

Keratin intermediate filament proteins – novel regulators of inflammation and immunity in skin

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Inflammation is a complex biological response, protecting the host and promoting homeostasis following mechanical, chemical or microbial insults. Epithelia are strategically located at the interface between the external and internal milieu where, collectively, their ability to establish a specialized and dynamic cytoarchitecture, to express an impressive array of relevant receptors and their associated signal transduction pathways, and to signal to resident and migrant cellular effectors of inflammation and immunity allows them to actively participate in the inception and subsequent regulation of inflammatory and immune responses (Segre, 2006; Swamy et al., 2010). The pace of progress in identifying new effectors and mechanisms in epithelial-based immunity has been fast and furious in recent years (Nestle et al., 2009; Swamy et al., 2010) in part as a result of a growing effort to understand the pathophysiology of relevant diseases and to foster the development of novel therapeutic strategies. In this issue, Roth et al. present evidence that adds to the emerging role for keratin cytoskeletal proteins in the regulation of epidermal immunity and skin barrier function (see this issue of *J. Cell. Sci.*, Roth et al. 5269–5279).

Keratins belong to the large family of intermediate filament (IF)-forming proteins. Two distinct subtypes of keratin, the acidic type I ($n=28$) and basic type II ($n=26$) keratins (Schweizer et al., 2006), assemble in a pairwise and obligatory fashion to form 10-nm-wide filaments. Keratin filaments are typically organized

into intricate networks integrated at sites of cell–cell and cell–matrix adhesion, as well as at the surface of the nucleus in epithelial cells (Coulombe and Lee, 2012). The differentiation-related and context-dependent regulation of keratin genes and proteins in epithelia is an evolutionarily conserved, defining property of these unique cytoskeletal elements. Genetic and biochemical evidence supports two main roles for keratins and other IF proteins: first, a general and context-independent role of providing mechanical support, the abrogation of which leads to fragile cells and accounts for a broad range of diseases and, second, the regulation of basic metabolic processes (e.g. cell survival, growth and death) in a context-specific manner that reflects interactions between IF proteins and key effectors of signaling pathways (Coulombe and Lee, 2012; Omary et al., 2004). The perturbation of such non-mechanical functions is likely to have a significant role in the modification of pathogenic traits in several types of human disease.

Roth et al. provide evidence for a new and unanticipated role of keratin I (Krt1) – the main differentiation-specific type II keratin expressed in the epidermis – in regulating innate immunity in the skin (Roth et al., 2012). Similar to mice that lack Krt10 – the type I partner of Krt1 (Reichelt and Magin, 2002) – *Krt1*-null mice do not show obvious signs of cell fragility in the suprabasal, differentiating layers of epidermis. However, unlike mice that are deficient in *Krt10*, *Krt1*-null mice exhibit a spectacular inflammatory disease phenotype with skin-specific and systemic components, further substantiating the now solid trend that the two members of a keratin filament can have distinct biological roles. Although the epidermis of newborn *Krt1*-null mice is intact and appears histologically normal at birth, the inside–out barrier function is grossly defective with a severe reduction of intact cornified envelopes, reflecting aberrant terminal differentiation and leading to completely penetrant neonatal lethality. Unexpectedly, the skin of unchallenged *Krt1*-null mice exhibits a perinatal upregulation of numerous pro-inflammatory and innate immunity-associated cytokine mRNA transcripts compared with normal skin. Additionally, the serum levels of the IL-1 superfamily proinflammatory cytokine IL-18 are significantly upregulated in *Krt1*-null mice, suggesting a role for Krt1 in regulating both local and systemic

inflammation. The mortality and diminution of cornified envelopes associated with the *Krt1*-null phenotype can both be rescued, in part, by pharmacologically or genetically blunting the expression of IL-18. Roth et al. go on to show that Krt1 participates in the regulation of IL-18 production and release in a keratinocyte-specific and inflammasome-dependent manner (Fig. 1).

Despite the lack of cell lysis, the compromised epidermal barrier and occasional presence of skin lesions in *Krt1*-null mice is reminiscent of epidermolytic hyperkeratosis (a disorder also known as bullous congenital ichthyosiform erythroderma), which arises in individuals who carry single, dominant-acting missense alleles in *KRT1* or *KRT10* (Omary et al., 2004). In addition, the authors draw parallels between the pro-inflammatory transcription profile observed in *Krt1*-null epidermis and that seen in atopic dermatitis (eczema) or psoriatic human skin, which both are characterized by a reduced expression of KRT1 and the concomitant upregulation of the wound-inducible keratins KRT6, KRT16 and KRT17 (Freedberg et al., 2001; Segre, 2006).

The study by Roth et al. in this issue adds significantly to a growing body of evidence highlighting a role in immunomodulation for select keratins in various epithelial contexts. In the simple epithelial lining of the mouse gut, for instance, loss of *Krt8* causes hyperplasia and colitis with increased T-cell recruitment to the colon and upregulation of T-helper (Th) 2 cytokines (Habtezion et al., 2005). Targeted deletion of *Krt5* in mouse leads to acute epidermal fragility along with massive, lethal skin blistering immediately after birth. This phenotype is remarkably similar to what occurs in epidermolysis bullosa simplex (EBS), a genetic disorder that results from function-abrogating mutations in either *KRT5* or its partner *KRT14* (Coulombe and Lee, 2012). Prior to birth, the skin of late-stage mouse *Krt5*-null embryos is mechanically sound *in utero*, but already shows elevated expression of the pro-inflammatory cytokines IL-6 and IL-1 β (Lu et al., 2007) and Ccl2 and Ccl20, correlating with an increased density of antigen-presenting Langerhans dendritic cells in their epidermis (Roth et al., 2009). Coincidentally, individuals that suffer from EBS as a result of mutations at the *KRT5* locus show an increased density of

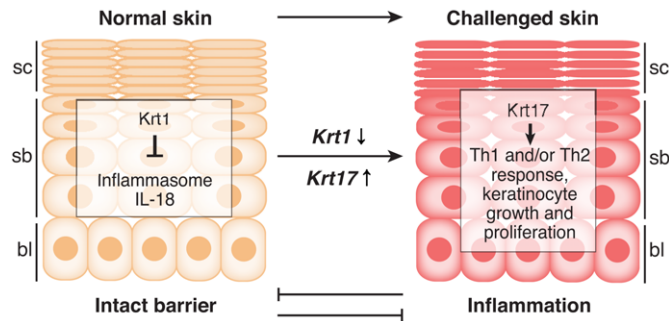


Fig. 1. Krt1 and Krt17 regulate epidermal immunity in a distinct and context-dependent manner.

Normal epidermis expresses *Krt1* in the differentiating suprabasal layers, where it contributes to skin barrier homeostasis, in part by preventing the activation of the inflammasome and the processing of IL-18 (Roth et al., 2012). Upon an acute or chronic challenge to the epidermal barrier (e.g. injury, environmental irritants or cancer), several genes, including *Krt17*, are robustly induced in basal and suprabasal keratinocytes at the expense of *Krt1* (and other differentiation-specific genes). *Krt17* actively modulates intracellular signaling pathways to stimulate protein synthesis (Kim et al., 2006) and to also polarize the inflammatory response towards Th1 and/or Th17 (DePianto et al., 2010), thereby promoting keratinocyte growth and proliferation. Inflammation and normal barrier function thus can antagonize one another. bl, basal layer; sb, suprabasal layers; sc, stratum corneum (barrier proper).

Langerhans cells in the epidermis, a phenomenon that is not seen in EBS cases that are associated with *KRT14* mutations or in *Krt14*-null mice (Roth et al., 2009). Finally, the genetic loss of *Krt17* results in a delay in the inception of basaloid tumors in *Gli2* transgenic mouse skin, which correlates with reduced tumor keratinocyte proliferation and a global cytokine switch, from what should be a Th1- and/or Th17-dominated to a Th2-dominated immune profile, in skin tumors (DePianto et al., 2010) (Fig. 1). In particular, pro-inflammatory cytokines such as Cxcl5, Cxcl9 and Cxcl11, which are produced by and mitogenic for keratinocytes and are otherwise known to have a role in the pathogenesis of basal cell carcinoma in humans (Lo et al., 2010), exhibit a *Krt17*-dependent and keratinocyte-autonomous regulation in mouse skin keratinocytes (DePianto et al., 2010).

Further validation for the role of select keratin proteins in the regulation of skin barrier function and inflammation originated from a systems genetics analysis of cancer susceptibility in mouse skin. Quigley et al. (Quigley et al., 2009) found that *Krt1*, *Krt6b* (which is co-regulated with *Krt17* in several settings), the obscure *Krt78* and, to a lesser extent, *Krt6a* and *Krt16*, are all part of a gene and linkage network that integrates barrier function, inflammation and susceptibility to two-step chemical carcinogenesis in mouse (of note, glabrous skin of *Krt16*-null mice exhibit a

compromised barrier function with minimal cell lysis) (Lessard and Coulombe, 2012). The absence of *Krt5* and *Krt17* from this 'barrier-inflammation-cancer' network is likely to be a reflection of their strong constitutive expression in skin tissue sites that have been analyzed in the study by Quigley and colleagues (Quigley et al., 2009). Additional members of this network, including *S100a8* and *Defb3*, are also markedly upregulated in *Krt1*-null skin.

Collectively, these studies open a new chapter in the rapidly evolving inventory of keratin function in skin epithelia: the regulation of inflammatory- and immune-based processes. The mechanistic relationship(s) between these various keratins and inflammation are likely to be complex. For instance, *Krt1* and *Krt17* have, broadly speaking, opposing roles in promoting inflammation in the epidermis, and tend to be regulated in opposite directions (with expression of *Krt17* up and *Krt1* down) in the context of psoriasis, non-melanoma skin cancer and related disease processes (Freedberg et al., 2001) (Fig. 1). Going forward, the mechanisms that govern the newly defined keratin-barrier-inflammation-disease axis need to be deciphered to achieve a deeper understanding of how keratins and the immune system collaborate at the biochemical, cellular and systemic levels towards the promotion of normal tissue homeostasis or of pathogenic processes.

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References

- Coulombe, P. A. and Lee, C. H. (2012). Defining keratin protein function in skin epithelia: epidermolysis bullosa simplex and its aftermath. *J. Invest. Dermatol.* **132**, 763-775.
- DePianto, D., Kerns, M. L., Dlugosz, A. A. and Coulombe, P. A. (2010). Keratin 17 promotes epithelial proliferation and tumor growth by polarizing the immune response in skin. *Nat. Genet.* **42**, 910-914.
- Freedberg, I. M., Tomic-Canic, M., Komine, M. and Blumenberg, M. (2001). Keratins and the keratinocyte activation cycle. *J. Invest. Dermatol.* **116**, 633-640.
- Habtezion, A., Toivola, D. M., Butcher, E. C. and Omary, M. B. (2005). Keratin-8-deficient mice develop chronic spontaneous Th2 colitis amenable to antibiotic treatment. *J. Cell Sci.* **118**, 1971-1980.
- Kim, S., Wong, P. and Coulombe, P. A. (2006). A keratin cytoskeletal protein regulates protein synthesis and epithelial cell growth. *Nature* **441**, 362-365.
- Lessard, J. C. and Coulombe, P. A. (2012). Keratin 16-null mice develop palmoplantar keratoderma, a hallmark feature of pachyonychia congenita and related disorders. *J. Invest. Dermatol.* **132**, 1384-1391.
- Lo, B. K., Yu, M., Zloty, D., Cowan, B., Shapiro, J. and McElwee, K. J. (2010). CXCR3/ligands are significantly involved in the tumorigenesis of basal cell carcinomas. *Am. J. Pathol.* **176**, 2435-2446.
- Lu, H., Chen, J., Planko, L., Zigrino, P., Klein-Hitpass, L. and Magin, T. M. (2007). Induction of inflammatory cytokines by a keratin mutation and their repression by a small molecule in a mouse model for EBS. *J. Invest. Dermatol.* **127**, 2781-2789.
- Nestle, F. O., Di Meglio, P., Qin, J. Z. and Nickoloff, B. J. (2009). Skin immune sentinels in health and disease. *Nat. Rev. Immunol.* **9**, 679-691.
- Omary, M. B., Coulombe, P. A. and McLean, W. H. (2004). Intermediate filament proteins and their associated diseases. *N. Engl. J. Med.* **351**, 2087-2100.
- Quigley, D. A., To, M. D., Pérez-Losada, J., Pelorosso, F. G., Mao, J. H., Nagase, H., Ginzinger, D. G. and Balmain, A. (2009). Genetic architecture of mouse skin inflammation and tumour susceptibility. *Nature* **458**, 505-508.
- Reichelt, J. and Magin, T. M. (2002). Hyperproliferation, induction of c-Myc and 14-3-3sigma, but no cell fragility in keratin-10-null mice. *J. Cell Sci.* **115**, 2639-2650.
- Roth, W., Reuter, U., Wohlenberg, C., Bruckner-Tuderman, L. and Magin, T. M. (2009). Cytokines as genetic modifiers in *K5*^{-/-} mice and in human epidermolysis bullosa simplex. *Hum. Mutat.* **30**, 832-841.
- Roth, W., Kumar, V., Beer, H.-D., Richter, M., Wohlenberg, C., Reuter, U., Thiering, S., Staratschek-Jox, A., Hofmann, A., Kreusch, et al. (2012). Keratin 1 maintains skin integrity and participates in an inflammatory network in skin via interleukin-18. *J. Cell Sci.* **125**, 5269-5279.
- Schweizer, J., Bowden, P. E., Coulombe, P. A., Langbein, L., Lane, E. B., Magin, T. M., Maltais, L., Omary, M. B., Parry, D. A., Rogers, M. A. et al. (2006). New consensus nomenclature for mammalian keratins. *J. Cell Biol.* **174**, 169-174.
- Segre, J. A. (2006). Epidermal barrier formation and recovery in skin disorders. *J. Clin. Invest.* **116**, 1150-1158.
- Swamy, M., Jamora, C., Havran, W. and Hayday, A. (2010). Epithelial decision makers: in search of the 'epimunome'. *Nat. Immunol.* **11**, 656-665.