase the pH of stratum corneum (SC). Increased pH in turn activates kallikreins (*Kik* and serine proteases), which inhibit lamellar body (LB) secretion and degrade corneodesmosomes (CD), leading to decreased SC integrity. Decreased LB secretion leads to decreased lamellar bilayers and a defective corneocyte lipid envelope (CLE), accounting in part for poor SC hydration.