Mast cells as targets of corticotropin-releasing factor and related peptides

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Several inflammatory skin conditions, including atopic dermatitis (AD) and psoriasis, are exacerbated by stress. Recent evidence suggests that crosstalk between mast cells, neurons and keratinocytes might be involved in such exacerbation. Mast cells are distributed widely in the skin, are present in increased numbers in AD and are located in close proximity to substance P- or neurotensin-containing neurons. Corticotropin-releasing factor (CRF), its structurally related peptide urocortin (Ucn) and their receptors are also present in the skin and their levels are increased following stress. Human mast cells synthesize and secrete both CRF and Ucn in response to immunoglobulin E receptor (FcεRI) crosslinking. Mast cells also express CRF receptors, activation of which leads to the selective release of cytokines and other pro-inflammatory mediators. Thus, we propose that CRF receptor antagonists could be used together with natural molecules, such as retinol and flavonoids, to inhibit mast cell activation and provide new therapeutic options for chronic inflammatory conditions exacerbated by stress.

Skin is the largest organ of the body and is in constant contact with the external environment. Common pathological states involving the skin include allergies, atopic dermatitis (AD) and psoriasis, whereas rare conditions of the skin include neurofibromatosis, scleroderma and systemic mastocytosis. Psychological factors increase the morbidity of allergic reactions and many dermatoses through activation of mast cells in the skin that lie in close proximity to a rich supply of sensory nerve endings [1]. Mast cells are derived from stem cells in the bone marrow and migrate into tissues where they are prominently located just below the dermal–epidermal junction; they mature, depending on the tissue, under the influence of stem cell factor (SCF), interleukin 3 (IL-3), IL-4 and IL-9 [2]. Mast cell infiltration and/or proliferation in the skin can be triggered by SCF released from fibroblasts and other immune cells, nerve growth factor (NGF) released from nerve endings, or RANTES (regulated on activation, normal T cells, expressed and secreted) [3]. Mast cells can also secrete SCF [4] and NGF [5], thus affecting their own growth and activation [6]. In addition to being activated by crosslinking of the immunoglobulin E (IgE) receptor (FcεRI), mast cells can also be activated by aggregated IgE through FcγRI, in a process that is augmented by the anaphylatoxin complement 3a (C3a) [7]. Mast cells can also enhance skin immune sensitization through an antigen-independent, but FcεRI-dependent, mechanism [8]. Moreover, increasing evidence indicates that mast cells release some of their mediators differentially without degranulation, as originally reported for 5-HT [9] and recently shown for IL-1, which induced selective release of IL-6 through a vesicular shuttle [10].

The cytokines expressed by mast cells are primarily pro-inflammatory or are necessary for innate immunity [e.g. IL-1, IL-6, IL-8 and tumor necrosis factor α (TNF-α) [2] (Table 1). In fact, mast cells are perhaps the largest contributor of pre-formed TNF-α in the body [11]. Both the expression and the synthesis of these cytokines, however, depend on: (i) the state of maturation of the mast cells; (ii) the location of mast cells within compartments of the same or different tissues [12]; and (iii) the type of cytokine(s) present during mast cell activation [12,13]. However, mast cells can also produce cytokines that are released from T helper 2 (Th2) cells (so-called Th2 cytokines), such as IL-4 and IL-13, which are present in increased levels in AD [14], and can facilitate the development of skin infections by inhibiting the production of anti-microbial peptides by keratinocytes [15].

In view of the ability of mast cells to alter their cytokine profile and secretory characteristics depending on the local tissue environment, they could have both physiological and pathological roles in, for example, innate and acquired immunity [2], inflammation [1], wound healing and tumor growth [16] (Figure 1).

A skin 'brain' and mast cells

It has often humorously been argued that the brain is an appendix of the skin! Recent evidence shows that many genes known to be expressed in the CNS are also

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Table 1. Mediators and diverse functions of mast cells[a]

Mediators	Main pathophysiological effects	Refs
Angiogenic factors		
Heparin, histamine, FGF-2, GM-CSF, IL-3, IL-8, PDGF, TGF-α, VEGF	Angiogenesis, mitogenesis, neovascularization, regulation of tissue perfusion, NGF stabilization, tumor growth and metastases	[16]
Growth and differentiation factors		
Chondroitin sulfate, CSF, EGF, FGF-2, 5, 7, 10, histamine, IL-3, IL-4, IL-8, NGF, PDGF, SCF, TGF-β, VEGF	Control of IgE synthesis, expression of MHC class II molecules, modulation of T- and B-cell responses, processing and presentation of antigens to T cells, tumor growth, wound healing	[2]
Inflammatory mediators		
Bradykinin, histamine, IL-1, IL-4, IL-6, IL-8, IL-13, LTB$_4$, LTC$_4$, PAF, PGD$_2$, 5-HT, TNF-α, tryptase	Acquired immunity to pathogens and bacterial cell-attachment blocking molecule secretion, FcϵRI-dependent immediate hypersensitivity reactions, initiation of immunity and host defenses, inflammation, leukocyte migration, leukocyte proliferation and activation, leukocyte chemotaxis, sensory nerve sensitization, platelet activation and 5-HT release, tumor cell apoptosis, tissue damage	[1]
Neuropeptides		
ACTH, CRF, urocortin, VIP	Inflammation, sensory nerve modulation, vasodilatation	[1]

[a]Abbreviations: ACTH, adrenocorticotropin hormone; CRF, corticotropin-releasing factor; CSF, colony stimulating factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; GM-CSF, granulocyte monocyte-colony stimulating factor; IL-3, interleukin 3; LTB$_4$, leukotriene B$_4$; NGF, nerve growth factor; PAF, platelet activating factor; PDGF, platelet-derived growth factor; PGD$_2$, prostaglandin D$_2$; SCF, stem cell factor; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial cell growth factor; VIP, vasoactive intestinal peptide.

expressed in vast epidermal domains of a hemichordate organism, suggesting the presence of a 'skin brain' [17]. Extensive neuroendocrinological associations in the skin have also been described [18]. Indeed, there are anatomical and functional interactions between peripheral nerves and mast cells in the skin [19]; studies suggest that such interactions are increased in AD, and plasma extravasation can be induced in the rat skin by antidromic stimulation of lumbosacral dorsal roots [20]. Neuronal stimulation, such as that occurring during stress, leads to secretion of many neuropeptides that can activate mast cells [1]. For example, substance P (SP) released from neurons and keratinocytes can participate in neurite–mast cell communication [21]. SP can either induce electrical responses in mast cells without degranulation [22] or lead to mast cell-dependent granulocyte infiltration directly through the synthesis of TNF-α or IL-8 by mast cells. Other neuropeptide triggers include NGF, neurotensin (NT), pituitary adenylate cyclase activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) [1]. Mast cell activation by neuropeptides appears to be mediated through specific receptors, such as receptors for NT [23] or tachykinin NK$_1$ receptors [24]. However, recent studies have shown that acute stress can activate dura mast cells and increase vascular permeability through activation of NK$_1$ receptors, without the requirement of SP [25]; these findings implicate the involvement of NK$_1$ receptor agonists other than SP (e.g. hemokinin) [26]. Neuropeptides can also activate mast cells in a receptor-independent manner by activating G proteins directly. Regardless of the mechanism of activation, mast cell-derived vasoactive, pro-inflammatory and neurosensitizing molecules could act on keratinocytes, endothelial cells or nerve endings to liberate additional molecules and lead to chronic inflammation and neuropathic hypersensitivity or pain (Figure 1). For example, the unique mast cell mediator tryptase can stimulate protease-activated receptor 2 (PAR2) and lead to widespread inflammation, such as

in psoriatic skin [27], and induce hyperexcitability of submucosal neurons [28].

The skin also contains the main components of a functional equivalent of the hypothalamic–pituitary–adrenal (HPA) axis [18]. Corticotropin-releasing factor (CRF) regulates the HPA axis through two main types of receptors, CRF$_1$ and CRF$_2$ receptors, both of which have also been identified outside the brain. CRF itself is also found outside the brain and has been postulated to have pro-inflammatory actions through the activation of mast cells [29]. Human mast cells were recently shown to be particularly rich in both CRF and the structurally related peptide urocortin (Ucn) [30], and express multiple CRF receptor isoforms [31], which suggests autocrine actions of CRF.

CRF and mast cell-dependent actions in the skin

There are fundamental differences between CRF signaling systems in human and rodent skin. Thus, although human skin expresses both CRF and Ucn mRNA, mouse skin cells produce only Ucn peptides; the most prevalent cutaneous stressor, UV radiation, can also stimulate CRF protein production by human skin cells [32–34]. The gene encoding CRF is not expressed in mouse skin but the peptide is delivered to the skin by nerve endings [35]. Furthermore, human epidermis expresses predominantly CRF$_1$ receptor mRNA, whereas CRF$_2$ receptor expression is restricted to some cells of the dermis (adnexal structures); this contrasts with mouse skin where both receptors are expressed equally [36]. Two additional forms of Ucn, Ucn-II and Ucn-III, have also been identified; both have potent stimulatory actions on CRF$_2$ receptors. Peripheral Ucn II mRNA in the mouse is expressed highly in skin and is apparently expressed equally in mouse and human skin [37].

Skin mast cells are juxtaposed to nerve endings during hair follicle formation and interestingly both CRF and CRF receptor expression in skin are associated with the hair cycle. Activation of CRF receptors could lead to local
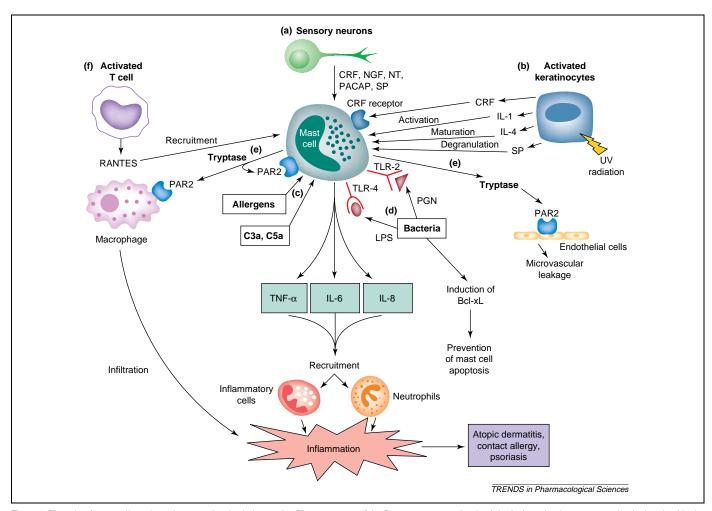
Figure 1. The role of mast cells and regulatory molecules in immunity. The upper part of the figure presents pathophysiological mechanisms proposed to be involved in the activation of skin mast cells. These include activation by: **(a)** sensory neurons that secrete several neuropeptides, such as corticotropin-releasing factor (CRF), nerve growth factor (NGF), neurotensin (NT), pituitary adenylate cyclase activating polypeptide (PACAP) and substance P (SP), that could activate mast cells alone or in association with each other; **(b)** keratinocytes activated by UV radiation to secrete CRF, SP and interleukin 1 (IL-1), which could all trigger mast cell activation and degranulation; in addition, IL-4 released from activated keratinocytes could induce maturation and upregulate CRF receptors and tachykinin NK_1 receptors on mast cells. Mast cells can, of course, be stimulated by **(c)** allergens through the immunoglobulin E receptor ($Fc\varepsilon RI$) and anaphylatoxins (complement fragments C3a and C5a), and by **(d)** bacteria through Toll-like receptor 2 (TLR-2) and TLR-4, which all (a–d) lead to the secretion of specific cytokines, chemokines and tumor necrosis factor α (TNF-α) from mast cells. Induction of Bcl-xL by bacteria prevents mast cell apoptosis. **(e)** Mast cell-derived tryptase can stimulate protease-activated receptor 2 (PAR2) on endothelial cells, leading to microvascular leakage, and could lead to further mast cell activation by a direct action on PAR2 on mast cells. This leads to the production of chemokines (IL-8) and cytokines (IL-6 and TNF-α) from mast cells and an inflammatory response. **(f)** In addition, activated T cells produce RANTES (regulated on activation, normal T cells, expressed and secreted), which recruits macrophages, mast cells and lymphocytes, but not neutrophils. Neutrophils are recruited by the CXC chemokine IL-8. The combined effect of these pro-inflammatory molecules, cells and receptors contributes to the development of atopic dermatitis, contact allergy and psoriasis. Abbreviations: LPS, lipopolysaccharide; PGN, peptidoglycan.

inflammation, as seen in alopecia, which is associated with increased numbers of activated mast cells. In fact, skin biopsies from affected scalp areas from patients with stress-induced alopecia areata exhibited increased expression of CRF_2 receptors only [38].

Acute stress can trigger mast cell degranulation [39] and lead to increased CRF peptide content [40] in rat skin. The former effect is blocked by depleting sensory nerves of their SP content by administration of capsaicin in neonates or by pretreatment with a NT receptor antagonist [1,39], implying the involvement of both SP and NT. Mouse dorsal root ganglia (DRG) express both CRF and NT precursor message; moreover, a NT receptor antagonist can block intradermal CRF-induced skin vascular permeability (J.M. Donelan *et al.*, unpublished). Intradermal administration of either CRF or Ucn activates skin mast cells and increases vascular permeability [29]. This effect is mast cell dependent because it does not occur in

mast cell-deficient mice, which have a mutation at the W/W^v c-kit locus. These effects are also receptor mediated because they are blocked by the CRF_1 receptor antagonist antalarmin [29,41]. These findings were confirmed independently both in the rat, where it was shown that the CRF_1 receptor was involved in stress-induced exacerbation of chronic contact dermatitis [42], and in human skin in which local administration of CRF induced vasodilatation [43] through mast cell-dependent mechanisms [44]. In addition to secreting CRF and Ucn [30], stimulated skin mast cells can also trigger the release of these peptides from DRG [45] and skin elements [18,40], further stimulating mast cells (Figure 2). Depending on the cell type and activation level, CRF could lead evidently to opposite results: CRF can stimulate nuclear factor κB (NF-κB) activity in human epidermal keratinocytes [46] but can inhibit the activity of NF-κB in human HaCaT keratinocytes [47]. Similarly, CRF induces NF-κB DNA
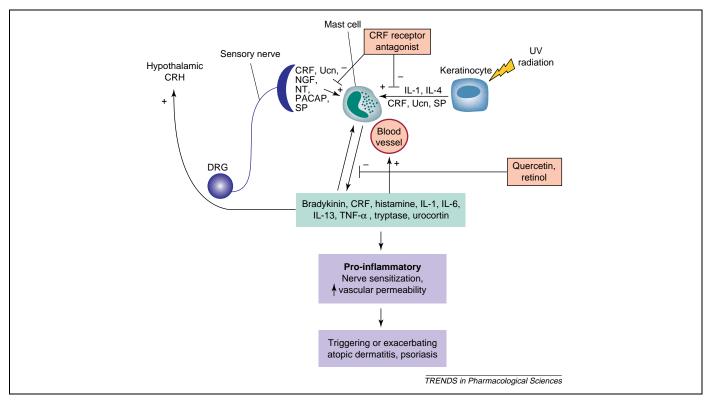
Figure 2. The hypothesized effect of stress on skin mast cells. Corticotropin-releasing factor (CRF) and urocortin (Ucn) secreted from dorsal root ganglia (DRG) or UV-activated keratinocytes stimulate mast cells alone or in association with other neuropeptides such as nerve growth factor (NGF), neurotensin (NT), pituitary adenylate cyclase activating polypeptide (PACAP) and substance P (SP). Mast cells then secrete CRF, histamine, interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), tryptase and Ucn (along with other mediators), which induce vascular permeability, leukocyte extravasation and inflammation. Histamine and cytokines can stimulate further local CRF and Ucn release by acting on the hypothalamus and on mast cells themselves. In addition, histamine, bradykinin and tryptase might further activate DRG. Pro-inflammatory mediators could then participate in skin physiology and pathology. CRF receptor antagonists could interfere with the pathological processes by blocking the action of CRF and related peptides, released from sensory neurons and activated keratinocytes, on mast cells. Natural molecules such as quercetin and retinol could inhibit mediator secretion from stimulated mast cells, preventing or limiting local inflammation.

binding activity in mouse thymocytes [48] but inhibits the same process in transfected pituitary AtT 20 cells [49]. Such opposing actions could be the result of the activation of different CRF receptors or CRF receptor isoforms. It was recently shown that CRF_{1e} receptors attenuated, whereas CRF_{1h} receptors amplified, $CRF_{1\alpha}$ receptor-induced cAMP production by Ucn in transfected COS cells [50]. Our preliminary results also indicate that activation of CRF_1 receptors leads to selective secretion of a growth factor whereas activation of CRF_2 receptors might lead to selective synthesis and release of a cytokine in human umbilical cord blood-derived cultured mast cells (J. Cao et al., unpublished).

Mast cells, keratinocytes and infections

Typically, chronic stress attenuates immune processes whereas acute stress appears to stimulate these processes. This latter effect appears to involve mast cell activation [29] and re-distribution of leukocytes from the blood to the skin, leading to enhanced delayed hypersensitivity reactions [1]. Recent findings indicate that mast cells might be crucial for defense against bacterial infections. For example, adherent Escherichia coli activates mouse mast cells in vitro, whereas W/W^v mast cell-deficient mice do not survive intraperitoneal inoculation with E. coli and cannot support leukocyte accumulation in lymph nodes [51]. Moreover, mast cell-deficient mice were shown to have an increased number of skin infections [15].

Mast cells appear to participate in bacterial infections through the activation of Toll-like receptors (TLR) (mammalian homologs of Drosophila Toll receptors), and have an important role in the innate immune response to bacterial challenge. TLR-4 is activated by E. coli lipopolysaccharide (LPS), whereas TLR-2 is activated by Staphylococcus aureus surface peptidoglycans (PGNs) [52]. Direct activation of TLR-2 on mast cells can provoke differential release of cytokines [53]. For example, one study showed that PGN, but not LPS, induced the generation of only IL-1β and leukotrienes from human mast cells [53], whereas other studies showed that both LPS and PGN induced significant release of TNF-α, IL-5, IL-10 and IL-13 from human cultured mast cells without degranulation.

Keratinocytes are probably the first cell type to encounter any infective microorganism or other environmental insult and lead to the stimulation of skin mast cells and activation of the local skin equivalent of the HPA axis. CRF and other stimuli can activate keratinocytes [35] to secrete IL-1, IL-6 and SP [18], which can further activate mast cells. Moreover, keratinocytes secrete IL-4, which induces functional NK_1 receptors on mast cells [24] and upregulates CRF_2 receptor expression on human mast cells [54]. Hypothalamic mast cells are located close to nerve endings that contain CRF and can be activated by acute stress [55]. Histamine, IL-1 and IL-6 derived

from skin mast cells can trigger further CRF release, leading to HPA activation, or can act as CRF-independent activators of the HPA axis [56] (Figure 2).

Conclusions and future directions

Mast cells have emerged recently as versatile effector cells in the regulation of numerous processes, including the regulation of immunity [2], inflammation, the blood–brain barrier [1] and cancer growth [16] (Figure 1). Skin and hypothalamic mast cells appear to have important physiological functions as sensors of stressful events with bidirectional regulation of the HPA axis; a local increase of the levels of CRF or Ucn in extracranial tissues under stress could adversely affect different disease states [1]. Perhaps a more appropriate name for CRF is 'stress-related factor' or 'stress-mediating factor' to indicate its multiple actions. Understanding CRF receptors [57] has led to the development of a variety of CRF receptor antagonists [58]; to date, many of these have been synthesized for the treatment of brain disorders, such as anxiety and Alzheimer's disease, but could be useful in the treatment of skin disorders, particularly if delivered locally. CRF receptor antagonists could be combined with natural substances that inhibit mast cells, such as retinoic acid (retinol) [59] or plant-derived flavonoids [60] (Figure 2), and provide new therapeutic options for skin conditions such as AD and psoriasis, both of which are made worse by stress.

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