REVIEW ARTICLE

Immune regulation in pathophysiology and targeted therapy for itch in atopic dermatitis

Chih-Hung Lee*

Department of Dermatology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan

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A B S T R A C T

Itch is an unpleasant perception that provokes one to desire to scratch. It results from the activation of free nerve endings by noxious stimuli in the skin. Atopic dermatitis (AD) is a prototypic inflammatory skin disease that always occurs with an intense itch. AD involves many components of skin-associated lymphoid tissue (SALT). As a disease with polarized T helper 2 cell activation, AD involves eosinophil infiltration and immunoglobulin E, interleukin (IL)-2, IL-4, IL-13, and IL-31 production. As a disease involving an impaired skin barrier, AD is characterized by the enhanced transepidermal entry of allergens and the production of thymic stromal lymphopoietin (TSLP) from epidermal keratinocytes, which worsen atopic march and disease progression. Both immune and epidermal events interact with cutaneous nerve components, including transient receptor potential (TRP) channels and opioid receptors, causing both the perception and propagation of itch from the skin to the brain. In addition to treating itch through TRP channels and opioid receptors, it might be possible to target the various cellular components of SALT, including keratinocytes, eosinophils, and soluble factors, such as IL-31, IL-4, IL-13, IL-31, and TSLP.

Introduction

Atopic dermatitis (AD) is a common chronic relapsing disease with intense itch. AD usually accompanies a personal or familial history of allergic diseases, including allergic rhinitis, asthma, and allergic conjunctivitis.1 Itch is the cardinal symptom of AD. It severely interferes with the life quality of patients and their caregivers and it may impair school and work performance and trigger anxiety and depression.2 The prevalence of AD is estimated to be 6–9% in Taiwan.3 While the exact pathogenesis of AD remains to be investigated, both impairment of the skin barriers and aberrant immune activation play significant roles in its pathogenesis.4,5

Itch perception: From skin initiation to brain activation

Itch is a unique perception that provokes one to desire to scratch to get rid of noxious stimuli.6 Cough, which may be induced by noxious chemicals or particles, is a similar action used to expel such stimuli. The epidermis is innervated by small unmyelinated C fiber. The perception of itch is transmitted from the peripheral free nerve endings (C fiber) in the epidermis back to the neuron body in the dorsal root ganglion located at the spinal cord. Subsequently, a synapse in the spinal cord transmits the signal in the contralateral spinothalamic tract to the thalamus, and eventually the signal radiates to the cortical neurons.7 In skin, the propagation of itch could result from both inflammatory and noninflammatory diseases. AD is a prototypic inflammatory skin disease that is always accompanied by intense itching.8 People affected by other inflammatory skin diseases, such as lupus erythematosus and pityriasis lichenoides, may or may not experience itch. Among the noninflammatory skin diseases, some diseases (e.g., uremic pruritus) may cause itch, but others (e.g., stable vitiligo) may not. In diseases with inflammatory conditions, immune factors may play a significant role in the initiation of itch. On
the other hand, in those diseases with noninflammatory conditions, neurogenic factors may play a more significant role in itch pathophysiology.

Several functional imaging studies have been performed to observe the functional activation of itch signals in the brain. However, different diseases associated with itching can cause differences in the activation of the brain cortex. Furthermore, histamine-induced or non-histamine-induced itch causes differences in the chronological and topological activation of the brain cortex. Based on the principle of near-infrared spectroscopy, one of our previous studies demonstrated that brain cortex activation, reflected by changes in oxygenated and deoxygenated hemoglobin levels, in histamine-induced itch is distinctly different from push–pull gauge-induced pain.10

**Impaired skin barrier and itch perception**

AD is characterized by the impairment of the skin barrier. The epidermis is an intact tissue that protects the human body from harsh outside environments and is fully organized with ordered differentiated keratinocytes and has a densely packed corneal layer equipped with “brick and mortar.” However, the impaired skin barrier in AD allows the entry of potential allergens, which subsequently aggravate the inflammatory responses and thereby further deteriorate the impaired skin barrier, creating a vicious cycle. Filaggrin, an important protein that binds to keratins associated with keratinocyte differentiation, is important to the integrity of the skin barrier. In AD, approximately half of the patients are affected by the mutations in filaggrin.12 The loss or mutation of filaggrin, which decreases the skin’s ability to hold water, impairs skin barrier function. On the other hand, enhancing the barrier function of the skin increases the therapeutic effects on AD. We skin barrier function. On the other hand, enhancing the barrier function of the skin increases the therapeutic effects on AD.13

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**Skin-associated lymphoid tissue**

Before we discuss the role of aberrant immune responses in the pathophysiology of AD, the topic of skin-associated lymphoid tissue (SALT) needs to be reviewed. In the gastrointestinal tract, lymphoid structures are present in some submucosal areas, which are also referred to as mucosa-associated lymphoid tissue (MALT).10 MALT is specialized in the appropriate antigen presentation independently of the secondary lymphoid organs (lymph nodes). This is evidenced by the fact that MALT lymphoma can derive directly from several gastrointestinal mucosal tissues, independently of its associated lymph nodes. Noting similarities in antigen presentation and T cell trafficking from skin to lymph nodes, Streilein et al.17 introduced the term “skin-associated lymphoid tissue” (SALT) and proposed that SALT acts as an integrated immunosurveillance system for the skin. Like MALT lymphoma, SALT by itself can develop several cutaneous lymphomas with different lymphoid cell origins independent of the lymph nodes. SALT is comprised of: (1) epidermal Langerhans cells; (2) T cells; (3) keratinocytes; and (4) a set of draining lymph nodes, along with endothelial cells and several dermal cells.18 Ono and Kabashima19 proposed the term “inducible SALT” as they found that the interleukin (IL)-1α produced by keratinocytes activated perivascular macrophages, which attracted dermal dendritic cells via CXCL2 signaling under an inflammatory condition in a model of contact dermatitis. We then discuss the role of several lineages of immune cells of SALT in the pathophysiology of itch in AD, a proinflammatory disease with dynamic innate and adaptive immune responses.

**Role of cellular and soluble components of SALT in AD**

**Eosinophils**

The association between eosinophil infiltration and itch perception is well known with regard to scabies infestation.20 Patients with scabies experience intense itching, and their skin is densely infiltrated by eosinophils. Patients with bullous pemphigoid also experience a significant itch and have moderate eosinophil-rich dermal inflammation. Eosinophil-deficient mice (PHIL mice) have impaired hapten-induced hypersensitivity responses, reduced scratching behavior, and decreased PGP9.5 stained nerve endings. However, there remains some debate as to whether eosinophils and itch might have just a serendipitous relationship via the interactions of immunoglobulin E (IgE) and mast cells.20

**Fibroblasts**

Keloid scarring occurs with a proliferation of fibroblasts and an unregulated deposition of extracellular matrix, and there is often intense itch as the disease progresses. Keloid skin scars have more nerve fibers. It has also been noted that dermal fibroblasts secrete artemin, a neurotrophic factor, in response to substance P and that these artemin-expressing fibroblasts are increased in AD skin.23

**Keratinocytes**

The extent of skin innervations depend on the balance between nerve elongation factors [e.g., nerve growth factor (NGF)] and nerve repulsion factors (e.g., semaphorin 3A), both of which can be produced by epidermal keratinocytes. The blood concentration of NGF correlates well to itch intensity in patients with AD, while epidermal semaphorin 3A levels are low in AD patients who also have increased epidermal nerve density. Furthermore, in a previous study of keratinocytes, we found that IL-31 induces stromal interaction molecule 1 (STIM1) activation, signal transducer and activator of transcription 3 (STAT3) phosphorylation, and beta-endorphin release, and in another study, we found that the blood levels of beta-endorphins correlate well to subjective itch intensity.14

Thymic stromal lymphopoietin (TSLP), another cytokine produced by keratinocytes, plays an important role in the pathophysiology of AD. Expression of TSLP is increased in keratinocytes in AD. TSLP contributes to the progression of atopic march in a variety of disorders ranging from the sensitization of AD in skin to the development of asthma and other allergic diseases.20 In mice, overexpression of TSLP induces AD-like skin.21 Keratinocyte signaling through TSLP to immune cells may play an important role in AD. Keratinocyte-derived TSLP directly activates cutaneous sensory neurons to promote itch perception.22 In fact, the signaling from the Ca²⁺ release-activated Ca²⁺ channel (ORA1), which interacts with STIM1 in the cell membrane of keratinocytes, can induce the production of TSLP, which then activates transient receptor potential cation channel, subfamily A, member 1 (TRPA1) and thereby induces itch.26 Undoubtedly, keratinocytes play a pivotal role in the initiation of itch. In fact, one of our previous international studies found an association between the genetic predisposition of ORA1 and the development of AD. That study found an association between the single gene polymorphism of ORA1, a calcium channel protein, and the development and severity of AD in both Japanese and Taiwanese populations.29
**Mast cells**

Histamine is a well-known pruritogen released from mast cells in response to IgE crosslinking and degranulation in urticaria. It might be possible to use traditional antihistamines to target H1 or H2 receptors when treating itch associated with a variety of diseases, but itch accompanying AD is probably unrelated to histamine because it is not satisfactorily relieved by anti-H1 and anti-H2 histamine. Further efforts, however, have been made to target another histamine receptor, H4R, to treat itch. A phase 2a study in Japan demonstrated that the H4 antagonist (JNJ-39758979) alleviates itch in patients with AD. In addition, the use of omalizumab as an anti-IgE agent reduces the clinical severity of AD in patients with very high IgE, suggesting that the therapeutic benefit of anti-IgE results not only from the neutralization of IgE but also from the downregulation of high-affinity IgE-Fc receptors on basophils and mast cells.

**T cells and cytokines**

**IL-2**
High-dose IL-2, which has been used as an immunotherapy agent against metastatic renal cell carcinoma and malignant melanoma, induces severe itch. Injection of IL-2 into healthy controls or into patients with AD induces a 48–72 hour itch. Cyclosporin, a calcineurin inhibitor, decreases the synthesis of IL-2 and reduces itch in patients with AD. However, whether the antipruritic effects of cyclosporin are mediated via its decrease of IL-2 or via its other immunosuppressive effects in AD remains controversial.

**IL-31**
The transgenic overexpression of the cytokine IL-31 in lymphocytes induces severe pruritus and atopic-lik dermatitis in mice. IL-31, expressed preferentially in T helper 2 (Th2) cells, activates a heterodimeric receptor composed of IL-31 receptor A (IL-31RA) and oncostatin M receptor in keratinocytes and nerve endings. The blood levels of IL-31 are increased in many patients with pruritic skin diseases, including AD, cutaneous T cell lymphoma, urticarial pruritus, chronic urticaria, and prurigo nodularis. Increased expressions of IL-31RA and IL-31 are observed in the epidermis of patients with AD. Furthermore, the blood level of IL-31 is correlated to disease severity in patients with AD. Consistent with these findings, IL-31 induces the activation of STAT3, followed by the activation of STAT3 and the release of beta-endorphins from keratinocytes in peripheral skin. In the central processing of itch, dorsal root ganglion neurons co-express TRPV1 and IL-31R. IL-31-induced itch requires TRPV1 and TRPA1. Interestingly, IL-31 delays the onset of pruritus by hours, suggesting that the itch induction by IL-31 may occur through an indirect mechanism rather than through cutaneous receptor activation. Together, these findings suggest that IL-31 may be targeted in the treatment of itch. In fact, a Phase 1 clinical trial is being conducted to test the effect of anti-IL-31 antibody (NCT01614756).

**IL-4 and IL-13**
The receptors of IL-4 and IL-13 have a common subunit. As Th2 cytokines, IL-4 and IL-13 are important in the development of AD. The transgenic overexpression of IL-4 or IL-13 in mice causes a severely itchy atopic disease-like phenotype. IL-4 and IL-13 are increased in AD skin. In blood, the level of IL-13 is increased in AD and correlated with disease severity. A mouse study showed that IL-13 induces pruritus in AD at least in part through the activation of TRPA1. Treatment with the monoclonal antibody against IL-4, dupilumab, reduces itch perception in AD by more than half. In summary, both cytokines may mediate the development of itch to some extent.

**Nerve components in itch perception**

**Transient receptor potential channels**

Transient receptor potential (TRP) channels, expressed in various cells, including keratinocytes, melanocytes, and nerves in the skin, may act in sensory processing and other biological functions. Several classes of TRP proteins, including TRPV1, TRPA1, and TRPM8, are implicated in itch.

TRPV1 expression is increased in AD skin lesions, and its activation promotes both immune responses and itch by secreting soluble factors. Amagai et al reported that in conventional NC/Tnd mice with AD, TRPV1 stimulation reduces scratching behavior, and suggested that it might be used as a TRPV1 modulator in the treatment of atopic itch. However, one recent clinical trial investigating the effects of topical TRPV1 inhibitor (SB705498) failed to demonstrate its efficacy on the relief of itch induced by histamine or cowhage.

TRPA1 is a known transducer of histamine-independent itch. It is present in sensory nerves, mast cells, and keratinocytes. TRPA1, which is increased in AD, mediated pruritus in an IL-13-induced AD model. However, the biological effects of TRPA1 on the itch of AD are complicated. On the one hand, TRPA1 transduces itch signals; on the other hand, its activation promotes barrier recovery. As mentioned above, IL-31-mediated itch requires TRPV1 and TRPA1.

TRPM8 is expressed in both A-delta fibers and C fibers. Administration of substance P attenuates the scratching activity induced by menthol, a TRPM8 agonist. In a subset of patients, itch is transmitted through A-delta fibers but not commonly through unmyelinated C fibers. The different components of nerve fibers might explain the diversity of the itch that patients experience.

**Opioid receptors**

Morphine, a strong analgesic, induces or enhances itch in a significant portion of healthy individuals. However, μ-opiate receptor antagonists inhibit itch but not pain. One of our previous studies found an association between the blood levels of beta-endorphins and itch intensity in patients with AD. Morphine-induced itch may be related to the fact that morphine binds to the μ-opiate receptor isoform MOR1D, which heterodimerizes with the gastrin-releasing peptide receptor coexpressed in itch-signaling spinal neurons. The κ-opioid receptor TRK-820 (nalftafurine) inhibits pruritogen-evoked scratching, suggesting that the κ-opioid receptor may play a putative role in the modulation of itch. TRK-820 has been proven effective in relieving intractable itch in patients on renal dialysis.

**Treatment strategy for alleviating inflammation and itch**

Physicians have known for many years that antihistamines barely alleviate itch in patients with AD with active itching. A burst use of systemic steroid, which abolishes the most upstream cause of inflammation, is particularly useful in controlling the acute flare and itch of AD. In the subacute phase, the calcineurin inhibitor cyclosporin is also helpful in controlling inflammatory itch. Many monoclonal biologics are being developed or have been developed to treat inflammation-related itch in AD (Table 1). To name a few, dupilumab, a monoclonal antibody against IL-4, markedly and rapidly improves itching in AD patients, and the monoclonal antibody against IL-13, lebrikizumab, for which an AD trial is
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Conclusions

The itch in AD always occurs concurrently with inflammation and the desire to scratch. This vicious cycle of scratch, inflammation, and itch make the itch associated with AD a troublesome clinical problem. Any of the components discussed in this review could be interrupted to treat itch or its associated inflammation in AD. The delicate regulation of the various components of SATL leads to the dynamic homeostasis of immune responses in skin. Studies targeting the components of SATL, including keratinocytes, eosinophils, and soluble factors, such as IL-31, IL-4, IL-13, and TSLP, might shed new light on the development of treatment strategies for inflammatory itch in AD.

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