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Allergic Contact Dermatitis: A Model of Inflammatory Itch and Pain in Human and Mouse

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Abstract

This chapter is an overview of published observations from our laboratory on the psychophysics and neurobiology of the persistent itch and pain of allergic contact dermatitis (ACD). ACD is a clinically significant problem with many features characteristic of other pruritic disorders. Our approach was to produce ACD experimentally in humans and in the mouse. The goal was to use the mouse as an animal model for investigating the peripheral neural mechanisms of itch and pain of ACD in humans. Humans and mice were each sensitized by cutaneous topical application of squaric acid dibutyl ester, a hapten not encountered in the environment. Subsequent challenge at another cutaneous site produced local inflammation ("ACD") with humans reporting persistent itch (lasting up to a week) and mice exhibiting persistent itch- and pain-like behaviors directed toward the ACD site. Enhanced mechanically evoked itch and pain in surrounding skin in humans were reversibly blocked by numbing the ACD site with cold, suggesting dependence on ongoing activity from the site. In mice, in vivo recordings revealed spontaneous activity in a subset of pruriceptive, mechanoheat-sensitive nociceptors with unmyelinated axons innervating the ACD site. These and a larger subpopulation of acutely dissociated small-diameter neurons innervating the ACD site exhibited an upregulation of the receptor CXCR3 and excitatory responses to one of its ligands, the chemokine CXCL10 (IP-10) that contributes to the pathogenesis of ACD. Preliminary findings point to possible therapeutic targets that could be investigated in inflammatory itch disorders in humans.

Keywords

Pain; Itch; Dermatitis; Nociceptor

2.1 Introduction

Persistent itch accompanying diseases of the skin and other organs can significantly impair the quality of life. The action potential activity in cutaneous nociceptors signals the presence, magnitude, and time course of chemical stimuli that elicit itch in humans and itch-like behavior in animals (LaMotte et al. 2014 for review). Nociceptors differ in their capacity to respond to different pruritic chemicals. For example, histamine elicits higher

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discharge rates in nociceptors that do not respond to noxious mechanical stimuli than those that do, whereas the reverse is true for the protease of cowhage (*Mucuna pruriens*) that evokes a histamine-independent itch (Schmelz et al. 1997; Johanek et al. 2008; Namer et al. 2008; LaMotte et al. 2009). In these experiments, the chemically evoked itch and nociceptor responses are typically transient (e.g. lasting less than half an hour). Less is known of nociceptor activity accompanying pathological itch that persists for days or longer.

One type of persistent itch is produced by allergic contact dermatitis (ACD), a disorder that affects a significant percentage of the population (Alikhan and Maibach 2014). ACD is a type IV delayed hypersensitivity reaction mediated by T lymphocytes specific for a substance, typically a chemical called a hapten, to which the individual has become sensitized. Immunological mechanisms of ACD have been intensively investigated (Christensen and Haase 2012), but studies of itch and nociceptive sensations accompanying ACD and the activity of cutaneous nociceptors within the area of inflammation are lacking. The present studies were designed to characterize the inflammatory itch and pain sensations accompanying ACD that is experimentally produced in humans using the contact sensitizer squaric acid dibutyl ester (SADBE). This hapten is not encountered in the environment but has been safely used in the clinic as an immunotherapeutic agent (Micali et al. 1996; Silverberg et al. 2000). Using the same model in mice, we observed that ACD evoked site-directed spontaneous itch- and pain-like behaviors and enhanced the excitability of certain cutaneous nociceptors.

2.2 ACD Produced a Persistent Itch and Enhanced Stimulus-Evoked Itch and Nociceptive Sensations

Eight healthy volunteers, four males and four females, participated in the study as described (Pall et al. 2015). Each was trained to use the generalized Labeled Magnitude Scale (gLMS) (Bartoshuk et al. 2004; Green et al. 1996; LaMotte et al. 2009) and then tested for their ratings of the perceived intensities of itch, pricking/ stinging, and burning in response to cowhage spicules and to one or more other pruritic chemicals. Then each subject was sensitized to the hapten squaric acid dibutyl ester (SADBE) in acetone vehicle to filter paper lined Finn chamber, 1.2 cm, applied to the lower back for 48 h. Other than a slight erythema, the skin site was asymptomatic. Two weeks later, a Finn chamber was applied to each volar forearm for a duration of 6 h, one chamber containing SADBE in acetone and the other only acetone as a vehicle control. The subjects took home copies of the gLMS scale and were instructed to mark on the scale their ratings of the perceived intensity of the greatest magnitude of itch, pricking/stinging, and burning occurring at the site of each Finn chamber for successive intervals of time starting after chamber removal and continuing up to 144 h. The subjects were instructed not to scratch or rub the affected skin.

All subjects experienced a skin reaction at the application site for SADBE but not vehicle alone. The reaction was characterized by erythema starting at 6 h and subsequently edema, vesiculations, and an increase in skin thickness, which reached a peak at 72 h. Similarly, there developed spontaneous itch at the SADBE challenge but not at the vehicle site. The itch reached a peak mean magnitude at 48 h and gradually disappeared within a week. Three

subjects reported occasional nociceptive sensations, and these were of lesser magnitude than the itch.

Within and surrounding the site of ACD, the skin exhibited abnormal sensations (dysesthesias) to mechanical stimuli manifested as itch to lightly stroking with a cotton swab (alloknesis) and greater than normal pain and itch (hyperalgesia and hyperkinesis) to indentations with von Frey filaments with tip diameters of 200 and 50 um, respectively. The borders of each area of dysesthesia were marked on the skin. The subjects then rated the magnitude of pain and itch evoked by the two types of von Frey filaments applied to multiple sites within the areas of hyperalgesia.

An ice-cold probe (1 cm diameter) was applied to the ACD site. When the skin under the probe became numb to mechanically evoked pricking, any ongoing spontaneous itch disappeared, the areas of dysesthesia were significantly reduced or eliminated, and the pricking evoked itch and pain within these former areas reduced to normal values. Upon rewarming the ACD site to normal skin temperature, the areas of dysesthesias and enhanced ratings of pricking-evoked itch and pain returned to former values. We hypothesized that the dysesthesias were dependent on ongoing neuronal activity that a) originated at the ACD site and b) was reduced or eliminated when the skin was anesthetized by cooling.

In three additional experiments, we tested whether ACD altered stimulus-evoked itch or nociceptive sensations in response to stimuli delivered to the site of ACD (vs. vehicle-treated site). In the first experiment, subjects were asked to judge the maximal perceived intensity of pricking pain evoked by von Frey stimuli, each having the same tip diameter of 200 um but differing in bending forces (5 to 180 mN). All but the lowest force elicited a significantly greater mean magnitude of pricking pain on the ACD vs. the control site.

In a second experiment, subjects rated the maximal itch, pricking, and burning elicited by noxious heat stimuli of 41 to 51 °C, each of 6 s duration and delivered on a base temperature of 38 °C. Not only did stimulus temperatures of 45 °C or greater evoke greater pricking pain on the ACD (vs control-) site, but each heat stimulus elicited itch as well. In contrast, itch was rarely reported during heat stimulation of the control site.

In the third experiment, subjects were asked to rate the perceived intensity of itch, pricking, and burning every 30 s after injection of a pruritic chemical into either the ACD or control sites: Histamine, bovine adrenal medulla (BAM8-22) peptide, or beta-alanine. Each pruritic chemical normally elicited a dominant sensation of itch that was accompanied by sensations (typically weaker) of pricking/ stinging and burning. But in comparison with ratings obtained from the control site, the peak magnitude of itch and the area under the curve plotting itch rating vs. time were each significantly greater in response to each pruritogen injected in the site of ACD. In contrast, there were no significant differences in the ratings of pricking or burning for the ACD vs. control sites.

Taken together, these psychophysical findings provide a preliminary characterization of the persistent abnormal itch and pain sensations that accompany ACD in humans. The findings are useful in cross-species comparisons between sensations in humans and both sensory behavior and underlying neural mechanisms observed in animals.

2.3 ACD Enhanced Itch- and Pain-Like Behaviors in Mice

The following procedures and findings are described in Qu et al. (2014). Wild-type C57BL/6 mice were sensitized to SADBE with a daily topical application of the hapten in a vehicle of acetone to abdominal skin for three consecutive days. Five days later, for different groups of mice, either the cheek or the calf of the hind paw was challenged with a topical application of SADBE ("ACD mice") or acetone alone (control mice) on two consecutive days. Behaviors directed toward the site of chemical application were video recorded for a 2-h period before the first challenge and again 24 h after the first and again 24 h after the second challenge. Similar to the spontaneous sensations reported by humans during ACD, spontaneous itch-like and pain-like behaviors of mice were directed toward the site of SADBE challenge and not toward the site of the application of vehicle alone. In comparison with control values obtained either in the prechallenge phase or on the vehicle site, the number of bouts of scratching the cheek with the hind limb (itch-like behavior) and wiping the check with the forelimb (pain-like behavior) significantly increased 24 h after the first or the second challenge. Similarly, for the calf, there was a significant increase in itch-like "biting" behavior (scraping or "scratching" the skin with the teeth) and also licking (pain-like behavior) directed toward the SADBE but not vehicle-application sites. Thus, both mice and humans exhibited spontaneous itch and nociceptive sensations or behaviors directed toward the ACD site. We hypothesize that one reason why mice exhibited more spontaneous pain (behavior) than humans (sensory reports) is because only the mice were allowed to scratch the ACD site thereby exacerbating the injury.

The effects of ACD on itch- and pain-like behaviors evoked by intradermal injection of a pruritic or algesic chemical into the cheek were tested by Fu et al. 2014. When injected into the vehicle (acetone-treated) site in control mice, histamine and BAM8-22 each elicited significantly more scratching in comparison with the effects of a saline injection but not more wiping. In contrast, the algesic chemical bradykinin evoked significantly more wiping but not scratching in comparison with saline. These findings are consistent with the sensory reports of humans, that is, histamine and BAM8-22 are primarily pruritic (Sikand et al. 2011) and bradykinin algesic (Hosogi et al. 2006). The similarities between sensory reports of humans and site- directed responses of mice support the validity of using the mouse as an animal model for studying neural mechanisms of itch and pain in humans. On the other hand, the minor nociceptive sensations of pricking/stinging and burning that humans report as accompanying an itch to histamine or BAM8-22 did not cause site-directed nociceptive behaviors in mice. Thus, the present sensory measures in mice do not reflect a one-to-one correspondence with all the qualities of sensation reported by humans in response to a pruritic chemical applied to normal skin.

When injected into the site of ACD, BAM8-22 evoked significantly more scratching (but not more wiping) than it did after injection into a vehicle-challenged site in accordance with findings we obtained from humans. However, ACD had no effect on either scratching or wiping responses to histamine, in contrast to our finding of the increased itch reported by humans. Again, while some behavioral findings translate from mice to humans, others may not.

Bradykinin elicited significantly more scratching (but not more wiping) when injected into the ACD (vs. acetone-control) site. Although we have yet to try bradykinin after ACD in humans, the findings of scratching accompanying wiping after ACD in mice are reminiscent of the reports of experimental studies of patients with atopic dermatitis. These patients report that mildly painful stimuli such as bradykinin applied to lesion sites elicit itch in addition to pain sensations (Hosogi et al. 2006).

2.4 ACD Enhanced the Excitability of Cutaneous Mechanosensitive C-nociceptors in Mice

Chemical stimuli that elicit itch sensation in humans or itch-like behavior in animals elicit action potential activity in certain types of cutaneous nociceptors (for review, LaMotte et al. 2014). These sensory neurons typically also encode the intensities of noxious cutaneous stimuli that elicit different ratings of pain in humans, suggesting that the same types of nociceptor may encode both pruritic and algesic stimuli. For example, unmyelinated peripheral nerve fibers (C-fibers) with cutaneous nociceptors are activated by certain histamine-independent pruritic agents such as cowhage, beta-alanine, or BAM8-22 (Han et al. 2013; Johanek et al. 2008; Liu et al. 2012; Ma et al. 2012; Namer et al. 2008; Wooten et al. 2014). This type of C-nociceptor also encodes with graded frequencies of discharge to the temperature of noxious heat or the force of punctate mechanical indentation that can elicit different incidences and magnitude of pain when applied to the human skin (Torebjork et al. 1984; Wang et al. 2015; Ziegler et al. 1999). In general, it appears that a smaller proportion of nociceptors are activated and/or the discharge frequencies are lower in response to a pruritic vs. a painful stimulus (e.g. Ma et al. 2012; Wooten et al. 2014). These differences in encoding of pruritic vs. algesic stimuli appear to hold as well in the responses of projection neurons in the spinal dorsal horn (Davidson et al. 2012).

Experiments were designed to test whether mechanosensitive C-nociceptive neurons innervating an area of ACD (neurons of “ACD mice”) were more excitable than those terminating in healthy, vehicle-treated skin (neurons of “control mice”) (for details, refer to Qu et al. 2014). Enhanced excitability after ACD might contribute to increased discharges to external stimulation or to spontaneous activity in the absence of stimulation and therefore increase the likelihood of generating site- directed itch- or pain-like behaviors or sensation. Electrophysiological recordings were obtained from the cell bodies of cutaneous mechanosensitive C-nociceptors, visually identified in transgenic mice by their expression of a green fluorescent protein (GFP) marker. In one type of mouse, the GFP was present in neurons that expressed the MrgprA3 receptor for chloroquine and also typically expressed receptors for capsaicin (TRPV1), histamine (H1), and BAM8-22 (MrgprC11) (Han et al. 2013). In the other type of transgenic mouse, the GFP was present in neurons that expressed the MrgprD receptor for beta-alanine (Zylka et al. 2005) and that do not normally express TRPV1, H1, or MrgprC11 (Han et al. 2013). These two types of neurons each innervate the stratum granulosum of the epidermis (Han et al. 2013; Zylka et al. 2005) and constitute the majority of mechanosensitive C-nociceptors innervating the hairy skin of the mouse (Imamachi et al. 2009).

GFP-labeled neurons of ACD and control mice were electrophysiologically recorded in vitro and in vivo (Qu et al. 2014). In the in vitro studies, whole-cell patch-clamp recordings were obtained from acutely dissociated cell bodies of GFP- labeled neurons that, for separate groups of each type of transgenic mouse, innervated either skin with ACD ("ACD mice) or vehicle-treated skin ("control mice"). In comparison with neurons from control mice and under current-clamp recording, both MrgprA3+ neurons and MrgprD+ neurons exhibited significant signs of increased membrane excitability. These signs included a more depolarized resting membrane potential, a decreased threshold current for an action potential (rheobase), and more action potentials evoked by a stimulus current that was twice rheobase. Thus, ACD made these neurons more likely to respond to a near-threshold stimulus and to fire more action potentials to a suprathreshold stimulus.

As no significant differences were observed in input resistance between ACD and control neurons, the next experiment examined the possibility that increased neuronal excitability after ACD might be accompanied by an increased expression of voltage-gated sodium currents. Using different voltage stimulus protocols under voltage clamp recording, it was found that in comparison with responses of control neurons, both MrgprA3+ and MrgprD+ neurons from ACD mice exhibited significant increases in the peak amplitudes of both tetrodotoxin-sensitive and tetrodotoxin- resistant sodium currents (Qu et al. 2014). If this increased magnitude of sodium current is present at the cutaneous nerve endings of these C-nociceptors, then action potentials might be more easily evoked by natural stimulation or might even occur spontaneously thereby eliciting spontaneous itch- and pain-related behaviors directed toward the ACD site.

To examine the possibility that these Mrgpr mechanosensitive C-nociceptors at the ACD site might exhibit signs of hyperexcitability such as spontaneous activity in the intact animal, action potentials were extracellularly from their cell bodies in the intact DRG (Qu et al. 2014). For ACD or control transgenic mice, the functional properties of the cutaneous receptive fields were characterized for MrgprD+ or MrgprA3+ neurons innervating the chemically treated skin 24 h after the second challenge. Each type of neuron was identified as a mechanosensitive C-nociceptor. Some of the MrgprD+ neurons and all of the MrgprA3+ neurons were also responsive to noxious heat. In control mice, none of these C-nociceptors exhibited any ongoing (spontaneous) activity in the absence of applied cutaneous stimuli. But for neurons in ACD mice with receptive fields in the area of dermatitis, there was a low rate of ongoing activity in 43 % of the MrgprA3+ neurons (vs. only 5 % of MrgprD+ types) and some neurons of either Mrg type exhibited abnormally high and long discharges to heat or to punctate mechanical noxious stimulation.

Taken together, these electrophysiological findings support the hypothesis that ACD causes an increase in the incidence of spontaneous activity in a subpopulation of cutaneous C-nociceptors, secondary to enhanced sodium current and membrane excitability, which, in turn, may trigger spontaneous itch- and pain-like behaviors directed toward the site of inflammation.

2.5 ACD Upregulates CXCR3 Chemokine Receptor Signaling in Cutaneous C-nociceptors

Some of the chemical mediators that orchestrate the inflammation during the challenge or elicitation phase of ACD might also act to increase nociceptor excitability. When humans and mice are exposed to the hapten to which they were previously sensitized, keratinocytes produce cytokines including tumor necrosis factor alpha (TNF-alpha) and the chemokine CXCL10 (IP10), a ligand for CXCR3 which is expressed on activated T-helper type 1 (Th1) cells. These chemical stimuli facilitate the migration of cytotoxic CD8+ Th1 cells that are specific for the antigen (a hapten- protein complex) and that cause apoptosis of the antigen presenting cells in the challenged skin.

Based on the capacity of TNF-alpha to be retrogradely transported to the DRG (Shubayev and Myers 2001) and its effect of increasing TTX-resistant sodium currents when applied to dissociated DRG neurons (Jin and Gereau 2006), it is possible that TNF-alpha could act to increase voltage-gated sodium current both at the nerve terminals and at the somas of nociceptive neurons innervating the area of ACD. This hypothesis remains to be tested for the neuronal effects of ACD.

After SADBE challenge, acutely dissociated small-diameter DRG neurons that innervated the area of ACD upregulated the expression of mRNA and protein for CXCR3 and exhibited responses to CXCL10 – responses rarely and weakly seen in control neurons (for full details see Qu et al. 2015). The neurons innervating the inflamed skin (ACD neurons) exhibited a calcium response to CXCL10 that was blocked by prior delivery of an antagonist for the ligand’s receptor, CXCR3. These neurons included but were not confined to those that expressed MrgprA3 or MrgprD. During electrophysiological recordings, CXCL10 evoked a membrane depolarization and action potentials in ACD neurons but not in control neurons. In behavioral studies, systemic delivery of a selective antagonist of CXCR3 decreased the incidence of itch- but not pain-like behaviors directed toward the ACD site. Also CXCL10 elicited itch-like site-directed behaviors when injected into the ACD site but evoked no significant itch- or pain-like behaviors when injected into vehicle- challenged skin (Qu et al. 2015).

In other studies, CXCL10 was upregulated in DRG neurons in rats after an experimentally induced inflammation of the ganglion (Strong et al. 2012) or after a demyelination of the nerve (Bhangoo et al. 2007) and was upregulated in DRG neurons from human after infection of the ganglion with varicellazoster virus (Steain et al. 2011). It remains to be determined whether the activation of neurons expressing message or protein for CXC10 would release CXCL10 from DRG neurons. If so, the chemokine might facilitate the attraction of CXCR3-expressing lymphocytes and activate these cells as well as itch- and pain-mediating nociceptive neurons that express CXCR3 after ACD. Clearly there is much work to be done to fully understand the role of this chemokine receptor in nociceptor physiology during ACD given the complexities of chemokine biology (Van Raemdonck et al. 2015). In addition, there are probably multiple inflammatory mediators that may modulate the excitability of cutaneous nociceptors with C-fibers and probably also certain nociceptors with myelinated axons. The advantage of the ACD model is that it can be

experimentally applied in humans and animals. Behavioral and cellular physiological findings in animals can be related more easily to sensory measurements in humans using similar experimental stimuli and protocols. This interspecies comparison should facilitate the discovery of molecular targets for treating persistent itch and pain in humans.

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