Immunoregulatory Effects of Neuropeptides on Endothelial Cells: Relevance to Dermatological Disorders

Devina Mehta Richard D. Granstein

Department of Dermatology, Weill Cornell Medicine, New York, NY, USA

Keywords

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Abstract

Many skin diseases, including psoriasis and atopic dermatitis, have a neurogenic component. In this regard, bidirectional interactions between components of the nervous system and multiple target cells in the skin and elsewhere have been receiving increasing attention. Neuropeptides released by sensory nerves that innervate the skin can directly modulate functions of keratinocytes, Langerhans cells, dermal dendritic cells, mast cells, dermal microvascular endothelial cells and infiltrating immune cells. As a result, neuropeptides and neuropeptide receptors participate in a complex, interdependent network of mediators that modulate the skin immune system, skin inflammation, and wound healing. In this review, we will focus on recent studies demonstrating the roles of α -melanocyte-stimulating hormone, calcitonin gene-related peptide, substance P, somatostatin, vasoactive intestinal peptide, pituitary adenylate cyclase-activating peptide, and nerve growth factor in modulating inflammation and immunity in the skin through their effects on dermal microvascular endothelial cells.

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Introduction

Inflammatory skin diseases are prevalent worldwide and have a serious impact on the quality of life of patients. However, treatment options remain limited with corticosteroids being the main topical treatment option. Considerable progress has been made in the past few years in understanding functions of the different cell types in the skin and the mechanisms by which they function to maintain immune homeostasis. Extensive crosstalk between these different cells, nerves and neuropeptides of the skin regulates local immune responses to maintain and restore homeostasis. The identification of neuropeptides as active components of the immunoregulatory network and their roles in the pathogenesis of skin inflammation are now well appreciated. Endothelial cells, which make up the lining of blood vessels and lymphatics in the dermis, play an important role in the cutaneous immune system. These include not only the recruitment and delivery of inflammatory cells to the skin, but also the release of cytokines and chemokines. Additionally, endothelial cells are present in secondary lymphoid organs [1], including skin-draining lymph nodes, and have immunoregulatory effects at those sites [2]. Blood vessels in the skin are intimately associated with sensory and sympathetic nerves as

are the secondary lymphoid organs [3–5]. Thus, an anatomical substrate is present for products of nerves to interact with endothelial cells in the skin and draining lymph nodes. Neuropeptides in the skin and lymph nodes are synthesized and released predominantly by small unmyelinated afferent neurons called C fibers and to a lesser extent by small myelinated A δ fibers [6, 7]. In addition, nonnerve cells in the skin can make some neuropeptides, as discussed below. Dermal endothelial cells are directly implicated as active components of the neuroregulatory network which has been implicated in the pathogenesis of inflammatory skin disorders. In this review, we discuss recent findings that highlight the complex regulatory roles of neuropeptides in skin immune responses and their potential role in chronic inflammation.

α -Melanocyte-Stimulating Hormone

α -Melanocyte-stimulating hormone (α -MSH) is a tri-decapeptide that is generated from the precursor hormone proopiomelanocortin via proteolytic cleavage [8]. Proopiomelanocortin generation is controlled by mediators such as corticotrophin-releasing hormone, exogenous noxious stimuli (ultraviolet irradiation, microbial agents), and pro-inflammatory cytokines (interleukin [IL]-1, tumor necrosis factor [TNF]- α as well as others) [9]. Proopiomelanocortin is produced by many cells in the skin including keratinocytes, Langerhans cells, melanocytes, and endothelial cells; after production it is cleaved to form adrenocorticotrophin hormone which upon proteolytic cleavage forms α -MSH [10].

α -MSH has long been known to have anti-inflammatory functions in the skin [11]. This anti-inflammatory role has been defined experimentally and confirmed through animal models of inflammation. Its anti-inflammatory effects on cells are broad – including inhibition of neutrophil migration, downregulation of pro-inflammatory cytokine production by mononuclear cells and keratinocytes, and downregulation of endothelial cell adhesion factors [11].

Previous studies have shown that α -MSH suppresses the sensitization and elicitation phases of the cutaneous immune response to the hapten sensitizers dinitrofluorobenzene and oxazolone and induces hapten-specific tolerance [12]. More importantly, it was discovered that this suppressed response could be abrogated by administration of antibodies against IL-10, strongly suggesting a vital role for IL-10 in the anti-inflammatory activities of α -MSH [13]. Interestingly, administration of α -MSH to

the central nervous system also suppressed the response in the skin to these haptens, and the response was abrogated by spinal cord transection [14]. This suggests involvement of descending anti-inflammatory neurogenic pathways.

It has recently been discovered that α -MSH downregulates IL-1, TNF- α , and lipopolysaccharide-induced expression of the adhesion molecules E-selectin, vascular cell adhesion molecule 1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1) by dermal microvascular endothelial cells through inhibiting the nuclear translocation of the transcription factor nuclear factor- κ B [10]. In vitro studies have shown this effect is dependent upon α -MSH binding to the melanocortin-1 receptor [15]. These adhesion molecules are crucial for diapedesis and activation of leukocytes [16]. An in vivo study using a model of lipopolysaccharide-induced cutaneous vasculitis (local Schwartzman reaction) showed that a single intraperitoneal injection of α -MSH was able to suppress the extravasation, inflammation, and hemorrhagic vascular damage typically expected, through its downregulation of vascular E-selectin and VCAM-1 [17].

Another recent finding is the role that α -MSH plays in angiogenesis. The increased vascularization and the angiogenesis that accompany chronic inflammation prolong and intensify the inflammatory response, which has researchers intrigued. It is believed that angiogenesis sustains inflammation by delivering oxygen and nutrients to support the increased metabolic needs of cells at sites of inflammation [18]. It has recently been shown that α -MSH inhibits angiogenesis by attenuating vascular endothelial growth factor (VEGF) signaling in endothelial cells [19]. This study showed that the binding of α -MSH to melanocortin receptors 1 and 2 caused inhibition of metalloproteinase secretion, inhibition of endothelial cell migration, and inhibition of tube formation of endothelial cells. It was further elucidated that α -MSH dose-dependently decreased expression of angiogenic VEGF and the VEGF receptor 2 (VEGFR-2) within and on the surface of endothelial cells. It is known that VEGFR-2 is the main receptor regulating the angiogenic function of VEGF in endothelial cells. Furthermore, it was shown that the downstream effector of VEGF, Akt, was inactivated. Of note, excessive VEGF supply did not substantially reverse the α -MSH-induced inhibition of angiogenesis in vitro [19]. This suggests that while inhibition of the VEGF/VEGFR-2/Akt pathway may contribute, there are other mechanisms involved in α -MSH inhibition of angiogenesis left to be discovered.

In vivo studies support the concept that α -MSH inhibition of VEGF/VEGFR-2/Akt plays a significant role in its antiangiogenic and anti-inflammatory effects. Murine models of inflammatory bowel disease showed that disease activity and tissue injury were suppressed by antiangiogenic agents or genetic overexpression of soluble VEGFR-1 (which is inhibitory in nature) and exacerbated by administration or genetic overexpression of VEGF-A [20]. In vivo studies have also demonstrated that pro-opiomelanocortin gene delivery reduced tumor angiogenesis in melanoma models [21].

Other recent in vivo studies confirmed the anti-inflammatory effect of α -MSH in animal models of noncutaneous inflammatory disorders such as asthma, inflammatory bowel disease, rheumatoid arthritis, ocular inflammation, and brain inflammation [14, 22–26]. Due to the overwhelming evidence that α -MSH is a potent anti-inflammatory mediator in both cutaneous and systemic diseases, significant efforts are being made to develop α -MSH analogues to deliver as a topical or systemic drug. The current focus is on the use of the α -MSH C-terminal peptide KPV or its derivative K(D)PT as these tripeptides do not have the melanotrophic effect of α -MSH, thus avoiding the potential side effect of undesired pigmentation [27]. However, further studies are needed to confirm that these tripeptides have equivalent potency of anti-inflammatory action.

Somatostatin

Somatostatin (SST) is a cyclic peptide secreted from neurons in the central and peripheral nervous system, monocytes in the skin [28], various cells within the gastrointestinal tract, the retina, and the pancreatic islets of Langerhans [29]. It travels systemically to exert a wide range of effects on the endocrine, gastrointestinal, cardiovascular, and nervous systems [30, 31]. SST has 5 receptors (SSTR_{1–5}) that not only vary in expression throughout the human body and skin, but also mediate different effects. For example, SSTR₂ and SSTR₅ are predominant in endocrine tissues [32] and SSTR₃ is the predominant receptor on endothelial cells [33]. The varying effects of these receptors are still an area of ongoing research with no clear consensus on the precise functions of each of the receptor subtypes. Additionally, SST receptors have been shown to have different levels of expression in different layers of the skin of patients with healthy skin, atopic dermatitis, and psoriasis [34]. However, it is still unclear what significance these differences in receptor expression have in pathological states.

SST is most known for its inhibition of pituitary and gastrointestinal hormone release, which makes it a first-line treatment for acromegaly and neuroendocrine tumors. SST analogues are thought to be effective for these tumors due not only to the inhibition of hormone release, but also to direct inhibition of cell proliferation. This antiproliferative effect is believed to be secondary to activation of SSTR₃ [35] which activates phosphotyrosine phosphatase activity to interfere with growth factor receptor signaling by dephosphorylating the growth factor-bound receptor [36].

It is also well known that SST plays a role in inflammation, which is predominantly inhibitory. SST and the analogue angiopeptin were shown to decrease the adhesion of monocytes to unstimulated and IL-1-stimulated endothelial cells by a cyclic adenosine monophosphate-dependent mechanism [37]. Exogenous administration of SST has been shown to inhibit vasodilation and plasma protein extravasation in response to neurogenic stimulation [38] through actions on its receptors. It has also been shown to inhibit the release of substance P [39] and calcitonin gene-related peptide (CGRP) [40] by nerve endings in the peripheral and central nervous system, thus, also limiting a pro-inflammatory vasodilation and plasma extravasation response. Additionally, SST has been shown to inhibit the secretion of interferon- γ from T cells [41], IgG and IgA from B cells [42], and the degranulation of mast cells [43]. Cortistatin, a neuropeptide named for its predominantly cerebral cortical expression, is structurally closely related to SST. It has been shown to be capable of binding SSTRs and shares many of the functional properties of SST [44]. It has been found to have equivalent inhibition potency for neurogenic inflammation but greater effects on the inhibition of cellular inflammation [40].

Recently, another effect of SST, which may play a role in its antitumor and anti-inflammatory effect, was elucidated. SST was shown to inhibit angiogenesis through binding to the SSTR₃ on endothelial cells, which led to downstream inhibition of the growth factor-stimulated, mitogen-activated protein kinase pathway and endothelial nitric oxide synthase activity [29]. This mechanism was elucidated in a study examining the ability of SST to inhibit the growth of established Kaposi's sarcoma (a highly vascularized lesion typically associated with HIV/AIDS) tumors in a murine model [33].

The majority of the SST analogues on the market today, such as octreotide and lanreotide, are utilized for hormone-dependent tumors or variceal bleeding and have significant receptor affinity for SSTR₂ [45]. How-

ever, interest is now growing in developing different analogues having actions preferentially at different receptor subtypes so that they may be used for the treatment of inflammatory disorders and diabetes-related conditions such as retinopathy and neuropathy [46]. More work elucidating the precise role and function of each SSTR subtype will be required to advance this goal.

Calcitonin Gene-Related Peptide

CGRP, a sensory neuropeptide, has been shown to have several important immunoregulatory effects. It is released from C and A δ nerve fibers in the skin and colocalizes with substance P in perivascular sensory nerves [28]. Initially it was noted that CGRP inhibited antigen presentation for certain immune responses [47]. However, it was subsequently found that exposure of Langerhans cells to CGRP not only inhibits antigen presentation for Th1-type immunity; it enhances presentation for Th2 responses [48]. CGRP also inhibits the activation of nuclear factor- κ B in human endothelial cells and the release of certain pro-inflammatory chemokines (IL-8, chemokine CCL2, chemokine CXCL1) in response to lipopolysaccharide [49], as well as increases the release of the inhibitory cytokine IL-10 from trigeminal glia [50] and macrophages [51]. It has also been found that CGRP suppresses the maturation of Langerhans cells, potentially through the suppression of the expression of chemokine receptor 2, a chemokine receptor speculated to play a role in the maturation and migration of Langerhans cells [52]. Another possibility is that CGRP inhibits Langerhans cell maturation and migration through induction of IL-10, which can in turn downregulate costimulatory molecules and the cytokines IL-12 and interferon- γ [53, 54]. This theory is supported by the observation that epidermal Langerhans cell migration is enhanced in IL-10 knockout mice [55].

The role of CGRP is quite complex and includes immunoregulatory activities with a shift from a Th1 response to a Th2 and Th17 response. In vitro, exposure of Langerhans cells to CGRP led to inhibition of antigen presentation for a Th1 response, while presentation for Th2 response was enhanced as evidenced by an increase in IL-4 (suppresses Th1 response) and decreased interferon- γ (supports Th1 response) [48]. Additionally, the stimulated production of Th1 chemokines CXCL9 and CXCL10 by Langerhans cells was reduced while the production of Th2 chemokines CCL17 and CCL22 was increased [48]. These results are supported by a similar

experiment in which receptor activity-modifying protein-1 knockout mice (this protein being a crucial component of the CGRP receptor) were used to support the finding that CGRP suppressed a Th1 contact hypersensitivity response, induced by the Th1 hapten sensitizer 2,4,6-trinitrochlorobenzene, and enhanced a Th2 contact hypersensitivity response, induced by the Th2 hapten sensitizer fluorescein isothiocyanate [52].

Recently, it has been found that CGRP may regulate the outcome of antigen presentation by Langerhans cells or bone marrow-derived dendritic cells to responsive T cells through actions on dermal microvascular endothelial cells. Evidence indicated that CGRP exposure increased IL-6 release from dermal microvascular endothelial cells and this, in turn, caused a shift in the outcome of antigen presentation towards inducing a Th17 response with increased IL-17A [56]. This was accompanied by inhibition of interferon- γ , IL-4, IL-22 expression and decreased levels of T-bet (transcription factor associated with Th1 response) and GATA3 (transcription factor associated with Th2 response) by responding T cells while retinoic acid receptor-related orphan receptor γ t mRNA (transcription factor associated with Th17 response) was increased. These findings were supported by in vivo studies where immunization at the site of intradermally administered CGRP led to a similar bias in CD4 $^{+}$ T cells from draining lymph node cells towards IL-17A and away from interferon- γ [56]. This mechanism may play a role in the expression of skin disorders in which Th17 cells have a pathophysiological role, such as psoriasis.

It has long been known that CGRP induces endothelium-dependent vasodilation through the activation of nitric oxide synthase and thus release of nitric oxide [57]. This vasodilation supports the delivery of immune cells to the site of inflammation. However, it has recently been discovered that CGRP also induces endothelial cell proliferation in a model of capsaicin-induced neurogenic inflammation [58]. As discussed above, persistent angiogenesis is a characteristic of chronic inflammation. An in vivo study showed that CGRP stimulated angiogenesis directly by activating CGRP receptors as endothelial cell proliferation was blunted by CGRP receptor antagonists [58]. Of note, these experiments examined blunted synovial endothelial cell proliferation following intra-articular CGRP antagonist injection, and further studies are needed to clarify whether a similar effect is seen in the dermal microvasculature. Similar blunting of endothelial cell proliferation was found in the synovium with administration of substance P and neurokinin 1 receptor (NK1R) antagonists [59].

There is considerable evidence suggesting that CGRP plays a significant role in the pathogenesis of psoriasis. It has been demonstrated that in psoriatic lesions there are increased numbers of CGRP-containing nerves in the epidermis [60], and endothelial cells in involved skin stain positive for CGRP on their surface [61]. Additionally, patients with psoriasis have higher plasma CGRP levels [62] which correlate with severity of the disease [63]. Also, in a murine model of psoriasiform dermatitis, in which the angiopoietin receptor Tie2 is overexpressed in keratinocytes, denervation of skin results in loss of the psoriasiform phenotype but CGRP and substance P administration each blunt the effects of denervation [64]. One possible mechanism under investigation is based on the observation that, in a murine model of psoriatic dermatitis induced by application of the Toll-like receptor 7 agonist imiquimod to murine skin, nociceptive neurons drive IL-23 release from dermal dendritic cells that then induce $\gamma\delta$ T cells to produce IL-17, resulting in psoriasiform skin inflammation [65]. There is evidence that nerve-derived CGRP induces that IL-23 release. Kashem et al. [66] describe a similar pathway in which *Candida albicans* acts as a stimulus for CGRP release from sensory neurons, which in turn binds receptors on CD301b+ dermal dendritic cells causing release of IL-23 that then induces $\gamma\delta$ T cells to produce IL-17. This study investigated the critical role of CGRP in *C. albicans* immunity, as IL-17 is required for resistance to this pathogen [67, 68]. They also found that ablation of sensory neurons increased susceptibility to *C. albicans* infection and was reversed by exogenous addition of CGRP [66].

CGRP has also been implicated in the pathogenesis of atopic dermatitis, which is, in part, a Th2-mediated disease. This is supported by evidence that CGRP-bearing nerve fibers are increased in lesions of atopic dermatitis [69] and affected individuals have greater circulating levels of CGRP [70].

Additional research on the role of CGRP on inflammatory skin disease is ongoing in our laboratory. Further elucidation of the role CGRP plays in these diseases might suggest novel therapeutic targets for inflammatory skin disorders such as psoriasis and atopic dermatitis.

Substance P

Substance P is a neuropeptide that belongs to the tachykinin neuropeptide family that includes neurokinin A and neurokinin B [71]. It plays an important role in the nervous system as a neurotransmitter and is thought to

generally enhance cellular immunity. Substance P mediates the majority of its effects through the NK1R which can be found throughout the nervous system and in peripheral tissues [72]. In addition to cutaneous nerve fibers, substance P is synthesized and released from mast cells, monocytes, and eosinophils within the skin [28]. Substance P, along with IL-17A, has been shown to be significantly elevated in the plasma of eczema patients [73]. Additionally, the percentages of substance P and NK1R-expressing peripheral blood leukocytes (including monocytes, helper T cells, natural killer T cells, and basophils) were elevated in eczema patients as were the number of substance P-expressing mast cells in lesional skin [73].

Previous studies have shown that substance P induces mast cell degranulation and release of histamine [74] and TNF- α [75]. It has also been shown that substance P induces dermal microvascular endothelial cells to express ICAM-1 and VCAM-1 through binding to NK1R, which in turn activates the expression of the transcription factor nuclear factor- κ B [76]. More recently, studies have shown that substance P also induces the expression of another endothelial cell adhesion molecule, P-selectin, in a dose-dependent manner [77]. Endothelial P-selectin is thought to play a critical role in leukocyte, especially eosinophil, recruitment [78]. High levels of P-selectin expression on dermal vessels in lesional skin have been observed in urticaria and atopic dermatitis and, to a lesser extent, in psoriasis vulgaris and pustulosis palmaris et plantaris. Substance P was found to induce P-selectin expression through increased translocation of the transcription factor *signal transducer and activator of transcription 6* (STAT6) into the nuclei of dermal microvascular endothelial cells. Treatment of these cells with STAT6 decoy oligodeoxynucleotides significantly inhibited P-selectin expression, thus indicating that STAT6 plays a key role in this mechanism [77]. Of note, STAT6 is a transcription factor in IL-4/IL-13 signaling pathways, which have been implicated in atopic diseases including atopic dermatitis [79].

Similar to CGRP, it has long been known that substance P induces endothelium-dependent vasodilation through the activation of nitric oxide synthase and thus release of nitric oxide [57]. Substance P also appears to play a role in angiogenesis, as in vivo studies examining impaired wound healing in diabetics found that subcutaneous injection of substance P into both normal mice [80] and type 2 diabetic model mice [81] accelerated wound closing through enhanced angiogenesis [80, 81] and myofibroblast-mediated wound contracture [81]. It is thought

that the angiogenesis is in part the result of substance P-induced production and migration of endothelial cell progenitor cells from the bone marrow into injured peripheral tissue [82], as well as dermal microvascular endothelial cell proliferation [83]. The observation that substance P improves wound healing has been made previously [84] but the ability to induce angiogenesis may, in part, be responsible for this phenomenon. Also, as discussed above, the connection between angiogenesis and chronic inflammation has come to light.

Pituitary Adenylate Cyclase-Activating Peptide/ Vasoactive Intestinal Peptide

Pituitary adenylate cyclase-activating peptide (PACAP) is a regulatory peptide that belongs to the vasoactive intestinal peptide (VIP)/secretin family [85]. PACAP and VIP are structurally related peptides which are synthesized and released by immunoreactive fibers in the skin [86–88] as well as mast cells and polymorphonuclear leukocytes [28]. PACAP and VIP exert their effects through PACAP/VIP G protein-coupled receptors, VIP 1 and 2 receptors (VPAC1R, VPAC2R, respectively) and pituitary adenylate cyclase-activating polypeptide type 1 receptor (PAC1R). While VPAC1R and VPAC2R have high affinity for both VIP and PACAP, the PAC1R is selective for PACAP. These receptors have varying levels of expression throughout the skin. Endothelial cells were found to express both VPAC1R and VPAC2R [89] and PACAP-immunoreactive nerve fibers are found in close proximity to dermal blood vessels [90] and mast cells [91]. Of particular interest, VPAC1R expression was not seen in the dermal microvascular endothelial cells of healthy skin but was significant in dermal microvascular endothelial cells of atopic dermatitis and urticaria patients [91]. Additionally, PACAP concentration was increased in the skin of psoriasis and urticaria patients [90], and serum VIP levels are increased in patients with atopic dermatitis [92] and psoriasis [57]. Both VIP and PACAP were significantly elevated in the lesional skin of all subtypes of rosacea patients [93].

PACAP and VIP are thought to be involved in the vasoregulation of normal and diseased skin [94]. Specifically, it appears that PACAP and VIP contribute to the vasodilation and edema seen in the early phase of inflammation in an effort to recruit leukocytes to the site of inflammation [91]. When PACAP was administered intradermally into normal human skin, a dose-dependent and significant wheal and flare reaction developed within

minutes [91]. This finding is consistent with previous studies that showed PACAP-mediated vasodilation and plasma extravasation in rodents after injection into the skin resulting in edema and erythema [95]. Also, when administered intravenously to humans, PACAP caused a significant flushing response of the upper trunk and face within minutes but no systemic side effects (hypotension, tachycardia, diarrhea, increased bowel sounds). This suggests that the cutaneous vascular system in human skin has increased sensitivity to PACAP [91] versus the systemic vasculature [91]. However, systemic PACAP did induce hyperthermia, which is thought to be due to a cyclooxygenase-dependent central pathway [96].

In vitro studies also showed that PACAP treatment led to a pronounced increase in endothelial cell proliferation with expansion in the number of vessel cross-sections in vitro, thus indicating a proangiogenic effect of PACAP [91]. VIP has also been found to increase VEGF expression in endothelial cells and promotes proliferation and angiogenesis [97, 98].

Both PACAP and VIP bias Langerhans cell antigen presentation to responsive T cells towards Th17 and Th2 responses [99]. It was found that PACAP/VIP treatment of Langerhans cells prior to in vitro antigen presentation to CD4⁺ T cells enhances presentation for a Th17 response with increased IL-17A production and also increases the Th2 response with enhanced IL-4 production along with enhanced expression of receptor-related orphan receptor γ t and GATA3. These changes are accompanied by decreased Th1 type immunity with decreased production of interferon- γ and decreased expression of T-bet [99]. Additionally, separate in vivo studies showed that mice overexpressing VPAC2R in CD4⁺ T cells exhibit a Th2 preference while mice deficient in the receptor show a preference for Th1 response [100, 101]. However, the mechanism has not been further elucidated so the sequence of events by which PACAP and VIP produce these effects in vivo or whether they involve endothelial cells remains unknown. Figure 1 summarizes the current understanding of the effects of the neuropeptides CGRP, PACAP, and VIP on biasing Langerhans cell antigen presentation.

Nerve Growth Factor

Nerve growth factor is a neuropeptide that is part of a family of protein growth factors, collectively called neurotrophins. Other members of this family include brain-derived neurotrophic factor, neurotrophin 3, and neuro-

Fig. 1. Summary of the current understanding of the immunoregulatory effects of the neuropeptides CGRP, PACAP, and VIP on biasing Langerhans cell antigen presentation towards a Th2 and Th17 response and away from a Th1 response. CGRP, calcitonin gene-related peptide; IL-6, interleukin-6; PACAP, pituitary adenylate cyclase-activating peptide; Th1, T helper cell response type 1; Th2, T helper cell response type 2; Th17, T helper cell response type 17; VIP, vasoactive intestinal peptide.

phin 4/5. Neurotrophins were originally described as critical trophic factors involved in the regulation of neuronal development, survival, and function [102–105]. All members of the family share a similar structure and genes, but the specificity to their biological actions is conferred through their specificity for differing receptors. While they share affinity for the p75 receptor (proform), they have specificity for differing Trk receptors in their mature form (A, B, C) [105], which are generated from the pro-form upon a posttranscriptional proteolytic step [106]. In the skin, the main producer of nerve growth factor are keratinocytes [107], although fibroblasts [108, 109] and endothelial cells [110, 111] have also been shown to synthesize and release nerve growth factor. Nerve growth factor receptors have been seen in the skin [112] as well as primary and secondary lymphoid organs [113, 114].

Neurotrophins are now thought to also play an immunoregulatory role and have been implicated in the pathophysiology of many allergic conditions including allergic rhinitis, asthma, and atopic dermatitis. Elevated nerve growth factor levels are seen in these conditions [115–117], and nerve growth factor has been shown to mediate bronchial hyperresponsiveness and airway inflammation closely resembling asthma in animal studies [118, 119]. Brain-derived neurotrophic factor has been found to correlate with atopic dermatitis severity in human patients, as well as serum eosinophil cationic protein (a protein released upon eosinophil degranulation) and total serum IgE levels in these patients [120]. Additionally, elevated expression of nerve growth factor has been seen in peripheral blood eosinophils of human patients [121] and in the diseased epidermis and dermis in the NC/Nga

mouse model of atopic dermatitis [122]. Also of note, the onset of the pruritic lesions of atopic dermatitis was associated with increased TrkA receptor (specific for nerve growth factor) expression on keratinocytes [112].

Neurotrophins have also been detected within vascular endothelial cells [123], and neurotrophin receptors are expressed on the intima and adventitia [124]. Neurotrophins have been found to promote endothelial barrier permeability, plasma extravasation, cellular infiltration, and the release of pro-inflammatory cytokines [125]. Specifically, brain-derived neurotrophic factor and nerve growth factor have been found to increase nitric oxide production, endothelial cell proliferation and survival [126] and intensify endothelin-1-induced contractions [127].

Interestingly, neurotrophin production is affected by ultraviolet radiation, a known inflammatory stimulus. Ultraviolet B radiation is a stimulus for increased nerve growth factor release from keratinocytes and results in mast cell degranulation [128]. It is thought that this effect is indirect and is actually due to nerve growth factor-induced release of CGRP and substance P from peripheral sensory C fibers [129] which then induce the degranulation of mast cells.

Conclusions and Future Perspectives

The various mechanisms by which neuropeptides are involved in the induction and maintenance of autoimmune diseases are being intensively investigated. It is well established that some neuropeptides possess pro-inflammatory properties whereas others inhibit inflammation (Table 1).

Table 1. Summary of the current understanding of neuropeptide immunoregulatory effects on endothelial cells

	Neuropeptide effects on endothelial cells	Model system
α -MSH	↓expression of endothelial cell adhesion factors (E-selectin, ICAM-1, VCAM-1) ↓angiogenesis via decreased VEGF/VEGFR-2/Akt signaling	in vitro, in vivo (H) [17] in vivo, ex vivo (M) [19]
SST, cortistatin	↓adhesion of monocytes to endothelial cells ↓vasodilation and plasma protein extravasation via decreased endothelial NOS ↓angiogenesis via decreased MAPK signaling	in vitro (M) [37] in vitro (M) [33] in vitro (M) [33]
CGRP	↑antigen presentation for Th17 responses but ↓antigen presentation for Th2 responses via effects on endothelial cells ↑vasodilation via increased endothelial NOS ↑angiogenesis via increased endothelial cell proliferation	in vitro, in vivo (M) [56] in vitro (H) [57] in vivo (M) [58]
SP	↑expression of endothelial cell adhesion factors (P-selectin, ICAM-1, VCAM-1) ↑vasodilation via increased endothelial NOS ↑angiogenesis via increased endothelial cell proliferation	in vitro (H) [76, 77] in vitro (H) [57] in vivo (M) [80, 81]
PACAP	↑vasodilation (mechanism unclear) ↑angiogenesis (mechanism unclear) ↑antigen presentation for Th2 and Th17 responses	in vivo (H) [91] in vivo (H) [91] in vitro, in vivo (M) [98]
VIP	↑angiogenesis via increased VEGF expression ↑antigen presentation for Th2 and Th17 response	in vivo (M) [97] in vitro (M) [98]
NGF	↑angiogenesis via increased endothelial cell proliferation ↑expression of endothelial cell adhesion factor ICAM-1 ↑endothelial barrier permeability via increased endothelin-1-induced contractions	in vitro (H) [125] in vitro (H) [125] in vitro (M) [126]

H, human; M, mouse; α -MSH, α -melanocyte-stimulating hormone; SST, somatostatin; CGRP, calcitonin gene-related peptide; SP, substance P; PACAP, pituitary adenylate cyclase-activating peptide; VIP, vasoactive intestinal peptide; NGF, nerve growth factor; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor 2; NOS, nitric oxide synthase; MAPK, mitogen-activated protein kinase.

The finding that inflammatory skin disorders such as psoriasis and atopic dermatitis have altered levels of the neuropeptides discussed above in either the lesional skin or plasma of diseased patients support the concept of a pathophysiological role for these neuropeptides in inflammatory skin disorders. Despite the significant progress in our understanding of neuropeptide-induced signaling in endothelial cells, a full understanding of neuropeptide actions and their roles in inflammatory skin diseases have yet to be fully elucidated. Future translational and clinical investigations are needed to also define the role and contribution of impaired endothelial cell function in immune-mediated skin diseases. Finally, there is a growing emphasis on the relationship between the complexity and diversity of the skin microbiota and health/disease, including skin disorders. It will also be important to study effects of neuropeptides on host-mi-

croorganism interactions in controlling local immune responses. With the use of α -MSH and SST analogues for the possible treatment of cutaneous inflammatory disorders already being investigated, further understanding of neuropeptide actions and their roles in inflammatory skin diseases may allow for the development of additional neuropeptide-specific therapeutics and the development of novel neuroimmune-modulating drugs.

Key Message

Neuropeptides modulate cutaneous immunity through actions on endothelial cells illustrating a pathway by which nerves may influence inflammatory and immune-mediated skin disorders.

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Author Contributions

D.M. wrote the first draft, and R.D.G. edited, proofread the paper, and rewrote sections.

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References

- 1 Podgrabinska S, Braun P, Velasco P, Kloos B, Pepper MS, Skobe M. Molecular characterization of lymphatic endothelial cells. *Proc Natl Acad Sci USA*. 2002 Dec;99(25):16069–74.
- 2 Kedl RM, Tamburini BA. Antigen archiving by lymph node stroma: A novel function for the lymphatic endothelium. *Eur J Immunol*. 2015 Oct;45(10):2721–9.
- 3 Dalsgaard CJ, Jonsson CE, Hökfelt T, Cuello AC. Localization of substance P-immunoreactive nerve fibers in the human digital skin. *Experientia*. 1983 Sep;39(9):1018–20.
- 4 Dalsgaard CJ, Björklund H, Jonsson CE, Hermansson A, Dahl D. Distribution of neurofilament-immunoreactive nerve fibers in human skin. *Histochemistry*. 1984;81(2):111–4.
- 5 Bellinger DL, Lorton D, Felten SY, Felten DL. Innervation of lymphoid organs and implications in development, aging, and autoimmunity. *Int J Immunopharmacol*. 1992 Apr;14(3):329–44.
- 6 Lawson SN. Peptides and cutaneous polymodal nociceptor neurones. *Prog Brain Res*. 1996;113:369–85.
- 7 Fink T, Weihe E. Multiple neuropeptides in nerves supplying mammalian lymph nodes: messenger candidates for sensory and autonomic neuroimmunomodulation? *Neurosci Lett*. 1988 Jul;90(1-2):39–44.
- 8 Rousseau K, Kauser S, Pritchard LE, Warhurst A, Oliver RL, Slominski A, et al. Pro-opiomelanocortin (POMC), the ACTH/melanocortin precursor, is secreted by human epidermal keratinocytes and melanocytes and stimulates melanogenesis. *FASEB J*. 2007 Jun;21(8):1844–56.
- 9 Böhm M, Luger TA, Tobin DJ, García-Borrón JC. Melanocortin receptor ligands: new horizons for skin biology and clinical dermatology. *J Invest Dermatol*. 2006 Sep;126(9):1966–75.
- 10 Brzoska T, Luger TA, Maaser C, Abels C, Böhm M. Alpha-melanocyte-stimulating hormone and related tripeptides: biochemistry, antiinflammatory and protective effects in vitro and in vivo, and future perspectives for the treatment of immune-mediated inflammatory diseases. *Endocr Rev*. 2008 Aug;29(5):581–602.
- 11 Redondo P, García-Foncillas J, Okroujnov I, Bandrés E. Alpha-MSH regulates interleukin-10 expression by human keratinocytes. *Arch Dermatol Res*. 1998 Aug;290(8):425–8.
- 12 Rheins LA, Coteleur AL, Kleier RS, Hoppenjans WB, Saunderson DN, Nordlund JJ. Alpha-melanocyte stimulating hormone modulates contact hypersensitivity responsiveness in C57/BL6 mice. *J Invest Dermatol*. 1989 Oct;93(4):511–7.
- 13 Grabbe S, Bhardwaj RS, Mahnke K, Simon MM, Schwarz T, Luger TA. alpha-Melanocyte-stimulating hormone induces hapten-specific tolerance in mice. *J Immunol*. 1996 Jan;156(2):473–8.
- 14 Lipton JM, Ceriani G, Macaluso A, McCoy D, Carnes K, Biltz J, et al. Antiinflammatory effects of the neuropeptide alpha-MSH in acute, chronic, and systemic inflammation. *Ann NY Acad Sci*. 1994 Nov;741(1):137–48.
- 15 Yang Y, Zhang W, Meng L, Yu H, Lu N, Fu G, et al. Alpha-melanocyte stimulating hormone inhibits monocytes adhesion to vascular endothelium. *Exp Biol Med (Maywood)*. 2015 Nov;240(11):1537–42.
- 16 Muller WA. Getting leukocytes to the site of inflammation. *Vet Pathol*. 2013 Jan;50(1):7–22.
- 17 Scholzen TE, Sunderkötter C, Kalden DH, Brzoska T, Fastrich M, Fisbeck T, et al. Alpha-melanocyte stimulating hormone prevents lipopolysaccharide-induced vasculitis by down-regulating endothelial cell adhesion molecule expression. *Endocrinology*. 2003 Jan;144(1):360–70.
- 18 Costa C, Incio J, Soares R. Angiogenesis and chronic inflammation: cause or consequence? *Angiogenesis*. 2007;10(3):149–66.
- 19 Weng WT, Huang SC, Ma YL, Chan HH, Lin SW, Wu JC, et al. α -Melanocyte-stimulating hormone inhibits angiogenesis through attenuation of VEGF/VEGFR2 signaling pathway. *Biochim Biophys Acta*. 2014 Jun;1840(6):1850–60.
- 20 Cromer WE, Mathis JM, Granger DN, Chaitanya GV, Alexander JS. Role of the endothelium in inflammatory bowel diseases. *World J Gastroenterol*. 2011 Feb;17(5):578–93.
- 21 Liu GS, Tsai HE, Weng WT, Liu LF, Weng CH, Chuang MR, et al. Systemic pro-opiomelanocortin expression induces melanogenic differentiation and inhibits tumor angiogenesis in established mouse melanoma. *Hum Gene Ther*. 2011 Mar;22(3):325–35.
- 22 Han D, Tian Y, Zhang M, Zhou Z, Lu J. Prevention and treatment of experimental autoimmune encephalomyelitis with recombinant adeno-associated virus-mediated alpha-melanocyte-stimulating hormone-transduced PLP139-151-specific T cells. *Gene Ther*. 2007 Mar;14(5):383–95.
- 23 Ceriani G, Macaluso A, Catania A, Lipton JM. Central neurogenic antiinflammatory action of alpha-MSH: modulation of peripheral inflammation induced by cytokines and other mediators of inflammation. *Neuroendocrinology*. 1994 Feb;59(2):138–43.
- 24 Nishida T, Miyata S, Itoh Y, Mizuki N, Ohgami K, Shiratori K, et al. Anti-inflammatory effects of alpha-melanocyte-stimulating hormone against rat endotoxin-induced uveitis and the time course of inflammatory agents in aqueous humor. *Int Immunopharmacol*. 2004 Aug;4(8):1059–66.

- 25 Cai S, Yang Q, Hou M, Han Q, Zhang H, Wang J, et al. A-Melanocyte-Stimulating Hormone Protects Early Diabetic Retina from Blood-Retinal Barrier Breakdown and Vascular Leakage via MC4R. *Cell Physiol Biochem*. 2018;45(2):505–22.
- 26 Myklicki N, Herrmann AM, Schwab N, Deenen R, Sparwasser T, Limmer A, et al. Melanocortin-1 receptor activation is neuroprotective in mouse models of neuroinflammatory disease. *Sci Transl Med*. 2016 Oct; 8(362):362ra146.
- 27 Hadley ME, Dorr RT. Melanocortin peptide therapeutics: historical milestones, clinical studies and commercialization. *Peptides*. 2006 Apr;27(4):921–30.
- 28 Scholzen T, Armstrong CA, Bunnett NW, Luger TA, Olerud JE, Ansel JC. Neuropeptides in the skin: interactions between the neuroendocrine and the skin immune systems. *Exp Dermatol*. 1998 Apr-Jun;7(2-3): 81–96.
- 29 Kumar U, Grant M. Somatostatin and somatostatin receptors. *Results Probl Cell Differ*. 2010;50:137–84.
- 30 ten Bokum AM, Hofland LJ, van Hagen PM. Somatostatin and somatostatin receptors in the immune system: a review. *Eur Cytokine Netw*. 2000 Jun;11(2):161–76.
- 31 Pintér E, Helyes Z, Szolcsányi J. Inhibitory effect of somatostatin on inflammation and nociception. *Pharmacol Ther*. 2006 Nov;112(2): 440–56.
- 32 Patel YC. Somatostatin and its receptor family. *Front Neuroendocrinol*. 1999 Jul;20(3): 157–98.
- 33 Florio T, Morini M, Villa V, Arena S, Corsaro A, Thellung S, et al. Somatostatin inhibits tumor angiogenesis and growth via somatostatin receptor-3-mediated regulation of endothelial nitric oxide synthase and mitogen-activated protein kinase activities. *Endocrinology*. 2003 Apr;144(4):1574–84.
- 34 Hagströmer L, Emtestam L, Stridsberg M, Talme T. Expression pattern of somatostatin receptor subtypes 1–5 in human skin: an immunohistochemical study of healthy subjects and patients with psoriasis or atopic dermatitis. *Exp Dermatol*. 2006 Dec;15(12):950–7.
- 35 Zatelli MC, Tagliati F, Taylor JE, Piccin D, Culler MD, degli Uberti EC. Somatostatin, but not somatostatin receptor subtypes 2 and 5 selective agonists, inhibits calcitonin secretion and gene expression in the human medullary thyroid carcinoma cell line, TT. *Horm Metab Res*. 2002 May;34(5):229–33.
- 36 Pan MG, Florio T, Stork PJ. G protein activation of a hormone-stimulated phosphatase in human tumor cells. *Science*. 1992 May; 256(5060):1215–7.
- 37 Leszczynski D, Josephs MD, Fournier RS, Foegh ML. Angiopeptin, the octapeptide analogue of somatostatin, decreases rat heart endothelial cell adhesiveness for mononuclear cells. *Regul Pept*. 1993 Feb;43(3):131–40.
- 38 Lembeck F, Donnerer J, Barthó L. Inhibition of neurogenic vasodilation and plasma extravasation by substance P antagonists, somatostatin and [D-Met2, Pro5]enkephalinamide. *Eur J Pharmacol*. 1982 Nov;85(2): 171–6.
- 39 Sicuteri F, Rainò L, Geppetti P. Substance P and endogenous opioids: how and where they could play a role in cluster headache. *Cephalalgia*. 1983 Aug;3(1_suppl Suppl 1):143–5.
- 40 Markovics A, Szoke É, Sándor K, Börzsei R, Bagoly T, Kemény Á, et al. Comparison of the anti-inflammatory and anti-nociceptive effects of cortistatin-14 and somatostatin-14 in distinct in vitro and in vivo model systems. *J Mol Neurosci*. 2012 Jan;46(1):40–50.
- 41 Muscettola M, Grasso G. Neuropeptide modulation of interferon gamma production. *Int J Neurosci*. 1990 Apr;51(3-4):189–91.
- 42 Scicchitano R, Dazin P, Bienenstock J, Payan DG, Stanis AM. The murine IgA-secreting plasmacytoma MOPC-315 expresses somatostatin receptors. *J Immunol*. 1988 Aug; 141(3):937–41.
- 43 Saavedra Y, Vergara P. Somatostatin inhibits intestinal mucosal mast cell degranulation in normal conditions and during mast cell hyperplasia. *Regul Pept*. 2003 Mar;111(1-3):67–75.
- 44 Criado JR, Li H, Jiang X, Spina M, Huitrón-Reséndiz S, Liapakis G, et al. Structural and compositional determinants of cortistatin activity. *J Neurosci Res*. 1999 Jun;56(6):611–9.
- 45 Machado-Alba JE, Machado-Duque ME. Prescription patterns of long-acting somatostatin analogues. *SAGE Open Med*. 2017 Mar;5: 2050312117694795.
- 46 Grant MB, Caballero S. Somatostatin analogues as drug therapies for retinopathies. *Drugs Today (Barc)*. 2002 Nov;38(11):783–91.
- 47 Hosoi J, Murphy GF, Egan CL, Lerner EA, Grabbe S, Asahina A, et al. Regulation of Langerhans cell function by nerves containing calcitonin gene-related peptide. *Nature*. 1993 May;363(6425):159–63.
- 48 Ding W, Stohl LL, Wagner JA, Granstein RD. Calcitonin gene-related peptide biases Langerhans cells toward Th2-type immunity. *J Immunol*. 2008 Nov;181(9):6020–6.
- 49 Huang J, Stohl LL, Zhou X, Ding W, Granstein RD. Calcitonin gene-related peptide inhibits chemokine production by human dermal microvascular endothelial cells. *Brain Behav Immun*. 2011 May;25(4):787–99.
- 50 Vause CV, Durham PL. Calcitonin gene-related peptide differentially regulates gene and protein expression in trigeminal glia cells: findings from array analysis. *Neurosci Lett*. 2010 Apr;473(3):163–7.
- 51 Baliu-Piqué M, Jusek G, Holzmann B. Neuroimmunological communication via CGRP promotes the development of a regulatory phenotype in TLR4-stimulated macrophages. *Eur J Immunol*. 2014 Dec;44(12):3708–16.
- 52 Mikami N, Matsushita H, Kato T, Kawasaki R, Sawazaki T, Kishimoto T, et al. Calcitonin gene-related peptide is an important regulator of cutaneous immunity: effect on dendritic cell and T cell functions. *J Immunol*. 2011 Jun;186(12):6886–93.
- 53 Fox FE, Kubin M, Cassin M, Niu Z, Hosoi J, Torii H, et al. Calcitonin gene-related peptide inhibits proliferation and antigen presentation by human peripheral blood mononuclear cells: effects on B7, interleukin 10, and interleukin 12. *J Invest Dermatol*. 1997 Jan;108(1): 43–8.
- 54 Torii H, Hosoi J, Beissert S, Xu S, Fox FE, Asahina A, et al. Regulation of cytokine expression in macrophages and the Langerhans cell-like line XS52 by calcitonin gene-related peptide. *J Leukoc Biol*. 1997 Feb;61(2):216–23.
- 55 Wang B, Zhuang L, Fujisawa H, Shinder GA, Feliciani C, Shivji GM, et al. Enhanced epidermal Langerhans cell migration in IL-10 knockout mice. *J Immunol*. 1999 Jan;162(1): 277–83.
- 56 Ding W, Stohl LL, Xu L, Zhou XK, Manni M, Wagner JA, et al. Calcitonin gene-related peptide-exposed endothelial cells bias antigen presentation to CD4+ T cells toward a Th17 response. *J Immunol*. 2016 Mar;196(5):2181–94.
- 57 Bull HA, Hotherhall J, Chowdhury N, Cohen J, Dowd PM. Neuropeptides induce release of nitric oxide from human dermal microvascular endothelial cells. *J Invest Dermatol*. 1996 Apr;106(4):655–60.
- 58 Ashraf S, Mapp PI, Walsh DA. Contributions of angiogenesis to inflammation, joint damage, and pain in a rat model of osteoarthritis. *Arthritis Rheum*. 2011 Sep;63(9):2700–10.
- 59 Alexander SP, Mathie A, Peters JA. Guide to Receptors and Channels (GRAC), 5th edition. *Br J Pharmacol*. 2011 Nov;164 Suppl 1:S1–324.
- 60 Jiang WY, Raychaudhuri SP, Farber EM. Double-labeled immunofluorescence study of cutaneous nerves in psoriasis. *Int J Dermatol*. 1998 Aug;37(8):572–4.
- 61 He Y, Ding G, Wang X, Zhu T, Fan S. Calcitonin gene-related peptide in Langerhans cells in psoriatic plaque lesions. *Chin Med J (Engl)*. 2000 Aug;113(8):747–51.
- 62 Reich A, Orda A, Wiśnicka B, Szepietowski JC. Plasma concentration of selected neuropeptides in patients suffering from psoriasis. *Exp Dermatol*. 2007 May;16(5):421–8.
- 63 Gerbaud P, Guibourdenche J, Jarray R, Conti M, Palmic P, Leclerc-Mercier S, et al. APN/CD13 is over-expressed by psoriatic fibroblasts and is modulated by CGRP and IL-4 but not by retinoic acid treatment. *J Cell Physiol*. 2018 Feb;233(2):958–67.
- 64 Ostrowski SM, Belkadi A, Loyd CM, Diaconu D, Ward NL. Cutaneous denervation of psoriasisiform mouse skin improves acanthosis and inflammation in a sensory neuropeptide-dependent manner. *J Invest Dermatol*. 2011 Jul;131(7):1530–8.

- 65 Rioli-Blanco L, Ordovas-Montanes J, Perro M, Naval E, Thiriot A, Alvarez D, et al. Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation. *Nature*. 2014 Jun;510(7503):157–61.
- 66 Kashem SW, Riedl MS, Yao C, Honda CN, Vulchanova L, Kaplan DH. Nociceptive sensory fibers drive interleukin-23 production from CD301b+ dermal dendritic cells and drive protective cutaneous immunity. *Immunity*. 2015 Sep;43(3):515–26.
- 67 Gladiator A, Wangler N, Trautwein-Weidner K, LeibundGut-Landmann S. Cutting edge: IL-17-secreting innate lymphoid cells are essential for host defense against fungal infection. *J Immunol*. 2013 Jan;190(2):521–5.
- 68 Conti HR, Shen F, Nayyar N, Stocum E, Sun JN, Lindemann MJ, et al. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. *J Exp Med*. 2009 Feb;206(2):299–311.
- 69 Järvikallio A, Harvima IT, Naukkarinen A. Mast cells, nerves and neuropeptides in atopic dermatitis and nummular eczema. *Arch Dermatol Res*. 2003 Apr;295(1):2–7.
- 70 Hodeib A, El-Samad ZA, Hanafy H, El-Latief AA, El-Bendary A, Abu-Raya A. Nerve growth factor, neuropeptides and cutaneous nerves in atopic dermatitis. *Indian J Dermatol*. 2010 Apr-Jun;55(2):135–9.
- 71 Pennefather JN, Lecci A, Candenas ML, Patak E, Pinto FM, Maggi CA. Tachykinins and tachykinin receptors: a growing family. *Life Sci*. 2004 Feb;74(12):1445–63.
- 72 Garcia-Recio S, Gascón P. Biological and pharmacological aspects of the NK1-receptor. *BioMed Res Int*. 2015;2015:495704.
- 73 Zhan M, Zheng W, Jiang Q, Zhao Z, Wang Z, Wang J, et al. Upregulated expression of substance P (SP) and NK1R in eczema and SP-induced mast cell accumulation. *Cell Biol Toxicol*. 2017 Aug;33(4):389–405.
- 74 Ebertz JM, Hirshman CA, Kettelkamp NS, Uno H, Hanifin JM. Substance P-induced histamine release in human cutaneous mast cells. *J Invest Dermatol*. 1987 Jun;88(6):682–5.
- 75 Ansel JC, Kaynard AH, Armstrong CA, Ole-rud J, Bunnett N, Payan D. Skin-nervous system interactions. *J Invest Dermatol*. 1996 Jan;106(1):198–204.
- 76 Quinlan KL, Song IS, Naik SM, Letran EL, Olerud JE, Bunnett NW, et al. VCAM-1 expression on human dermal microvascular endothelial cells is directly and specifically up-regulated by substance P. *J Immunol*. 1999 Feb;162(3):1656–61.
- 77 Miyazaki Y, Satoh T, Nishioka K, Yokozeki H. STAT-6-mediated control of P-selectin by substance P and interleukin-4 in human dermal endothelial cells. *Am J Pathol*. 2006 Aug;169(2):697–707.
- 78 Satoh T, Kaneko M, Wu MH, Yokozeki H, Nishioka K. Contribution of selectin ligands to eosinophil recruitment into the skin of patients with atopic dermatitis. *Eur J Immunol*. 2002 May;32(5):1274–81.
- 79 Gandhi NA, Pirozzi G, Graham NM. Commonality of the IL-4/IL-13 pathway in atopic diseases. *Expert Rev Clin Immunol*. 2017 May;13(5):425–37.
- 80 Um J, Jung N, Chin S, Cho Y, Choi S, Park KS. Substance P enhances EPC mobilization for accelerated wound healing. *Wound Repair Regen*. 2016 Mar;24(2):402–10.
- 81 Um J, Yu J, Park KS. Substance P accelerates wound healing in type 2 diabetic mice through endothelial progenitor cell mobilization and Yes-associated protein activation. *Mol Med Rep*. 2017 May;15(5):3035–40.
- 82 Amadesi S, Reni C, Katore R, Meloni M, Oikawa A, Beltrami AP, Avolio E, Cesselli D, Fortunato O, Spinetti G, Ascione R, Cangiano E, Valgimigli M, Hunt SP, Emanuelli C, Madeddu P. Role for substance P-based nociceptive signaling in progenitor cell activation and angiogenesis during ischemia in mice and in human subjects. *Circulation*. 2012 Apr;125(14):1774–86, S1–19. doi:10.1161/CIRCULATIONAHA.111.089763
- 83 Ejaz A, LoGerfo FW, Khabbaz K, Pradhan L. Expression of Neuropeptide Y, Substance P, and their receptors in the right atrium of diabetic patients. *Clin Transl Sci*. 2011 Oct;4(5):346–50.
- 84 Delgado AV, McManus AT, Chambers JP. Exogenous administration of Substance P enhances wound healing in a novel skin-injury model. *Exp Biol Med (Maywood)*. 2005 Apr;230(4):271–80.
- 85 Miyata A, Arimura A, Dahl RR, Minamino N, Uehara A, Jiang L, et al. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem Biophys Res Commun*. 1989 Oct;164(1):567–74.
- 86 Schulze E, Witt M, Fink T, Hofer A, Funk RH. Immunohistochemical detection of human skin nerve fibers. *Acta Histochem*. 1997 Aug;99(3):301–9.
- 87 Savage MV, Brengelmann GL, Buchan AM, Freund PR. Cystic fibrosis, vasoactive intestinal polypeptide, and active cutaneous vasodilation. *J Appl Physiol (1985)*. 1990 Dec;69(6):2149–54.
- 88 Groneberg DA, Welker P, Fischer TC, Dinh QT, Grützkau A, Peiser C, et al. Down-regulation of vasoactive intestinal polypeptide receptor expression in atopic dermatitis. *J Allergy Clin Immunol*. 2003 May;111(5):1099–105.
- 89 Odum L, Petersen LJ, Skov PS, Ebskov LB. Pituitary adenylate cyclase activating polypeptide (PACAP) is localized in human dermal neurons and causes histamine release from skin mast cells. *Inflamm Res*. 1998 Dec;47(12):488–92.
- 90 Steinhoff M, Corvera CU, Thoma MS, Kong W, McAlpine BE, Caughey GH, et al. Proteinase-activated receptor-2 in human skin: tissue distribution and activation of keratinocytes by mast cell tryptase. *Exp Dermatol*. 1999 Aug;8(4):282–94.
- 91 Seeliger S, Buddenkotte J, Schmidt-Choudhury A, Rosignoli C, Shpacovitch V, von Arnim U, et al. Pituitary adenylate cyclase activating polypeptide: an important vascular regulator in human skin in vivo. *Am J Pathol*. 2010 Nov;177(5):2563–75.
- 92 Hon KL, Lam MC, Leung TF, Wong KY, Chow CM, Fok TF, et al. Are age-specific high serum IgE levels associated with worse symptomatology in children with atopic dermatitis? *Int J Dermatol*. 2007 Dec;46(12):1258–62.
- 93 Schwab VD, Sulk M, Seeliger S, Nowak P, Aubert J, Mess C, et al. Neurovascular and neuroimmune aspects in the pathophysiology of rosacea. *J Invest Dermatol Symp Proc*. 2011 Dec;15(1):53–62.
- 94 Roosterman D, Goerge T, Schneider SW, Bunnett NW, Steinhoff M. Neuronal control of skin function: the skin as a neuroimmunoenocrine organ. *Physiol Rev*. 2006 Oct;86(4):1309–79.
- 95 Cardell LO, Stjärne P, Wagstaff SJ, Agustí C, Nadel JA. PACAP-induced plasma extravasation in rat skin. *Regul Pept*. 1997 Aug;71(2):67–71.
- 96 Pataki I, Adamik A, Jászberényi M, Mácsai M, Telegdy G. Pituitary adenylate cyclase-activating polypeptide induces hyperthermia in the rat. *Neuropharmacology*. 2000 Apr;39(7):1303–8.
- 97 Valdehita A, Carmena MJ, Collado B, Prieto JC, Bajo AM. Vasoactive intestinal peptide (VIP) increases vascular endothelial growth factor (VEGF) expression and secretion in human breast cancer cells. *Regul Pept*. 2007 Dec;144(1–3):101–8.
- 98 Collado B, Carmena MJ, Clemente C, Prieto JC, Bajo AM. Vasoactive intestinal peptide enhances growth and angiogenesis of human experimental prostate cancer in a xenograft model. *Peptides*. 2007 Sep;28(9):1896–901.
- 99 Ding W, Manni M, Stohl LL, Zhou XK, Wagner JA, Granstein RD. Pituitary adenylate cyclase-activating peptide and vasoactive intestinal polypeptide bias Langerhans cell Ag presentation toward Th17 cells. *Eur J Immunol*. 2012 Apr;42(4):901–11.
- 100 Goetzl EJ, Voice JK, Shen S, Dorsam G, Kong Y, West KM, et al. Enhanced delayed-type hypersensitivity and diminished immediate-type hypersensitivity in mice lacking the inducible VPAC(2) receptor for vasoactive intestinal peptide. *Proc Natl Acad Sci USA*. 2001 Nov;98(24):13854–9.
- 101 Delgado M, Ganea D. Vasoactive intestinal peptide: a neuropeptide with pleiotropic immune functions. *Amino Acids*. 2013 Jul;45(1):25–39.
- 102 Bibel M, Barde YA. Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. *Genes Dev*. 2000 Dec;14(23):2919–37.

- 103 Dechant G, Barde YA. The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system. *Nat Neurosci*. 2002 Nov;5(11):1131–6.
- 104 Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci*. 2001;24(1):677–736.
- 105 Lu B, Pang PT, Woo NH. The yin and yang of neurotrophin action. *Nat Rev Neurosci*. 2005 Aug;6(8):603–14.
- 106 Lee R, Kermani P, Teng KK, Hempstead BL. Regulation of cell survival by secreted pro-neurotrophins. *Science*. 2001 Nov;294(5548):1945–8.
- 107 Raychaudhuri SP, Jiang WY, Farber EM. Psoriatic keratinocytes express high levels of nerve growth factor. *Acta Derm Venereol*. 1998 Mar;78(2):84–6.
- 108 Hattori A, Iwasaki S, Murase K, Tsujimoto M, Sato M, Hayashi K, et al. Tumor necrosis factor is markedly synergistic with interleukin 1 and interferon-gamma in stimulating the production of nerve growth factor in fibroblasts. *FEBS Lett*. 1994 Mar;340(3):177–80.
- 109 Olgart C, Frossard N. Human lung fibroblasts secrete nerve growth factor: effect of inflammatory cytokines and glucocorticoids. *Eur Respir J*. 2001 Jul;18(1):115–21.
- 110 Tanaka A, Wakita U, Kambe N, Iwasaki T, Matsuda H. An autocrine function of nerve growth factor for cell cycle regulation of vascular endothelial cells. *Biochem Biophys Res Commun*. 2004 Jan;313(4):1009–14.
- 111 Moser KV, Reindl M, Blasig I, Humpel C. Brain capillary endothelial cells proliferate in response to NGF, express NGF receptors and secrete NGF after inflammation. *Brain Res*. 2004 Aug;1017(1-2):53–60.
- 112 Matsumura S, Terao M, Murota H, Katayama I. Th2 cytokines enhance TrkA expression, upregulate proliferation, and downregulate differentiation of keratinocytes. *J Dermatol Sci*. 2015 Jun;78(3):215–23.
- 113 Laurenzi MA, Barbany G, Timmusk T, Lindgren JA, Persson H. Expression of mRNA encoding neurotrophins and neurotrophin receptors in rat thymus, spleen tissue and immunocompetent cells. Regulation of neurotrophin-4 mRNA expression by mitogens and leukotriene B4. *Eur J Biochem*. 1994 Aug;223(3):733–41.
- 114 Ciriaco E, Dall'Aglio C, Hannestad J, Huerta JJ, Laurà R, Germanà G, et al. Localization of Trk neurotrophin receptor-like proteins in avian primary lymphoid organs (thymus and bursa of Fabricius). *J Neuroimmunol*. 1996 Sep;69(1-2):73–83.
- 115 Bonini S, Lambiase A, Bonini S, Angelucci F, Magrini L, Manni L, et al. Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. *Proc Natl Acad Sci USA*. 1996 Oct;93(20):10955–60.
- 116 Sanico AM, Stanisz AM, Gleeson TD, Bora S, Proud D, Bienenstock J, et al. Nerve growth factor expression and release in allergic inflammatory disease of the upper airways. *Am J Respir Crit Care Med*. 2000 May;161(5):1631–5.
- 117 Toyoda M, Nakamura M, Makino T, Hino T, Kagoura M, Morohashi M. Nerve growth factor and substance P are useful plasma markers of disease activity in atopic dermatitis. *Br J Dermatol*. 2002 Jul;147(1):71–9.
- 118 Braun A, Appel E, Baruch R, Herz U, Botchkarev V, Paus R, et al. Role of nerve growth factor in a mouse model of allergic airway inflammation and asthma. *Eur J Immunol*. 1998 Oct;28(10):3240–51.
- 119 Páth G, Braun A, Meents N, Kerzel S, Quarcoo D, Raap U, et al. Augmentation of allergic early-phase reaction by nerve growth factor. *Am J Respir Crit Care Med*. 2002 Sep;166(6):818–26.
- 120 Fölster-Holst R, Papakonstantinou E, Rüdric U, Buchner M, Pite H, Gehring M, et al. Childhood atopic dermatitis-Brain-derived neurotrophic factor correlates with serum eosinophil cationic protein and disease severity. *Allergy*. 2016 Jul;71(7):1062–5.
- 121 Toyoda M, Nakamura M, Makino T, Morohashi M. Localization and content of nerve growth factor in peripheral blood eosinophils of atopic dermatitis patients. *Clin Exp Allergy*. 2003 Jul;33(7):950–5.
- 122 Tominaga M, Ozawa S, Ogawa H, Takamori K. A hypothetical mechanism of intraepidermal neurite formation in NC/Nga mice with atopic dermatitis. *J Dermatol Sci*. 2007 Jun;46(3):199–210.
- 123 Nakahashi T, Fujimura H, Altar CA, Li J, Kambayashi J, Tandon NN, et al. Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. *FEBS Lett*. 2000 Mar;470(2):113–7.
- 124 Ricci A, Greco S, Amenta F, Bronzetti E, Felici L, Rossodivita I, et al. Neurotrophins and neurotrophin receptors in human pulmonary arteries. *J Vasc Res*. 2000 Sep-Oct;37(5):355–63.
- 125 Liezmann C, Klapp B, Peters EM. Stress, atopy and allergy: A re-evaluation from a psychoneuroimmunologic perspective. *Dermatoendocrinol*. 2011 Jan;3(1):37–40.
- 126 Raychaudhuri SK, Raychaudhuri SP, Weltman H, Farber EM. Effect of nerve growth factor on endothelial cell biology: proliferation and adherence molecule expression on human dermal microvascular endothelial cells. *Arch Dermatol Res*. 2001 Jun;293(6):291–5.
- 127 Kim H, Li Q, Hempstead BL, Madri JA. Paracrine and autocrine functions of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in brain-derived endothelial cells. *J Biol Chem*. 2004 Aug;279(32):33538–46.
- 128 Gillardon F, Moll I, Michel S, Benrath J, Weihe E, Zimmermann M. Calcitonin gene-related peptide and nitric oxide are involved in ultraviolet radiation-induced immunosuppression. *Eur J Pharmacol*. 1995 Dec;293(4):395–400.
- 129 Hart PH, Finlay-Jones JJ, Gorman S. Photo-neuroimmunology: modulation of the neuroimmune system by UV radiation. In: Granstein RD, Luger TA, editors. *Neuroimmunology of the skin: basic science to clinical practice*. Berlin: Springer; 2009. p. 125–36.