See related article on pg 782

Tightening the Epidermal Barrier with Atypical PKCs

Mitchell F. Denning[1]

The permeability barrier function of the epidermis is one of the most vital jobs performed by the skin; however, our understanding of the function and regulation of tight junctions in the epidermis remains limited. Helfrich *et al.* identify a key role for atypical protein kinase C (aPKC) activity, as a component of the Par3–Par6 polarity complex, in epidermal barrier function.

Journal of Investigative Dermatology (2007) **127**, 742–744. doi:10.1038/sj.jid.5700694

As the first line of defense against the outside world, the epidermis has an extensive arsenal of adhesive and sealing junctions that maintain physical integrity and act as a permeability barrier against water loss, foreign microbes, and toxins. The assembly of adhesive and sealing junctions is tightly linked to the stepwise epidermal differentiation program, which is also central to the barrier function of the epidermis via formation of the stratum corneum. Although our knowledge of the epidermal adhesive junctions (adherens junctions, desmosomes, hemi-desmosomes) is extensive, the existence and functional importance of the sealing tight junctions in the epidermis have only recently been appreciated (Furuse *et al.*, 2002). Tight junctions are the occluding junctions found in epithelial cells and are composed primarily of claudins, occludin, and ZO proteins. However, the permeability barrier function of the epidermis was historically attributed primarily to the stratum corneum, with its covalently cross-linked proteins and lipids forming a waxy impediment to water movement. Indeed, proper formation of cornified envelopes and extrusion of lamellar bodies are required to form the "bricks and mortar" of the stratum corneum (Elias, 2005). This complex process requires that the highly coordinated keratinocyte terminal differentiation program be properly executed. Support for this concept includes engineered deletions of differentiation proteins in the mouse (transglutaminase I, Klf4), or common inactivating mutations in humans (filaggrin), both of which disrupt the epidermal barrier function (Matsuki *et al.*, 1998; Segre *et al.*, 1999; Smith *et al.*, 2006). However, as is highlighted below, the role of tight junctions in the permeability barrier function in the epidermis warrants equal consideration.

> …an essential role for aPKC … in the establishment of the permeability barrier function in the granular layer.

The article by Helfrich and colleagues (2007, this issue) expands our understanding of tight junctions in the epidermal barrier by characterizing the localization of the polarity proteins Par3, Par6, and atypical protein kinase C (aPKCζ and aPKCι/λ) in mouse skin during wound healing and Ca^{2+}-induced differentiation *in vitro*. The authors find an essential role for aPKC, especially active aPKCι/λ, in the establishment of the permeability barrier function in the granular layer, the site of tight junctions. These findings have important implications for several cutaneous diseases with disrupted barrier function.

The permeability barrier function of simple epithelia is entirely dependent on tight junction integrity and is tightly linked to the formation of a tripartite polarity complex composed of aPKC, Par3, and Par6. Par6 functions as an adaptor protein linking aPKC and Cdc42 to Par3, and Par3 serves as a scaffolding protein recruiting the polarity complex to sites of tight junction assembly. The tight junctions in simple epithelial tissues perform two functions: (1) a barrier function to regulate paracellular transport, and (2) a fence function to divide or polarize basolateral from apical membrane resident proteins and lipids. In the epidermis, however, the polarization of junctional components is on a tissue level rather than on a cellular level, with tight junctions localized to the granular layer just beneath the stratum corneum. Strong evidence for the requirement for tight junctions in epidermal barrier function initially came from claudin-1-null mice (Furuse *et al.*, 2002). These mice died shortly after birth and had impaired epidermal barrier function despite biochemical and ultrastructurally normal stratum corneum.

The study by Helfrich *et al.* (2007) found that in newborn mouse skin, aPKCι/λ partially colocalized with Par3 and Par6 in the granular layer at sites of cell–cell contact. Furthermore, during Ca^{2+}-induced differentiation of keratinocytes *in vitro*, aPKC activity was necessary for the establishment of barrier function, and overexpression of aPKC accelerated and enhanced barrier function. Two highly homologous aPKC isoforms are expressed in the epidermis, aPKCζ and aPKCι/λ. As aPKCζ was not found in the granular layer, it was not directly implicated in normal epidermal barrier function; however, aPKCζ could compensate for aPKCι/λ function when

[1]*Loyola University Chicago, Cardinal Bernardin Cancer Center, Chicago, Illinois, USA*

Correspondence: Dr. Mitchell F. Denning, Loyola University Chicago, Cardinal Bernardin Cancer Center, 2160 S. First Avenue, Maywood, Illinois 60153, USA. E-mail: mdennin@lumc.edu

overexpressed *in vitro*. Consistent with a critical physiological role for aPKCι/λ, mice null for aPKCι/λ are embryonic lethal and have cell-polarity defects in ectodermal epithelia, whereas aPKCζ-null mice are viable despite having defects in NF-κB activation (Suzuki *et al.*, 2003; Leitges *et al.*, 2001). Taken together, these results indicate that aPKCζ and aPKCι/λ are capable of compensating for each other in polarity-associated barrier function but have different expression patterns and thus may perform separate functions *in vivo*.

Atypical PKCs were also found to be redistributed during wound healing, a process that disrupts cell polarity and barrier function. Atypical PKCs were lost from the granular layer junctional complexes 5–7 days after wounding, and although Par3 did not redistribute, Par6 became diffusely localized in the cytoplasm. By day 5, Par6 returned to the granular layer. One intriguing feature of these localization studies was the strong nuclear and basement membrane zone staining seen for aPKC in newborn mouse skin and in the epidermis during re-epithelialization. This pattern was not seen with isoform-specific antibodies against either aPKCζ or aPKCι/λ, and this different localization may be due to conformation-specific epitopes or epitope masking. This variability in antibody staining is a major impediment to PKC localization studies and illustrates that caution is needed in interpreting PKC localization in tissues and cells. Apical localization of aPKCζ in basal keratinocytes of mouse epidermis was also described as part of the polarity complex regulating asymmetric cell division, suggesting that aPKCζ may be an endogenous regulator of cell polarity in basal keratinocytes (Lechler and Fuchs, 2005).

The authors also developed a novel assay for transepithelial resistance that measures permeability barrier function during Ca^{2+}-induced keratinocyte differentiation *in vitro*. This system was validated by demonstration of recruitment of the tight junction protein ZO-1 and occludin, along with active aPKC, to sites of cell–cell contact, as well as a time-dependent rise in transepithelial resistance. This technical advance should find utility in a wide range of mutant keratinocytes with altered barrier function, especially in mouse models where embryonic or early postnatal lethality occurs, and in human diseases where barrier disruption is prominent (ichthyoses, psoriasis, atopic dermatitis). Keratinocytes can be cultured from patients or mice and induced to differentiate with Ca^{2+}, and the molecular features of the barrier defect studied in detail, with the use of this assay.

In addition to addressing important questions about the role of the cell-polarity proteins Par3, Par6, and aPKC in epidermal barrier function, this study raises several new questions. Central among these is how aPKC activity promotes barrier function. Because aPKC enzyme activity was required for barrier function, the real question becomes: what are the important aPKC substrates that regulate tight junction function? In general, PKCs have broad substrate specificity when assayed in solution; however, cofactor availability and binding/scaffolding proteins tightly regulate PKC localization in cells and therefore restrict substrate availability. In this regard, Par6 may help localize aPKC to junctional complexes where yet-to-be-identified junctional proteins can be phosphorylated. Although the inhibition of aPKC activity prevented the establishment of barrier function, it did not affect the recruitment of occludin to cell–cell contacts; this suggests that aPKC activity is required for the higher-order assembly and sealing functions of tight junctions. Indeed, only active aPKC extensively colocalized with ZO-1; total aPKC colocalization was much more limited. The identification of physiological aPKC substrates is a high-priority area in need of technical advances to move the field forward.

The link between epidermal differentiation and barrier function may help explain the activation mechanism of aPKC in the granular layer. PKCζ and PKCι/λ are "atypical" in that they are not activated by the canonical diacylglycerol or Ca^{2+} second messengers that activate classical and novel PKCs. Atypical PKCs are activated by a variety of other lipids, including ceramides and cholesterol sulfate, that are generated during granular layer differentiation and are essential for the permeability barrier function (Lozano *et al.*, 1994; Elias, 2005; Denning *et al.*, 1995). Thus the generation of these aPKC-activating ligands as a component of the epidermal differentiation program may integrate aPKCι/λ activation with tight junction function in the granular layer. A complementary role of stratum corneum lipids in epidermal barrier may be an aPKC signaling function in addition to the well-described hydrophobic function as "mortar" (Elias, 2005).

Finally, the study by Helfrich *et al.* (2007) has implications for several skin diseases characterized by deregulated epidermal permeability barrier. Common skin diseases such as psoriasis, ichthyosis vulgaris, atopic dermatitis, and contact dermatitis are all characterized by defects in epidermal permeability barrier. Although the permeability defect in these diseases may be in part due to disruption of the normal stratum corneum formation, perturbations in epidermal polarity and tight junction function should also be considered. In fact, the close association between two functions of epidermal differentiation in the stratum granulosum — generation of functional gap junctions and preparation of keratinocytes for the formation of functional stratum corneum — makes these two barrier mechanisms functionally interrelated and challenging to dissect.

CONFLICT OF INTEREST

The author states no conflict of interest.

REFERENCES

Denning MF, Kazanietz MG, Blumberg PM, Yuspa SH (1995) Cholesterol sulfate activates multiple protein kinase C isoenzymes and induces granular cell differentiation in cultured murine keratinocytes. *Cell Growth Differ* 6:1619–26

Elias PM (2005) Stratum corneum defensive functions: an integrated view. *J Invest Dermatol* 125:183–200

Furuse M, Hata M, Furuse K, Yoshida Y, Haratake A, Sugitani Y *et al.* (2002) Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J Cell Biol* 156:1099–111

Helfrich I, Schmitz A, Zigrino P, Michels C, Haase I, le Bivic A *et al.* (2007) Role of aPKC isoforms and their binding partners Par3 and Par6 in epidermal barrier formation. *J Invest Dermatol* 127:782–791

Lechler T, Fuchs E (2005) Asymmetric cell divisions promote stratification and differentiation of mammalian skin. *Nature* 437:275–80

Leitges M, Sanz L, Martin P, Duran A, Braun U, Garcia JF *et al.* (2001) Targeted disruption of the ζPKC gene results in the impairment of the NF-κB pathway. *Mol Cell* 8:771–80

Lozano J, Berra E, Municio MM, Diaz-Meco MT, Dominguez I, Sanz L *et al.* (1994) Protein kinase C ζ isoform is critical for κB-dependent promoter activation by sphingomyelinase. *J Biol Chem* 269:19200–2

Matsuki M, Yamashita F, Ishida-Yamamoto A, Yamada K, Kinoshita C, Fushiki S *et al.* (1998) Defective stratum corneum and early neonatal death in mice lacking the gene for transglutaminase 1 (keratinocyte transglutaminase). *Proc Natl Acad Sci USA* 95:1044–9

Segre JA, Bauer C, Fuchs E (1999) Klf4 is a transcription factor required for establishing the barrier function of the skin. *Nat Genet* 22:356–60

Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y *et al.* (2006) Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 38:337–42

Suzuki A, Akimoto K, Ohno S (2003) Protein kinase C λ/ι (PKCλ/ι): a PKC isotype essential for the development of multicellular organisms. *J Biochem (Tokyo)* 133:9–16

See related article on pg 802

Soluble Fas Ligand: Is It a Critical Mediator of Toxic Epidermal Necrolysis and Stevens–Johnson Syndrome?

Junko Murata[1] and Riichiro Abe[1]

Although soluble Fas ligand (sFasL) is an important candidate in toxic epidermal necrolysis (TEN) and Stevens–Johnson syndrome (SJS), Stur and colleagues report that elevated sFasL has been detected in maculopapular rashes. In addition to sFasL, other factors, including predisposing genetic factors, should also be investigated to determine their precise pathogenesis in TEN and SJS.

Journal of Investigative Dermatology (2007) **127**, 744–745. doi:10.1038/sj.jid.5700693

Toxic epidermal necrolysis (TEN) and Stevens–Johnson syndrome (SJS) are among the most severe cutaneous adverse reactions seen in humans. TEN and SJS are life-threatening diseases with a mortality rate approaching 25%. The main causes of these diseases are drug intake and, to a lesser extent, viral infection. In TEN and SJS patients' epidermis, marked keratinocyte apoptotic events are frequently observed. This keratinocyte apoptosis results in blister formation and widespread skin detachment. In contrast to TEN and SJS, maculopapular rashes (MPRs) are milder variants of cutaneous reactions also due to drug allergy or viral infection. Occasional apoptotic keratinocytes are sometimes observed histologically in MPR epidermis.

So far, numerous possible mediators of keratinocyte apoptosis have been suggested, such as peripheral cytotoxic T cells, inflammatory cytokines, nitric oxide, granzyme B, and perforin. For example, Nassif *et al.* (2002) showed that T lymphocytes present within TEN lesions may exhibit drug-specific cytotoxicity against autologous cells without restimulation. However, although TEN and SJS lesional skin contained few inflammatory cells, there were no other convincing ways to explain how keratinocyte apoptosis might occur. In addition, tumor necrosis factor-α, a potent apoptotic mediator, has been suggested as a critical factor in TEN and SJS (Paquet *et al.*, 1994). As increases in serum levels of tumor necrosis factor-α have been observed in various inflammatory diseases, it is unlikely that tumor necrosis factor-α alone is a specific mediator in TEN and SJS.

In 1998, Viard *et al.* (1998) reported that the activation of Fas through Fas ligand (FasL) is an important primary step leading to keratinocyte apoptosis in TEN. The generally held concept is that Fas and FasL are derived from keratinocytes, and that FasL expressed by keratinocytes causes keratinocyte apoptosis in TEN in either an autocrine or a paracrine fashion (Viard *et al.*, 1998).

Conversely, we demonstrated that the levels of soluble FasL (sFasL) in patients' sera were elevated in an initial phase and significantly declined between 3 and 6 days after the start of the disease course. We also showed that sFasL secretion from peripheral blood mononuclear cells could be induced after the causative drug stimulation *in vitro* (Abe *et al.*, 2003).

Clinically, there are some difficulties in diagnosing TEN and SJS. Because patients sometimes show only maculopapular eruptions without any mucosal involvement in the early stage of disease, it is quite difficult to distinguish TEN and SJS from MPR. It is also difficult to determine from a patient's clinical appearance and laboratory data alone whether the disease is due to drug intake or viral infection.

Stur and colleagues (2007, this issue) show that sFasL levels were elevated in sera from SJS patients but were low in TEN patients. In addition to this result, they detected high sFasL levels in sera from MPR patients. In particular, sFasL serum levels were elevated in drug-induced MPR; however, no significant increase was

[1]*Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan*

Correspondence: Dr. Riichiro Abe, Department of Dermatology, Hokkaido University Graduate School of Medicine, N 15 W 7, Kita-ku, Sapporo 060-8638, Japan. E-mail: aberi@med.hokudai.ac.jp