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Recapitulating Atopic Dermatitis in Three Dimensions: Cross Talk between Keratinocytes and Nerve Fibers

Carlo Pincelli¹ and Martin Steinhoff^{2,3}

Roggenkamp *et al.* (2013) report a significant advance in the *in vitro* reconstruction of atopic dermatitis (AD) by coculturing lesional human keratinocytes with sensory nerve fibers. This work has important implications for understanding the interaction between the resident skin cells and the peripheral nervous system in AD, and it sheds light on the pathways leading to the pruritus that typifies this disease.

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Atopic dermatitis, skin innervation, and neurogenic inflammation

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by epidermal barrier disruption, alterations in epidermal homeostasis, and increased amounts of Th-2-derived cytokines. AD is associated with severe pruritus, which greatly impairs patients' quality of life. The skin of patients with AD is richly innervated, with extensive sprouting of sensory nerve fibers, suggesting a close correlation between itch and the peripheral sensory nervous system. Although intimate contacts between the sensory nerve and skin cells have been well described, the mechanisms linking pruritus to increased innervation in AD is poorly defined. Addressing exacerbations of AD in

response to stress, several studies have demonstrated increased mast cell–nerve fiber contacts, alterations in the activities of immune-inflammatory cells, and the release of neuromediators by sensory nerve endings, leading to neurogenic inflammation and pruritus. In addition, skin cells also produce neural factors, and thus they can communicate with adjacent nerves to increase inflammatory and pruritic responses (Suárez *et al.*, 2012). This confirms that a psycho–neuro–immune pathway is—at least in part—operational in AD, whereas several therapeutic strategies are being developed to block this pathway. However, little is known about the peripheral involvement of sensory nerve fibers and their role in AD, in particular with respect to their interaction with

resident skin cells, and in the absence of stimuli from the central nervous system. The question arises whether involvement of sensory nerve fibers in AD is a secondary event following emotional stress and/or a primary peripheral pathway that has an important role in the pathogenesis of the disease.

Cutaneous sensory nerve fibers originate from the dorsal root ganglia where the cell bodies of these neurons are located. Peripheral nerve branches reach the dermis and move upward into the epidermis (Zylka *et al.*, 2005). Nerve growth factor (NGF) and the other members of the neurotrophin family are synthesized and released by keratinocytes and then transported “antidromically” to the dorsal root ganglia where they regulate the synthesis and differentiation of neurons, as well as the production and release of neuropeptides in the skin, resulting in neurogenic inflammation and/or pruritus. For example, calcitonin gene-related peptide (CGRP), substance P, or pituitary adenylate cyclase-activating peptide are expressed differently in several inflammatory dermatoses, where they contribute to the “so-called” neurogenic inflammation, either by acting directly on blood vessels or by stimulating mast cells to release histamine and other inflammatory mediators (Montaño *et al.*, 2010; Seeliger *et al.*, 2010).

Innervated skin in three-dimensional *in vitro* models

Roggenkamp *et al.* (2013, this issue) address an important question as to how sensory nerve fibers and resident skin cells, with special attention on keratinocytes, communicate with each other, as a critical mechanism in the pathogenesis of AD.

Thus far, our understanding has been hampered by methodological barriers, namely the absence of a reproducible model that would allow investigators to examine neuronal cells and resident skin cells of nonrodent origin simultaneously. In this issue, the authors used a novel approach that was based on an organotypic skin model in which human keratinocytes and fibroblasts were cocultured with neurons derived from porcine dorsal root ganglia. This three-dimensional skin equivalent was a major advance from the earlier *in vitro*

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Clinical Implications

- Three-dimensional models derived from skin-cultured cells can be manipulated to reproduce human skin diseases.
- Coculture of keratinocytes from patients with AD and sensory nerve fibers recapitulates atopic eczema and provides a model to understand pruritus that is associated with the disease.
- The three-dimensional atopic model can be used to investigate the role of the peripheral nervous system in the pathogenesis of AD and to test novel therapies.

models, as it was composed of air-exposed, isolated skin cells, allowing the investigators to study the biology of resident skin cells, as well as their interactions with sensory neurons under defined conditions. The model definitely resembles *in vivo* innervated skin with the presence of neurites in the dermis and in close contact with keratinocytes.

Although neurons clearly induce epidermal thickening, through the release

of CGRP, alterations in proliferation markers in this model should be evaluated with caution, given their different expression patterns in skin equivalents compared with normal skin, which sometimes render results difficult to interpret. Importantly, in some pictures of Roggenkamp's work, the epidermal basal layer is hardly visible. In any event, blocking experiments with CGRP and substance P antagonists, along with

the semiquantitative measurements of epidermal thickness and Ki-67+ cells, clearly confirm the effects of neuropeptide-releasing neurons on epidermal cells. However, the precise characteristics of the epidermal structure of this model compared with that of "real" skin are yet to be characterized.

Human skin equivalents generated from cultured cells have been used since many years to study epidermal homeostasis and to test for toxicity of drugs. More recently, this technology has been exploited to develop *in vitro* models of skin diseases, ranging from inflammatory dermatoses to cancer (Semlin *et al.*, 2011). Although *in vitro* skin disease models picture the pathological situation at a given point and are yet to be improved, they certainly will be able to replace animal models in many respects.

Along this line, Roggenkamp *et al.* attempted to reconstruct an *in vitro* model of AD by adding keratinocytes from atopic patients to the innervated porcine skin equivalent, which, indeed, resembles the human system more than the rodent. These investigators had already succeeded in showing that atopic keratinocytes express higher levels of NGF, and that they increase neurite outgrowth compared with healthy keratinocytes (Roggenkamp *et al.*, 2012). In this issue, these authors refined the *in vitro* innervated AD model by showing that atopic keratinocytes express increased levels of the CGRP receptor constituent Ramp1, and that elevated CGRP induces keratinocytes to increase their proliferation, resulting in epidermal hyperplasia and acanthosis in association with reduced expression of filaggrin, which typify AD lesional skin *in vivo*.

Taken together, these observations indicate that the innervated AD *in vitro* model partially recapitulates the pathophysiology of AD *in vivo*. Whether this can be extended to address how immune cells are involved in the neuro-immune communication between the resident skin cells and nerve cells is yet to be determined. The model also suggests—indirectly—that extensive epidermal fiber density might contribute to the pruritus of AD. Indeed, excessive sprouting of nerve fibers that release elevated amounts of neuropeptides in AD is accounted for by the increased

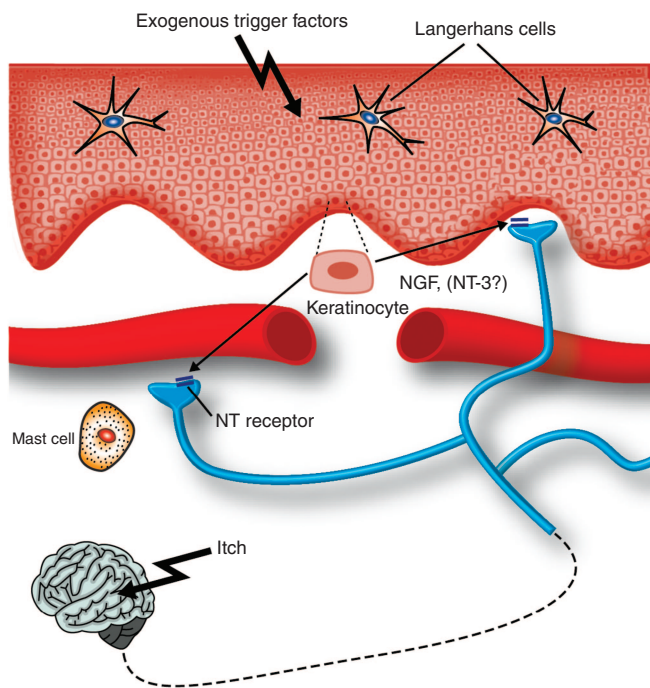


Figure 1. Atopic dermatitis (AD), nerve growth factor (NGF), and nerve fibers. In AD, exogenous trigger factors (e.g., mechanical stimuli, toxins, proteases, *Staphylococcus aureus*) can activate keratinocytes to release NGF and other inflammatory mediators. NGF activates its high-affinity receptor TrkA on sensory nerve endings in the epidermis and the dermis to mediate NGF-induced neural activation. Activation of sensory nerve endings results in transmission of a "pruritic" stimulus to the spinal cord and brain cortex resulting in "itch sensation" and scratching behavior. In addition, through an antidromal reflex the nerve endings release neuropeptides such as substance P, calcitonin gene-related peptide, or pituitary adenylate cyclase-activating peptide to mediate neurogenic inflammation in the skin. Neuromediators can modulate the function of mast cells, T cells, macrophages, or eosinophils, as well as certain dendritic cells. In a later phase, immune cells and keratinocytes may also communicate with sensory nerves to aggravate pruritus and the inflammatory condition.

production of NGF from keratinocytes. NGF, through its high-affinity receptor TrkA, seems to have a central role in pruritus (Indo, 2010). Moreover, histamine causes increased NGF levels in human keratinocytes (Kanda and Watanabe, 2003), and scratching behavior significantly correlates with high NGF levels (Yamaoka *et al.*, 2007). Thus, it may be speculated that NGF, released in high levels from keratinocytes in AD patients and further enhanced by CGRP in an autocrine manner, as well as neuropeptide-induced histamine and trypase release from mast cells, contribute to pruritus in AD. On one hand, it can be argued that, lacking the immunological component, this model cannot mimic all aspects of AD. On the other hand, pure immunological models thus far fail to integrate the recognized interplay between the epidermis and the nervous system. In addition, given the reduced expression of filaggrin, it is tempting to speculate that the first trigger in the pathogenesis of AD could take place in the dysregulated barrier, thus inducing an upregulation of NGF in keratinocytes, followed by the above described cascade of events eventually leading to keratinocyte proliferation and epidermal thickening, accompanied by further production of NGF giving rise to a vicious circle. This scenario would initially take place without primary intervention of the adaptive immune system that would subsequently amplify epidermal and nerve response. Vice versa, we are in substantial need of better *in vivo* models that explain the complex pathophysiology of how the adaptive immune system, represented mainly by the TH2-helper cell and IgE-producing B cells in AD, or even dendritic cells, regulate neuro-immune responses in AD, neurogenic inflammation, and pruritus, which are definitely a part of the pathophysiology of AD. Taken together, *in vivo* models that integrate the pathophysiological changes of the resident skin cells, the adaptive and innate immune system, and the skin nervous system are in great demand for understanding the “outside-in” versus the “inside-out” hypotheses in AD.

In sum, the AD model presented by Roggenkamp *et al.* is an important step,

one that can be improved even more to reproduce the disease pathophysiology of AD *in vivo*. In particular, it will be of great interest to evaluate the model after blocking the activity of NGF in the presence or absence of the immunological component. Finally, the model described by Roggenkamp *et al.* could be exploited to evaluate the efficacy of the treatment of AD and its related pruritus, with molecules other than antihistamines and immunosuppressants.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Photosensitivity in the Elderly—Think of Late-Onset Protoporphyrria

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Photosensitivity is the clinical hallmark of both erythropoietic protoporphyria (EPP) and X-linked dominant protoporphyria (XLDPP). Both disorders result from a hereditary dysfunction in heme biosynthesis. Disease onset is usually in early childhood. However, rare patients with late-onset EPP in association with a myeloproliferative disorder or myelodysplastic syndrome have been reported. In this issue, Livideanu *et al.* describe the first patient with late-onset XLDPP.

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Photosensitivity as the clinical hallmark of photodermatoses

Photosensitivity comprises any increase in cutaneous reactivity to sunlight. Sunlight is composed of ~50% visible,

40% infrared, and 10% UV radiation (Millard and Hawk, 2002). The biological effects of visible light (electromagnetic spectrum 400–800 nm) on the skin are mostly negligible. However, in

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