Review article

Eur J Dermatol 2008; 18 (2): 119-27

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Article accepted on 18/10/2007

The epidermis: a sensory tissue

The skin is an efficient barrier which protects our bodies from the external environment but it is also an important site for the perception of various stimuli. Sensory neurones of the peripheral nervous system send many primary afferent fibres to the skin. They pass through the dermis and penetrate the basement membrane to innervate epidermal cells or remain as free endings. Nerve fibres are clearly involved in somatosensation. However, they are not always so numerous, for example in distal parts of the limbs, and some kinds of sensors can be at a distance of hundreds of micrometers from each other. The skin can detect patterns at a very fine and smaller scale, which suggests that nerve terminals are helped by epidermal sensors. All epidermal cells (keratinocytes, melanocytes, Langerhans cells and Merkel cells) express sensor proteins and neuropeptides regulating the neuro-immuno-cutaneous system. Hence, they must play a part in the epidermal sensory system. This review will consider the epidermal components of this forefront sensory system and the stimulations they perceive. The epidermis can be considered a true sensory tissue where sensor proteins and neurone-like properties enable epidermal cells to participate in the skin surface perception through interactions with nerve fibres.

Key words: skin, receptors, epidermis, neuro-immuno-cutaneous system, peripheral nervous system, sensory neurone, perception

Over the last 15 or 20 years, numerous studies have shown an impressive number of interactions between the skin, immunity and the nervous system. These new data were so fascinating that they relegated to the middle distance the sensory function of the skin nervous system! Since some nerve endings and sensory receptors appear to be not so numerous in distal part of the body [1-5], the participation of epidermal cells is investigated. New studies reveal that interactions between the epidermis and nerve endings are involved in sensory functions and that the epidermis can be considered as a sensory organ.

Abbreviations: ASIC: Acid sensing ion channel; BDNF: Brain derived neurotrophic factor; BNC1: Brain Na+ channel 1; BLINaC: Brain-liver-intestine amiloride-sensitive Na+ channel; CGRP: Calcitonin gene-related peptide; Deg/ENaC: Degenerin/Epithelial sodium channel; DRASIC: Dorsal root acid-sensing ion channel; GDNF: Glial cell line derived neurotrophic factor; IB4: Isolectin B4 from Bandeiraea simplicifolia; LC: Langerhans cells; MC: Merkel cells; NGF: Nerve growth factor; NICS: Neuro-immunocutaneous system; NPY: Neuropeptide Y; NOMPC: No mechanoreceptor potential C; NT-3: Neurotrophin-3; ORS: Outer root sheath; POMC: Pro-opiomelanocortin; SAM: Slowly adapting mechanoreceptor; SP: Substance P; TRPA/M/V: Transient receptor potential Ankyrin-repeat/Melastatin/Vanilloid; TRPN: TRP cation channel subfamily N, analog to NOMPC; VIP: Vasoactive intestinal peptide

The neuro-immuno cutaneous system

It has been widely demonstrated that the skin is an organ of communication [6]. Epidermal cells connect the skin to the mind through a complex communication network, tightly related to the neuroendocrine and the immune systems [7-9]. Langerhans cells and mast cells are key cells to bridge the gap between neuroendocrine and immune systems in the skin [10, 11]. They take part in the endocrine system through the metabolism of vitamin D or the production of neurohormones [12-14]. They affect the permeability of blood vessels [15] and are implicated in wound healing [16], pruritus and other dermatological disorders like psoriasis [17-19]. Furthermore, epidermal cells act on the nervous system at local and central levels, so much so that 30 to 40% of dermatological patients also have psychological problems [20]. Epidermal cells are believed to modulate the sensory information of touch [21] or pain [22, 23]. After ultraviolet (UV) exposure, they lead to a decrease in the pain threshold [24, 25] and immunomodulatory effects through pro-opiomelanocortin (POMC)-peptide release [26]. Conversely, the brain can affect cutaneous functions in an efferent manner to stimulate target tissues; for example during neurogenic inflammation [27, 28]. Hence, the brain-epidermis connection is multi-directional and leads us to consider the integrated neuro-immuno-cutaneous system (NICS) [8, 25].
The NICS consists of a common language shared by sensory neurones, keratinocytes, melanocytes, Langerhans cells and Merkel cells, with the neuromediators as letters. These powerful molecules are widely involved in skin physiology and the response to a stimulus. Skin cells are

doi: 10.1684/ejd.2008.0348

able to recognize the relevant biological signals transmitted through neuromediators with high specificity because they synthesize the receptors themselves [12, 16]. Such neuro-endocrine capabilities are critical for the activity of the NICS. In the NICS, it is currently understood that substance P (SP) plays a key role in pain sensitization [29] and leads to mast cell degranulation [13], that POMC and derivatives are immunomodulators, that neurotrophines, like the nerve growth factor (NGF), are mitogenic proteins which also stimulate nerve fibre sprouting, regulate neuropeptides synthesis and probably take part in psoriasis [30] and that catecholamine acts as an inflammatory factor. Acetylcholine, calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and neuropeptide Y (NPY) seem to act differentially, depending on the skin environment. Therefore, the NICS acts locally, at the level of the neurogenic inflammation, but it is also considered to affect the whole organism via the endocrine and neurocrine pathways [31, 32]. Until now, the concept of NICS mainly described the effects of the nervous system on skin cells through the presence of synapses, neurotransmitters and specific receptors in the skin. We now know that the epidermis also appears at the forefront of the sensory system [33], as revealed by new data on the sensory abilities of epidermal cells.

Sensor proteins

Various sensor proteins are present on neurones of the peripheral nervous system, which are believed to be the unique transducers in skin perception. Nerve fibres are densely packed within the face and tactile areas like finger tips or ano-genital areas but the number of nerve endings decreases from the trunk to the distal parts of the limbs, without decreasing touch sensitivity [1]. Epidermal cells are thought to relay the signal transduction because they express many sensor proteins like those found in neurones. These proteins are mainly transmembrane proteins which allow transformation of stimuli like touch, osmotic pressure, temperature or chemical stimulations to biochemical intra-cellular messages *(table 1)* [5, 33]. Such neurone-like properties permit the whole epidermis to have sensory functions.

Among these sensor proteins, the transient receptor potential (TRP) family is the most important. TRP channels belong to a family of six transmembrane domain receptors which are divided into seven subtypes. TRPV1 (TRP vanilloid 1) is the most characterized receptor and probably the most expressed within the epidermis. TRPV1 is highly expressed in neurones involved in pain transmission and neurogenic inflammation (C and Aδ-fibres [34]) but also shows a strong immunoreactivity in keratinocytes from the upper and the basal layers of the epidermis *(figure 1)* [5, 35]. In humans, the temperature responsiveness ranges from – 10 to 60 °C. Pharmacological data are consistent with a major role of TRPV1 in the detection of temperatures over 42 °C and acidic conditions below a pH of 6.6 [36]. Another interesting property of TRPV1 is its ability to bind capsaicin, the molecule which confers spiciness to chili peppers, with high affinity. Thus TRPV1 activation evokes sensations ranging from warmth to burning pain, as well as piquant taste [35]. Consequences of its activation vary according to the context. Once activated by capsaicin, the TRPV1 channel first leads to calcium influx and neuropeptide release. But the lasting calcium influx, with too high intracellular calcium concentrations, leaves the neurone desensitized, thus it loses its ability to induce the release of neuropeptides such as SP, which is co-localized [37]. This is responsible for a transient insensitivity, which is exploited by dermatologists to induce analgesia or anti-inflammatory effects [38]. The heat-gated TRPV2 channel is strongly expressed in Aδ-fibres; it is activated for temperatures above 53 °C, for example in the case of burns, where it must be involved in the warning stimulation [39]. The TRPV3 channel is a camphor sensitive receptor found in sensory neurones and keratinocytes of the inner boundary of the epidermis. It is activated by heat from 31 °C to 39 °C [40]. This discrepancy in the results obtained may be due to the thermal history of the cell [39]. The TRPV4 channel, present in keratinocytes and Merkel cells, exhibits an apparent threshold of about 27 °C and reacts to hypo-

Table 1. Putative ion channels believed to be implied in somatosensation in mammals

Name	Physical stimuli	Chemical stimuli	Cells
TRPA1	Thermal, mechanical	Isothiocyanates, Ca^{2+}, icilin	C-fibres
TRPC1	Mechanical	Store-operated calcium channel	Mechanosensory neurones
TRPM8	Thermal	Menthol, icilin	C-fibres
TRPN1	Mechanical	None known	Hair cell, bristles
TRPV1	Thermal, osmotic	Capsaicin, proton, endocanabinoïds, Amandamide, protons, diphenyl compounds	C, Aδ-fibres, keratinocytes
TRPV2	Thermal, osmotic, mechanical	Diphenyl compounds	Aδ, Aβ-fibres, immune cells
TRPV3	Thermal	Camphor, carvacrol, diphenyl compounds	Keratinocytes, C-fibres
TRPV4	Thermal, osmotic cell swelling	Phorbol ester (4αPDD), epoxyeicosatrienoic acid	Keratinocytes, Merkel cells, Aδ and C-fibres
ASIC1	Mechanical	Protons	Aδ, Aβ and C-fibres
ASIC2	Mechanical	Protons	Aδ and Aβ-fibres
ASIC3	Mechanical	Protons	Aδ and Aβ-fibres
MEC4	Mechanical	None known	mechanosensory neurones
MEC 10	Mechanical	None known	mechanosensory neurones

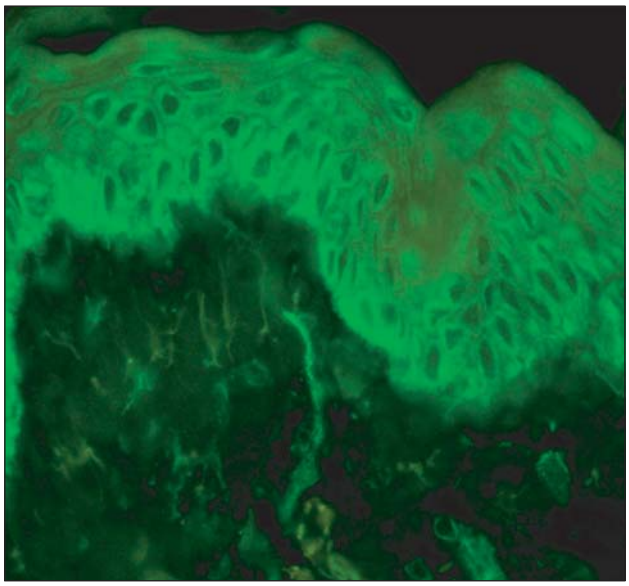

Figure 1. Expression of TRPV1 in frozen section of 10 µm of human epidermis. The capsaicin sensitive ion channel TRPV1, involved in the noxious perception, was highlighted using the rabbit polyclonal anti-VR1 (Santa Cruz, sc-20813, 1:100) revealed by the donkey-anti-rabbit FITC (Santa Cruz, sc-2090, 1:400). TRPV1 is expressed by sensory nerve fibres and the basal layer of keratinocytes.

osmolarity [41, 42]. Cold transduction is mainly ensured by the melastatin cation channel TRPM8, which is menthol-sensitive. This receptor gates at temperatures below 30 °C. TRPM8 is expressed almost exclusively in a subpopulation of C-fibres representing 10% of the sensory neurones [43]. TRPA1, a member of the TRP ankyrin-repeats family has been reported to be activated below 18 °C, so it may also participate in the cold responsive behaviour [44].

The molecular transduction mechanism of touch is largely unknown. Three models have arisen to explain touch perception. First, high speed channels convert stimuli into an electrical signal. This may occur in hair cells of the organ of Corti because of their remarkable transduction speed. In mammals, hair cells are the most commonly used model to study the molecular basis of the mechanotransduction. The second possibility is that the ion channels are tethered to the cytoskeleton or extracellular matrix. Membrane movements induce the opening of ion channels to generate electrical activity [45]. The 3rd possibility is that a mechanosensory protein initiates a second messenger cascade leading to the opening of the ion channels, thus producing depolarization [44].

To better understand the intimate mechanisms of touch, invertebrate models were used. Genetic screenings based on the light-touch machinery in *C. elegans* have led to the discovery of 2 proteins, MEC-4 and MEC-10, which belong to the Degenerin/Epithelial sodium channel family (Deg/ENaC). This family is characterized by common N and C terminals, two membrane-spanning sequences and a large extracellular loop with 14 conserved cysteins. The receptors are organized into homo- or heteromultimers of 4 to 9 subunits, forming nine voltage-insensitive Na^+ permeable channels in mammals. Thus the mechanosensitive Deg/ENaC is composed of α, β, γ and δ ENaC, the acid-sensing ion channel (ASIC), the brain Na^+ channel 1 (BNC1 or ASIC2), the dorsal root acid-sensing ion channel (DRASIC or ASIC3), the brain-liver-intestine amiloride-sensitive Na^+ channel (BLINaC) and the ASIC4, which is not proton-gated despite its name. Some of them are particular to cutaneous mechanosensory structures, including pacinian and Meissner corpuscles, lanceolate endings of hair follicles and the neurites contacting to Merkel cells [45]. The exact role of the Deg/ENaC family in mechanotransduction is not clear in mammals because many studies utilize invertebrate models where some genetic disruptions of these channels cause neonatal lethality. In mammals, the involvement of Deg/ENaC in the mechanotransduction was conveyed by their expression in many mechanosensory neurones of the dorsal root and trigeminal ganglia and hair cells of the inner ear. However, the electrophysiological properties of these channel are not yet consistent with transduction channels [46]. A possible role in the sensation of acid-evoked pain is also implicated in cardiac ischemia and cutaneous nociception. Due to their broad expression in the nervous system and their ability to sense acidification, it is possible that they regulate synaptic excitability [47].

Evidence for TRP family participation in touch has been found for several members: the Osm-9-like protein TRPV4, which rescues mechanosensory deficit in *C. elegans* [48], the stretch-sensitive ion channels TRPC1, gated by membrane deformation [49], TRPA1 whose mutation attenuates mechanical responsiveness [50] and even NOMPC (analogue to TRPN1 in Xenopus), implicated in the somatosensation of *Drosophila* and newly found in the vertebrate zebrafish, where it behaves as a mechanically-gated ion channel in sensory hair cells [51]. The participation of TRPV4 is probable because it is expressed in the Merkel cell-neurite complexes, anatomical structures composed of the association of mainly Aβ-fibres and Merkel cells, which play a key role in the slowly adapting type I mechanoreception [52]. However, TRPV4 is highly expressed in non-sensory tissues too. There, TRPV4 is believed to control the systemic fluid balance by its osmolarity-sensitive capability [53].

In addition to the TRPV family, purinergic receptors are also thought to participate in many cutaneous phenomena. They are involved in cell growth, differentiation, neuronal regeneration, wound healing, inflammation, etc [54]. They are also counted among the sensor proteins. Two types of receptors belong to this family, grouped according to the ligand they bind. P1 receptors bind adenosine and are divided into 4 subtypes, whereas P2 receptors, which bind ATP, ADP, and UTP, are divided into ionotropic P2X receptors and metabotropic G protein-coupled P2Y receptors. Keratinocytes express both the P2Y receptors, implicated in the mobilisation of intracellular calcium stores in response to noxious stimulation [55], and the P2X ion channel [56]. The latter is involved in the initiation of afferent signals on sensory neurones and plays a key role in sensing tissue-damaging and inflammatory stimuli [57]. Immunohistochemical investigation into Merkel cells has revealed expression of P2Y2 receptors, which could argue for a putative role of this channel in mechanoreception [58].

Sensory nerve endings

The peripheral nervous system innervating the skin originates from the dorsal root ganglia and the trigeminal ganglia. The neurites that they send into the skin form sub-

epidermal plexus from which some fibres cross the dermo-epidermal junction to innervate epidermal cells or to keep it free of targets *(figure 2)*. Nerve endings are diverse and can be classified according to many characteristics such as: diameter, the degree of myelinisation, the velocity at which action potentials travel along the fibres, or even the neuropeptides present at the nerve terminals and the information they transduce up to the central nervous system. Functional properties *(table 2)* are not strictly related to morphological aspects. However, it is currently accepted that cutaneous large myelinated Aβ-fibres of low-threshold are suited to be mechanoreceptors which feel pressure, stretch or hair movement. Unmyelinated C-fibres and lightly myelinated Aδ-fibres are often thermoreceptors which respond to heat and cold with different thresholds of activation. Nociceptors, containing opioid receptors, are mainly high-threshold C-fibres and Aδ-fibres which transduce painful sensations [3, 44]. A pruritus-specific pathway was recently defined. Pruritus is described as an unpleasant sensation provoking the desire to scratch. The pathway processing the itch is functionally and anatomically separate from the pain pathway. The itch pathway implies its own subgroup of peripheral, mainly mechano-insensitive, C-fibres in the skin. In the central nervous system, histaminergic spinal neurones transduce the itch sensation initiated by dedicated pruriceptors, to the thalamus. The pruriceptors are activated by histamine which consistently provokes pruritus, and rarely pain. However, other inflammatory molecules such as prostaglandin E2, serotonin, acetylcholine, bradykinin or even capsaicin may induce a moderate itching sensation [59]. Thus a complex interaction exists between the pain and the itch pathway. Scratching that induces pain is well-known to inhibit the pruritus and conversely, the inhibition of pain-processing by μ-opioïd can generate pruritus [60]. Therefore, the distinction between cutaneous fibres is not easy and disrupting criteria are frequently evoked, like nociceptive signalling, normally particular to Aδ and C-fibres, with the conductance speed of Aβ-neurones [61]. Further investigations have revealed that Aβ-fibres can phenotypically switch into fibres expressing SP; whereas normally, SP is only contained in a subpopulation of small C and Aδ-fibres involved in pain perception. This occurs following nerve injury [62] but also after inflammation [63]. Thus the peripheral endings of primary sensory neurones participate in neurotransmission. But they also participate in the immune response by the release of proinflammatory peptides, from unmyelinated C-fibres or myelinated Aδ-fibres, leading to the set of changes referred to as neurogenic inflammation [34].

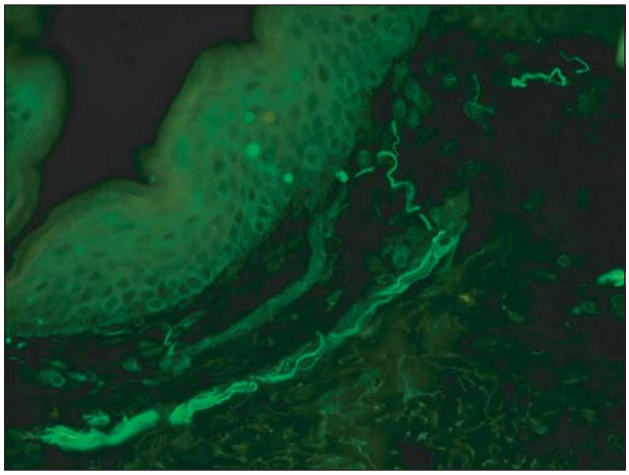

Figure 2. Immunofluorescence staining of neurofilaments in a cryofixed human foreskin epidermis. The rabbit-anti-pan-neurofilament monoclonal antibody (Biomol Int., NA1297, 1:100) revealed by the donkey-anti-rabbit FITC (Santa Cruz, sc-2090, 1:400) shows sensory nerve endings of the sub-epidermal plexus going upward the epidermis.

The ability of neurones to bind isolectin B4 (IB4) from Bandeiraea simplicifolia was also assessed with the aim of segregating subpopulations of sensors. In this way two kinds of nociceptors were identified, based on the binding of IB4 [64]. Those which bind IB4 are usually small diameter non-peptidergic neurones involved in acute pain [65]. However, only half of them seem to answer to noxious stimuli, with the remainder containing less mechanosensory C-fibres [66]. The polymodality of sensory endings hampers classification, but some overlapping characteristics were highlighted anyway. Within the epidermis, nerve viability and sensitivity can be modulated by neurotrophic factors secreted by epidermal cells. The responsiveness of each type of sensory neurone to these factors is fairly well-correlated to their class. Thus IB4-negative neurones containing SP and CGRP are NGF-responsive, small diameter nociceptors, whereas IB4-positive neurones, which lack such neuropeptides, respond to glial-derived neurotrophic factor (GDNF) [67]. Moreover, it was found that

Table 2. Physiological classification of cutaneous sensory endings

Type	Sub-type	Stimuli	Type of fibre
Mechanoreceptor	Type I	Quivering	Meissner, Aβ-fibres
		Touch	Merkel cells, Aβ-fibres, low-threshold C-fibres
	Type II	Vibration	Pacini, Aβ-fibres
		Pressure	Ruffini endings
Thermoreceptor	Cold	< 30 °C	C and Aδ-fibres
	Heat	32-48 °C	C-fibres predominantly
Nociceptors	Mechano	Significant pressure, inflammatory mediators, ischemia mediators	Aβ and Aδ-fibres
	Polymodal	Inflammatory mediators	C-fibres
Pruriceptors		Histamine, inflammatory mediators	Histaminergic C-fibres

NGF, produced in large quantities by keratinocytes *(figure 3)*, increases nociceptive-neurone survival [68, 69] while brain-derived neurotrophic factor (BDNF) decreases the activation threshold of mechanosensory Aβ-fibres [70], and finally neurotrophin-3 (NT3) enhances the innervation by slow adapting mechanosensory neurones [71, 72].
Once activated, cutaneous sensory neurones can of course induce action potentials, but also the release of neurotransmitters, which modulate inflammation, cell growth or pruritus. Such neuronal modulations of cutaneous properties regularly bring heterotrimeric G proteins into play at the beginning of the metabolic cascade, and endopeptidases at the end, for termination of the response degrading the messengers [73]. Finally, cutaneous neurites play a major role in the sensory behaviour, but there is much evidence suggesting a modulation of their sensitivity by epidermal cells [74, 75].

Keratinocytes

Keratinocytes play an important role as a forefront of the sensory system because they are equipped with sensing proteins similar to those found in neurones [33]. Keratinocytes express receptors like TRPV1 *(figure 1)*, TRPV3 and TRPV4 [76]. TRPV channels enable them to sense thermal and noxious stimuli and perhaps osmotic variation. The stimulation of these receptors is followed by the release of neuropeptides like SP, which can act as neurotransmitters onto target cells or modulators of epidermal functions. The ability of keratinocytes to interact with neurones has been demonstrated *in vitro*. In co-culture models, keratinocytes exhibit a strong trophic effect toward sensory neurones and close contact was found between these two elements [77-79]. The mechanism involved in signal transduction from keratinocytes to sensory neurones remains unclear. One hypothesis is that the signal goes through the purinergic receptors P2X2, P2X3 and P2Y2. It has been shown that ATP-activated cells can increase their intracellular calcium concentration, producing a calcium wave able to propagate to neighbouring cells. The ATP-dependant calcium waves so produced by keratinocytes can induce an increase in intracellular calcium concentration not only in adjacent keratinocytes, but also in sensory neurones [74]. Such events are interesting when keratinocytes are in such close contact with sensory neurones that synaptic transmission was considered [77, 78], but it may allow keratinocytes to communicate with neurones in the long-range too. Another putative pathway of communication from keratinocytes to neurones implicates the activation of bioactive substances like NGF or the inflammatory cytokine interleukins, IL-1α and IL-8, released subsequent to the receptor activation. These mediators are released upon activation of the keratinocytes by neuropeptides like SP, CGRP, VIP, galanin, and probably other proteins expressed by keratinocytes themselves [80]. Hence, the activation of one keratinocyte must lead to the activation of neighbouring cells in a paracrine manner, and finally by the depolarisation of nerve terminals. Thus, keratinocytes synthesize the key components which endow them to sense many physical variations and process the information perceived. The ion channels and neuropeptides originally found in the brain make the keratinocytes true partners for neurones.

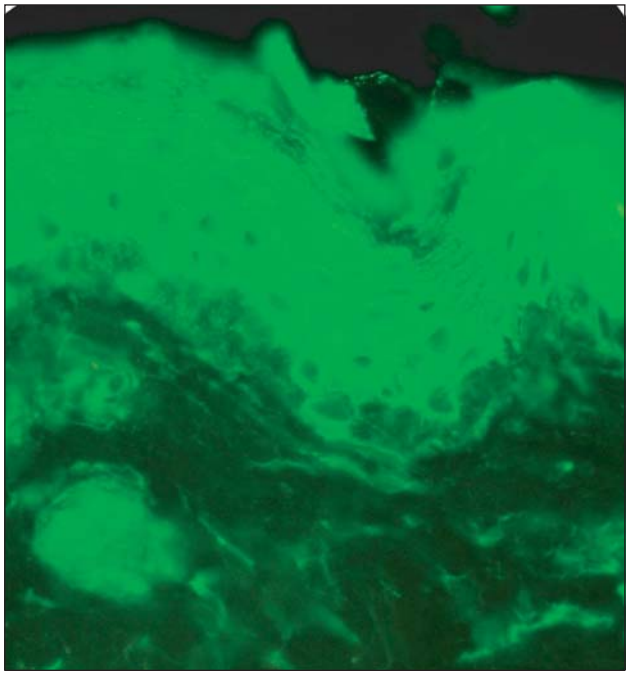

Figure 3. Expression of NGF in human foreskin epidermis. Immunofluorescence was performed using the rabbit polyclonal anti-NGF (Cedarlane, 1:100) revealed by the donkey-anti-rabbit FITC (Santa Cruz, sc-2090, 1:400). It elicits a strong expression of NGF within supra-basal keratinocytes up to the corneal layer, while the sensory nerve endings appear as thin neurites in the dermis.

Melanocytes

When the skin is exposed to the sun, melanocytes synthesize photoprotective melanin pigments with tyrsoinase, a key enzyme of the melanogenesis, and its homolog proteins, tyrosinase-related protein (TRP)-1 and TRP-2 [81]. UV radiation-stimulated melanocytes produce pro-opiomelanocortin (POMC), a precursor which, once cleaved by pro-hormone convertases, can give bioactive releasable peptides [26, 82]. Hence, α, β, γ-melanotropin, adrenocorticotropin, β, γ-lipotropin and β-endorphin [83], can activate melanogenesis, stimulate epidermal cell proliferation, induce melanocytes and Merkel cells to rise to a suprabasal location [84], have immunosuppressive and anti-inflammatory effects, probably through CGRP and interleukin-10, or can even elevate the intensity of the cutaneous innervations [82]. Melanocytes are often in close contact with sensory endings and electron microscopy has revealed a thickening of the apposing membrane, suggesting a synaptic communication [85]. Thus, the enhanced epidermal innervation might be due to proliferating melanocytes following UV radiation exposure, to maintain the connections.
Hence, melanocytes fully belong to the NICS and therefore appear to be sensory and regulatory cells for epidermis homeostasis [86]. Until now, melanocytes have never been clearly implicated in touch reception, thermal sensation or nociception. However, they are found in the outer root sheath (ORS), often as precursors or as poorly differentiated cells [87]. The ORS is the location of Merkel cells, where they are found in number, and therefore it is a place of mechanotransduction. In contrast, the function of bulbar melanocytes is more evident. They produce melanised granules toward keratinocytes of the bulb which will form

the pigmented hair shaft during the anagen phase of the hair cycle. The TRP receptors are present on melanocytes. In addition to retinal pigmented epithelium and brain, they express the melastatin cation channel TRPM1 [88] and the TRPM7 [89]. In contrast to the TRPV subfamily, TRPM1 and TRPM7 does not seem to sense physical conditions but rather act as a tumour suppressor [90] because it was found to be greatly decreased or lost in invasive melanoma [91], or in the detoxification of intermediate metabolites during melanogenesis [89], respectively. Few researchers have studied the ion channels expressed by melanocytes. Voltage-gated sodium and potassium channels were revealed to have an interesting rectifying potassium current, similar to those observed in neurones [92]. Nevertheless, melanocytes are not considered as excitable cells like Merkel cells, even if synaptic-like structures and excitable cell-specific ion channels are present.

Langerhans cells

Langerhans cells (LC) are antigen-presenting cells. After binding the antigen, LC migrate from the epidermis to the local lymph nodes to initiate protective immunity [93]. Until recently, LC were not known to express TRPV or TRPM channels but, like other dendritic cells, LC are sensitive to thermal stimulations like those occurring during fever or inflammation. A mild elevation of temperature enhances the immune potential of LC, the antigen-up take, their migration and their maturation [94]. Because sensor channels were not demonstrated to be present, the thermal perception by dendritic cells might involve sensory molecules with a second messenger cascade, rather than common thermo-sensitive ion channels. At least, LC express ionotropic ATP-specific P2X receptors, like monocyte-derived dendritic cells [95]. Activation of these receptors enhances the antigen-presenting function of LC and contact hypersensitivity in mice. Some metabotropic purinergic receptors P2Y also seem to be synthesized, as revealed by mRNA analysis, but only in an LC-like cell line [96]. Voltage-gated channels have not been found but mouse spleen dendritic cells, homologues to Langerhans cells, express voltage-gated potassium channels [97]. Like other cells of the NICS, LC express numerous neuropeptides and their receptors [7, 11]. This ability allows them to communicate with the cells of the NICS. For example, a close association between LC and Merkel cells was observed in hair follicles and below sebaceous glands [98]. Similarly, an intimate contact with sensory neurones was found [99]. These morphological associations strongly suggest a functional interaction. The Merkel cell-LC complex was not functionally investigated, while the CGRP released by nerve fibres innervating human LC inhibits their antigen-presenting function, thereby acting as an immunomodulator [10].

Merkel cells

Merkel cells (MC) are epidermal cells scattered in the basal layer of the epidermis and in the outer root sheath of hair follicles [14, 100]. They synthesize numerous neuropeptides inside dense core neurosecretory granules. The corresponding receptors are also present at the surface of MC, showing evidence for autocrine and paracrine functions [101]. The neuropeptide-containing granules are mainly located facing the low-threshold sensory neurones which supply nearly all epidermal MC. This fact highlights the tight interaction between the endocrine features of MC and neurones. Hence MC belong to the neuroendocrine cell family and they probably play a key role in the NICS. The cluster of MC with sensory neurones is named the Merkel cell-neurite complex *(figure 4)*. It constitutes the slowly adapting mechanoreceptor (SAM) reacting in nearby fashion and thus is named type 1 [102]. Conversely, Ruffini corpuscles within the dermis feel pressure in a wider area and are thus called type 2 SAM. The investigation of the exact role of MC in the perception of touch within the SAM-1 have produced conflicting results [103]. Either they are themselves mechanoreceptors which thereafter synaptically transduce the signal to sensory neurones [104, 105], or they only modulate the sensory function of neurones [106, 107]. Furthermore, the possibility that MC are not the trigger of the neuronal activity but rather the target of sensory neurones in an efferent signal, is still possible. In fact, the synaptic transmission between MC and neurones has only been implicated by molecular biology. It was found that MC express most of the proteins involved in vesicle trafficking and recycling [104], they have many components of the glutamatergic transmission machinery [108] and they bear P/Q-type voltage-gated calcium channels [109]. The latter are normally found in excitable cells and reveal synaptic capability, since quick calcium currents are believed to be involved in cell depolarisation and neurotransmitter release. Thus MC are the only excitable cells within the epidermis, in addition to neurones. Nevertheless there is still a lack of structural evidence of a synaptic connexion, identification of neurotransmitters and the

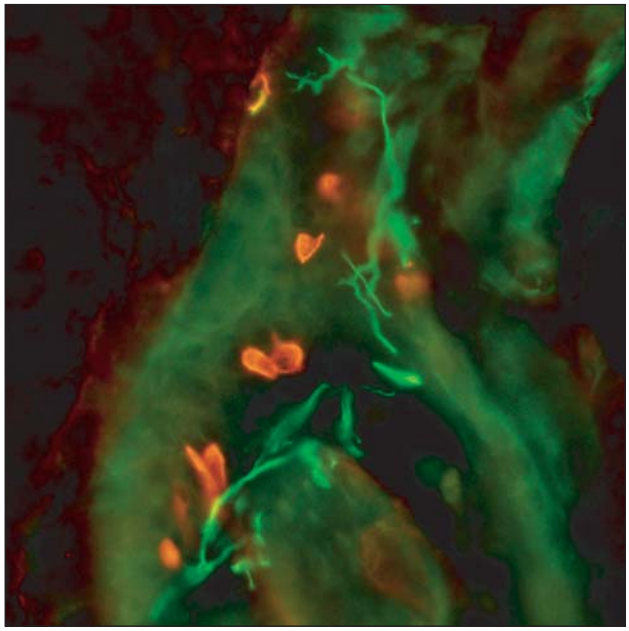

Figure 4. The close association of Merkel cells and sensory neurones forms the Merkel cell-neurite complex which acts as a slowly adapting type of mechanoreceptor. For the double immunofluorescence assay, we used a mouse monoclonal antibody against cytokeratin 20 (Progen, Ks20.2, 1:100), rabbit polyclonal anti-pan neurofilament (Cedarlane, 1:100), donkey anti-mouse TRITC-conjugated (Santa Cruz, sc-2300, 1:400) and donkey anti-rabbit FITC-conjugated (Santa Cruz, sc-2090, 1:400).

stimuli which activate MC [102, 110], and confirmation of synaptic transmission, and thus the neurosensory characteristics of MC.

Mechanoreceptive Aβ-neurones are the most represented subset of neurones supplying MC in the SAM-1. However, recent findings show that C and Aδ-fibres also innervate MC [21], demonstrating that the formation of the SAM is dependant on multiple neurotrophins and their receptors [111]. The presence of multiple nerve fibres in touch domes may suggest that MC can be implicated in many functions other than touch perception. Trophic roles onto sensory neurones, participation on the premise of the sub-epidermal plexus, keratinocytes proliferation and skin homeostasis are all expected, but we lack direct proof. Human epidermal MC do not express TRPV1 and SP and thus may not participate in the transduction of noxious stimulations. However, MC from the outer root sheath of hair follicles are different because they co-express them [33]. This could be explained by the need for hair follicles to keep an excellent sensitivity. In addition to their part in pain perception, TRPV1 and SP could be implicated in the maintenance of the homeostasis, as happens in hair cells of the organ of Corti [37, 112]. Hair follicles are among the most sensitive mechanoreceptors in the body and thus are frequently used to study mechanoreception. So MC from hair follicles would act in mechanosensation rather than in pain perception. Thus, it is important to be mindful of the polymodality of ion channels. Other ionic channels, like the osmotic receptor TRPV4 and the purinergic receptors P2Y2, are present on MC [42, 58]. Swelling-induced hypo-osmolarity may be able to activate MC through the TRPV4 receptor, while the P2Y2 receptors may mobilize the intracellular calcium required for cell excitability and neuropeptide release.

In our opinion, MC are excitable neurone-like cells which may respond to various stimuli. Few studies on MC are available, which can be explained by their minor representation in the epidermis. The discovery of one stretch-activated ion channel would support the idea that they are mechanosensory cells. The glutamatergic components present in MC (the mGluR5 receptor, subunits of the AMPA and NMDA receptors, VgluT1, 2 and 3 [58, 110, 113, 114]) reveal their capacity to modulate the excitability of neurones [63], rather than signal transduction. Furthermore, the glutamate receptors are more specific to post-synaptic elements than pre-synaptic ones [115]. However, they also should be capable of activating sensory neurones of the SAM following their depolarization and the release of their neurosecretory granules. Transduced information ranges from touch to hypo-osmolarity during inflammation. Hence, MC appear to be excitable cells enable to transduce stimuli toward several sensory nerve types and other epidermal cells, in a paracrine fashion. They act in touch perception directly or indirectly, but their involvement in other cutaneous functions remains to be seen.

Conclusion

The fundamental open question of whether epidermal cells transduce physical and chemical stimuli to nerve endings or if they only modulate the activity of sensory neurones, has been explored through the examination of sensory receptors. Ion channels have been discovered on epidermal cells: TRP, purinergic and Deg/ENa channels are putative transducers of touch, thermal sensation and nociception, as shown in invertebrate models and knockout mice. Thus they must start the signalling of the stimulus at the molecular level, based on their thermo-dynamical properties. Thereafter, the processes by which epidermal cells transmit the information to neurones remain to be explored. Merkel cells are excitable cells containing the molecular components of synaptic connections so they should transduce the stimuli synaptically. The mechanisms of communication between keratinocytes, Langerhans cells or melanocytes and sensory neurones are more mysterious. They are non-excitable cells with no molecular basis of synaptic connections. Paracrine function is supposed, but the mediator used to transmit rapid stimuli as fast as they occur must exhibit the characteristics of a neurotransmitter. It must be specific enough to carry a unique signal and quickly degraded to transmit a short stimulation. We have started to gain insight into this phenomenon so that some non-peptidic candidates are now being considered, like calcium, which can activate neighbouring cells, once released by keratinocytes.

Nowadays, we have no data about the role of epidermal sensor proteins in dermatological disorders. They are probably involved in all inflammatory diseases and may be implied in disorders of pigmentation or in skin dryness. In our opinion, they are the only molecules that could explain sensitive skin, as they can act by transforming physical OR chemical stimuli to inflammation.

Acceptance of the epidermis as a sensory and endocrine tissue as part of the NICS has increased, as some authors define skin as spread brain. However, the relationship between skin and brain, although fascinating, remains poorly understood. ■

Acknowledgements. *We thank the Laboratory of Pathology of the University Hospital of Brest for its help. Financial support: none. Conflict of interest: none.*

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