



The skin barrier, atopic dermatitis and allergy: a role for Langerhans cells?

Robin E. Callard¹ and John I. Harper^{1,2}

¹Immunobiology Unit, Institute of Child Health, University College London, London, UK, WC1N 1EH

²Dermatology Department, Great Ormond Street Hospital for Children NHS Trust, London, UK, WC1N 3JH

The skin barrier (stratum corneum) is a major factor for determining the nature of immune response to antigens presented at the skin surface. Genetic abnormalities in skin barrier function are associated with allergy and atopic dermatitis, and removal of the skin barrier by tape stripping results in dominant Th2 responses to protein antigens. Langerhans cells take up antigen applied to skin from which the skin barrier has been removed; they then migrate to draining lymph nodes and initiate typical Th2 responses. These observations lead us to propose that the high frequency of allergic disease in the industrialized world might be due to lifestyle choices that result in loss of integrity of the skin barrier such as excessive washing and exfoliation of the skin.

The skin barrier and Langerhans cells

The skin is a highly specialized structure that protects the host from harmful environmental toxins and infections. It has an outer epidermal layer of keratinocytes separated from the dermis by a basement membrane zone (BMZ) (Figure 1). Keratinocytes that make up the epidermis occur in stratified layers representing different stages of differentiation. The inner basal layer consists of stem cells adjacent to the BMZ. As the keratinocytes move upwards, they form the spinous layer, and then the granular layer or stratum granulosum. At the skin surface, keratinocytes form apoptotic structures enshrouded by a cornified envelope to become the stratum corneum. This outer layer or skin barrier provides mechanical strength, regulates water and salt loss and protects against exposure to harmful agents in the environment such as toxins and infectious microorganisms [1]. Formation of the stratum corneum involves layer-specific expression of keratins, production of the cornified cell envelope, extrusion of Odland bodies (vacuoles derived from Golgi bodies that store precursors of skin-specific lipids in the form of lamellar bilayer-lipid membranes) and formation of lipid lamellae [2]. Interspersed among the keratinocytes in the epithelium are specialized antigen-presenting Langerhans cells (LCs). These are members of the wider family of dendritic cells (DCs) but are phenotypically and functionally distinct from dermal DCs that reside in the dermis [3]. LCs form a contiguous network in the epidermis, where they serve as sentinels of the immune system by sampling antigens

that get through the stratum corneum and respond to signals, such as inflammatory cytokines, produced by the surrounding keratinocytes. They can then relay antigenic information to the adaptive immune system by migrating from the epidermis to draining lymph nodes (LNs). Interestingly, they occupy different sites in the draining LNs to dermal DCs [4] and there is recent evidence that Langerhans cells might not be antigen-presenting cells for immune responses to certain infectious agents [5].

Loss of skin barrier function is associated with allergy

There is increasing evidence that loss of skin barrier integrity is an important factor in the development of allergy. Netherton syndrome (NS) is a rare, autosomal recessive disorder characterized by a generalized inflammatory skin condition, a specific hair shaft abnormality called trichorrhexis invaginata and, invariably, atopic manifestations. NS is caused by loss-of-function mutations in *SPINK5* located on chromosome 5q31, which encodes the protein lympho-epithelial Kazal-type-related inhibitor (LEKTI) [6,7]. NS patients and mice with defective LEKTI expression have increased serine protease activity in the skin, particularly stratum corneum trypsin/chymotrypsin-like enzyme (SCTE/SCCE), with a concomitant reduced expression of desmosome proteins, particularly desmoglein I [8–10]. As a consequence, there is loss of stratum corneum cohesion affecting skin barrier function. NS shares several clinical features with atopic dermatitis (AD), a common chronic allergic skin disease characterized by dry, scaly skin, inflammation, increased trans-epidermal water loss, increased skin permeability to topical medications and susceptibility to allergy. About 60% of children with severe AD also have asthma and ~35% have allergic rhinitis (reviewed in Refs [11–13]). A G1258A polymorphism in *SPINK5* resulting in a Glu420Lys substitution in LEKTI was found to be highly associated with atopy and atopic dermatitis [14], and this polymorphism has also been found to disrupt normal expression of skin barrier proteins (Wei-Li Di, unpublished data). Furthermore, the G1258A *SPINK5* polymorphism has been reported to be associated with disease severity and food allergy in children with AD [15]. These findings establish a strong link between LEKTI, skin barrier function and atopy.

Several other genetic studies have also strongly implicated skin barrier function in AD. Genetic linkage

Corresponding author: Callard, R.E. (rcallard@ich.ucl.ac.uk).
Available online xxxxxx.

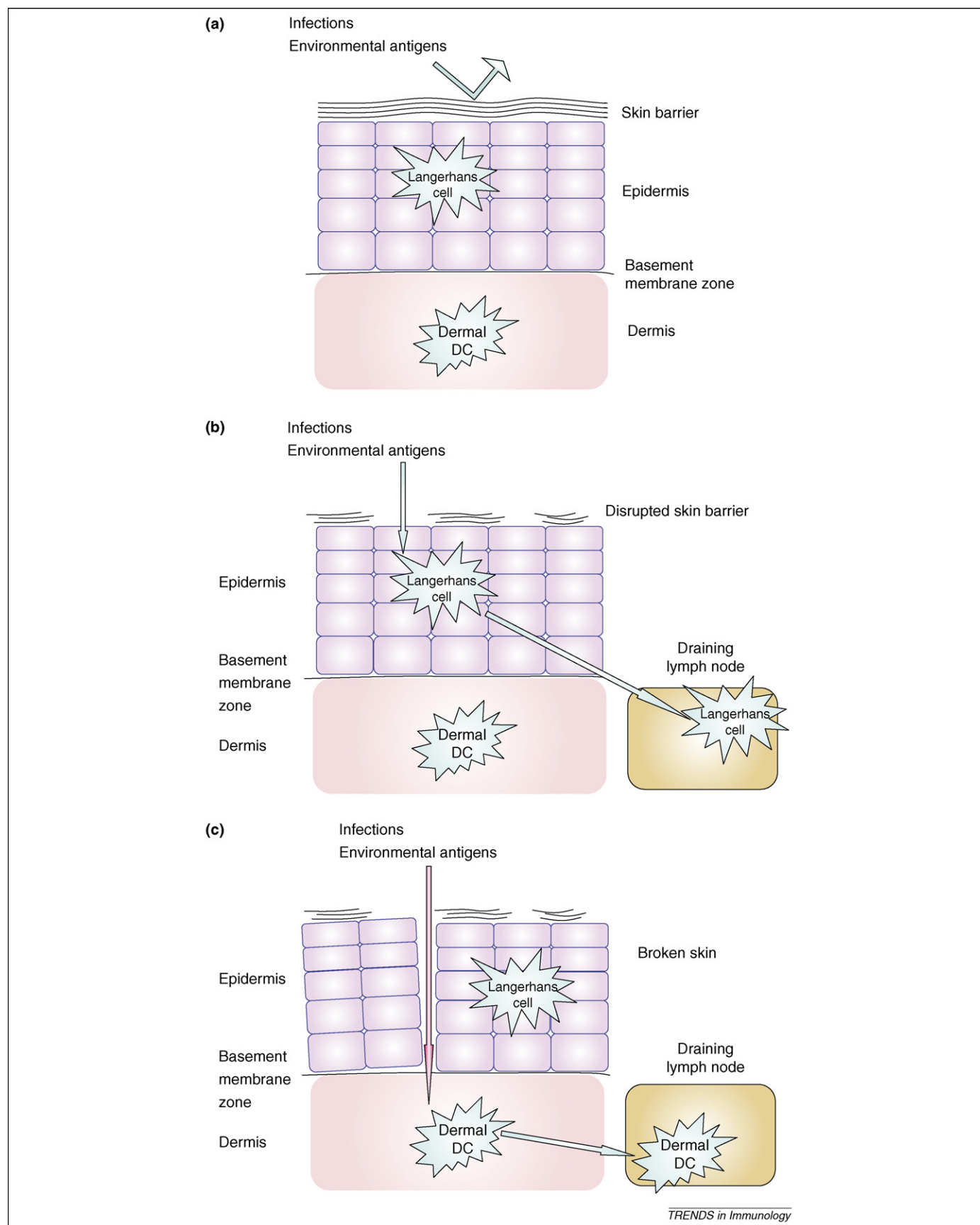


Figure 1. Skin exposure to environmental antigens. **(a)** The skin barrier (stratum corneum) protects the underlying epidermis and Langerhans cells from exposure to environmental chemicals and antigens. **(b)** If the stratum corneum is disrupted, antigens gain access to the epidermis. Here they can be taken up by Langerhans cells, which migrate to the draining lymph node and locate to the inner paracortex. In this scenario, T cells from the draining node respond to specific antigen and produce enhanced levels of IL-4 and decreased levels of IFN γ , typical of a Th2 response. **(c)** If the integrity of the epidermis is breached, antigen can be taken up by dermal DCs, which migrate to the draining lymph node and locate to the outer paracortex just beneath the B cell follicles. In this case, T cells make IFN γ , typical of a Th1 response.

of AD with 1q21 [16] is particularly notable because this region includes the epidermal differentiation complex (EDC) [17]. The EDC includes several gene clusters that encode proteins required for skin barrier function. Filaggrin is encoded in the EDC and is an essential component of the skin epidermis by helping aggregate keratin filaments in the apoptosed and flattened keratinocytes to form the skin barrier [1,18]. Loss-of-function variants in filaggrin have recently been described in AD [19]. Expression of other proteins important for the skin barrier encoded at the EDC region include loricrin and involucrin and S100 proteins, which have also been shown to be expressed abnormally in AD [20,21].

These genetic associations point strongly towards loss of skin barrier integrity contributing to the development of AD and allergy, but they do not give any indication of the immunological mechanisms involved. Allergy is associated with Th2 immune responses characterized by production of interleukin (IL)-4 and allergen-specific IgE [22]. There is abundant evidence that skin exposure to antigens can result in Th2-biased immune responses. For example, protein allergens elicit eczematous inflammatory reactions in the skin as indicated by the atopy patch reaction [23], and prolonged skin exposure to antigen in an occlusive patch applied to shaved skin can induce typical Th2 responses in mice. Animals sensitized in this way display systemic allergic responses and hyper-responsiveness to methacholine after inhalation of the sensitizing antigen ovalbumin [24,25]. The chemical hapten picryl chloride applied to shaved and barrier-disrupted skin can also elicit a Th2-dominant response that is clearly distinct from the response induced through intact skin [26]. Why Th2 responses are obtained in these examples of skin immunization is now becoming apparent. The consistent theme in these experiments is that antigen exposure after disruption of the skin barrier but without loss of epidermis integrity results in Th2 responses. This has now been further clarified in recent studies by Strid *et al.* who showed that aqueous solutions of protein antigens applied without any adjuvant to the epidermal surface after removal of the stratum corneum with adhesive tape induces primary antigen-specific immune responses that are strongly Th2 biased [27–29]. By contrast, the response elicited by subcutaneous immunization, where antigen would have direct access to the dermal dendritic cells, was predominantly Th1 in nature [27]. Epicutaneous application of antigen to skin stripped of the stratum corneum was able to override Th1 responses induced by antigen injected subcutaneously with Freund's complete adjuvant and switch the response to a Th2 response, clearly demonstrating the potency of the Th2 response obtained by this mode of immunization [29]. The Th2 response obtained in this way to type II collagen was also able to reverse the Th1 autoimmune disease collagen-induced arthritis [30]. In these experiments, the Th2 bias of the response was clearly evident by the high levels of IgG1 and IgE antibody with little or no IgG2a and by the elevated production of IL-4 and decreased production of interferon γ (IFN γ) by T cells from the draining LNs cultured with the immunizing antigen.

Does antigen uptake by Langerhans cells lead to allergy?

Why antigen applied to the skin epidermis in the absence of the stratum corneum induces such potent Th2 responses is not totally clear. One possibility is that antigen is taken up preferentially by LCs, which, in the absence of additional inflammatory or 'danger' signals, migrate to the draining LN and preferentially initiate a Th2 response. It is known that perturbation of the stratum corneum by, for example, mechanical tape stripping induces production of tumour necrosis factor α , IL-1 and granulocyte-macrophage colony-stimulating factor and migration of LCs to draining LNs [31–33]. Strid *et al.* showed clearly that fluorescein isothiocyanate (FITC)-labelled protein applied to the skin epidermis after removal of the stratum corneum was rapidly taken up by LCs, which then migrated to the draining LNs [27]. Within two hours, the majority of LCs had migrated away from the epidermis, whereas little or no migration occurred in response to removal of the stratum corneum and application of saline without antigen. Further analysis by flow cytometry showed clearly that the antigen-loaded LCs appeared in the draining LNs 18 h after the initial application of antigen. Although it was not possible to exclude migration of dermal DCs in addition to LCs under these conditions, antigen delivered directly into the dermis was found to give a weak Th1 response, suggesting that the dermal DCs are unlikely to stimulate a Th2 response.

T cells obtained from the draining LNs after immunization with antigen applied to barrier-disrupted skin proliferated in response to the immunizing antigen and secreted increased levels of IL-4 and decreased IFN γ consistent with a Th2 response [27]. This result suggests that LCs migrating to draining LNs could preferentially induce Th2 responses, although a role for dermal DCs cannot be completely excluded. Several observations are consistent with LCs having a distinct role from dermal DCs. Tagging DCs with green fluorescent protein under the control of the *langerin* gene selectively expressed by LCs has shown LC migration to draining LNs to peak at 4 days compared with the more rapid migration of dermal DCs, which appeared as early as 24 h [34]. Furthermore, LCs were found to be located in the inner paracortex, whereas dermal DCs located to the outer paracortex just beneath the B cell follicles [4,34]. The significance of the different sites occupied by the dermal DCs and LCs is not entirely clear but a recent study has suggested that the primary function of LCs might not be to induce adaptive immunity [5]. Moreover, LCs have a much reduced response to lipopolysaccharide and other microbial pathogen-associated molecular patterns, and seem to be less able to initiate an inflammatory response [35,36]. Significantly, T cells grown with cultured LCs make IL-4 and stimulate IgE production by B cells [37].

It is also possible that the local response to cutaneous barrier disruption could have a role in the Th2 dominance of the immune response. Removal of the stratum corneum by tape stripping results in some LC maturation [27,38] and in some studies results in emigration from the epidermis [38] but not in others [27]. IL-10 is absent in normal intact skin but is expressed in the epidermis soon after

barrier disruption [32]. IL-4 is also expressed in skin after barrier disruption and exposure to antigen [26,39]. Either might alter LC function to promote Th2 responses. These findings together suggest that reduced skin barrier function allows easier access of allergens to the Langerhans cells in the epidermis in a Th2-biasing cytokine environment, which then migrate to the draining LNs and induce a potent Th2 response.

Does our obsession with cleanliness and use of skincare products compromise the skin barrier and contribute to atopic dermatitis and allergy?

The hygiene hypothesis was proposed by Strachan in 1989 to explain the increase in susceptibility to allergic diseases [40]. This was based on a lack of early childhood exposure to infectious agents but there is still no clear epidemiological evidence to suggest that exposure to a specific infection reduces the risk of AD, and indeed, from clinical observation and several reports, infections can often trigger the onset of or exacerbate AD. A more plausible explanation is that a compromised skin barrier results in AD and an increased risk of allergy by direct sensitization through the skin [13]. Atopic individuals seem to have genetic defects in proteins that make up the skin barrier, but the increase in AD and allergy could also be related to damage to the skin as a consequence of a modern lifestyle, such as excessive washing with harsh soaps, the use of exfoliants as cosmetics to obtain 'healthy' skin, biological washing detergents containing proteases and even hard water causing dry skin. As a substitute for the hygiene hypothesis, we would propose that in genetically susceptible individuals (atopy affecting ~40%), a more probable explanation for the increase in AD and allergy in a world obsessed with cleanliness and skin beauty could be the loss of integrity of the skin barrier.

References

- Candi, E. *et al.* (2005) The cornified envelope: a model of cell death in the skin. *Nat. Rev. Mol. Cell Biol.* 6, 328–340
- Eckert, R.L. *et al.* (1997) The epidermal keratinocyte as a model for the study of gene regulation and cell differentiation. *Physiol. Rev.* 77, 397–424
- Valladeau, J. and Saeland, S. (2005) Cutaneous dendritic cells. *Semin. Immunol.* 17, 273–283
- Kissenpfennig, A. and Malissen, B. (2006) Langerhans cells – revisiting the paradigm using genetically engineered mice. *Trends Immunol.* 27, 132–139
- Allan, R.S. *et al.* (2003) Epidermal viral immunity induced by CD8 α^+ dendritic cells but not by Langerhans cells. *Science* 301, 1925–1928
- Chavanas, S. *et al.* (2000) Mutations in *SPINK5*, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat. Genet.* 25, 141–142
- Magert, H.J. *et al.* (1999) LEKTI, a novel 15-domain type of human serine proteinase inhibitor. *J. Biol. Chem.* 274, 21499–21502
- Descargues, P. *et al.* (2005) Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. *Nat. Genet.* 37, 56–65
- Ishida-Yamamoto, A. *et al.* (2005) LEKTI is localized in lamellar granules, separated from KLK5 and KLK7, and is secreted in the extracellular spaces of the superficial stratum granulosum. *J. Invest. Dermatol.* 124, 360–366
- Caubet, C. *et al.* (2004) Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/KLK7/hK7. *J. Invest. Dermatol.* 122, 1235–1244
- Cookson, W. (2004) The immunogenetics of asthma and eczema: a new focus on the epithelium. *Nat. Rev. Immunol.* 4, 978–988
- Morar, N. *et al.* (2006) The genetics of atopic dermatitis. *J. Allergy Clin. Immunol.* 118, 24–36
- Cork, M.J. *et al.* (2006) New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene–environment interactions. *J. Allergy Clin. Immunol.* 118, 3–23
- Walley, A.J. *et al.* (2001) Gene polymorphism in Netherton and common atopic disease. *Nat. Genet.* 29, 175–178
- Kusunoki, T. *et al.* (2005) SPINK5 polymorphism is associated with disease severity and food allergy in children with atopic dermatitis. *J. Allergy Clin. Immunol.* 115, 636–638
- Cookson, W.O. *et al.* (2001) Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. *Nat. Genet.* 27, 372–373
- Mischke, D. *et al.* (1996) Genes encoding structural proteins of epidermal cornification and S100 calcium-binding proteins form a gene complex ('epidermal differentiation complex') on human chromosome 1q21. *J. Invest. Dermatol.* 106, 989–992
- Hudson, T.J. (2006) Skin barrier function and allergic risk. *Nat. Genet.* 38, 399–400
- Palmer, C.N. *et al.* (2006) Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat. Genet.* 38, 441–446
- Sugiura, H. *et al.* (2005) Large-scale DNA microarray analysis of atopic skin lesions shows overexpression of an epidermal differentiation gene cluster in the alternative pathway and lack of protective gene expression in the cornified envelope. *Br. J. Dermatol.* 152, 146–149
- Seguchi, T. *et al.* (1996) Decreased expression of filaggrin in atopic skin. *Arch. Dermatol. Res.* 288, 442–446
- Romagnani, S. (1997) The Th1/Th2 paradigm. *Immunol. Today* 18, 263–268
- Darsow, U. *et al.* (1996) The atopy patch test: an increased rate of reactivity in patients who have an air-exposed pattern of atopic eczema. *Br. J. Dermatol.* 135, 182–186
- Wang, L.F. *et al.* (1996) Epicutaneous exposure of protein antigen induces a predominant Th2-like response with high IgE production in mice. *J. Immunol.* 156, 4077–4082
- Spergel, J.M. *et al.* (1998) Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. *J. Clin. Invest.* 101, 1614–1622
- Kondo, H. *et al.* (1998) Percutaneous sensitization with allergens through barrier-disrupted skin elicits a Th2-dominant cytokine response. *Eur. J. Immunol.* 28, 769–779
- Strid, J. *et al.* (2004) Disruption of the stratum corneum allows potent epicutaneous immunization with protein antigens resulting in a dominant systemic Th2 response. *Eur. J. Immunol.* 34, 2100–2109
- Strid, J. *et al.* (2005) Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization. *Clin. Exp. Allergy* 35, 757–766
- Strid, J. *et al.* (2006) Epicutaneous immunization converts subsequent and established antigen-specific T helper type 1 (Th1) to Th2-type responses. *Immunology* 119, 27–35
- Strid, J. *et al.* (2007) Epicutaneous immunization with type II collagen inhibits both onset and progression of chronic collagen-induced arthritis. *PLoS ONE* 2, e387 doi: 10.1371/journal.pone.0000387 (<http://www.plosone.org>)
- Wood, L.C. *et al.* (1992) Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. *J. Clin. Invest.* 90, 482–487
- Nickoloff, B.J. and Naidu, Y. (1994) Perturbation of epidermal barrier function correlates with initiation of cytokine cascade in human skin. *J. Am. Acad. Dermatol.* 30, 535–546
- Nishijima, T. *et al.* (1997) Altered permeability and disordered cutaneous immunoregulatory function in mice with acute barrier disruption. *J. Invest. Dermatol.* 109, 175–182
- Kissenpfennig, A. *et al.* (2005) Dynamics and function of Langerhans cells *in vivo*: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. *Immunity* 22, 643–654
- Larregina, A.T. and Falo, L.D. (2005) Changing paradigms in cutaneous immunology: adapting with dendritic cells. *J. Invest. Dermatol.* 124, 1–12
- Asahina, A. and Tamaki, K. (2006) Role of Langerhans cells in cutaneous protective immunity: is the reappraisal necessary? *J. Dermatol. Sci.* 44, 1–9

- 37 Hauser, C. *et al.* (1989) T helper cells grown with hapten-modified cultured Langerhans' cells produce interleukin 4 and stimulate IgE production by B cells. *Eur. J. Immunol.* 19, 245–251
- 38 Holzmann, S. *et al.* (2004) A model system using tape stripping for characterization of Langerhans cell-precursors *in vivo*. *J. Invest. Dermatol.* 122, 1165–1174
- 39 Matsui, K. and Nishikawa, A. (2002) Lipoteichoic acid from *Staphylococcus aureus* induces Th2-prone dermatitis in mice sensitized percutaneously with an allergen. *Clin. Exp. Allergy* 32, 783–788
- 40 Strachan, D.P. (1989) Hay fever, hygiene, and household size. *BMJ* 299, 1259–1260