Abnormalities in Itch Sensation and Skin Barrier Function in Atopic NC/Tnd Mice

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Received April 30, 2013

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by dryness and itchy skin. Genetic factors as well as other factors, including abnormality in skin barrier function, hypersensitivity of itch sensory nerves, and dysfunction of the immune system, strongly affect the onset and exacerbation of AD. Recently, it has become clear that itch sensation is closely related to pain sensation. By using NC/Tnd mice, a unique spontaneous animal model for human AD, we found abnormalities in sensitivity against external stimuli as compared to two standard strains, BALB/c and B6 mice. Particularly, in conventional NC/Tnd mice with AD, stimulation against transient receptor potential (TRP) V1 reduced the scratching behavior, suggesting the possibility of a TRPV1 modulator in the treatment of atopic itch. The review outlines observations regarding itch sensation and skin barrier function in NC/Tnd mice by using a novel itch quantification system for the laboratory animals, which may bring great progress in the future study of itch.

Key words atopic dermatitis; itch; NC/Tnd mouse; SCLABA-Real®

1. INTRODUCTION

Atopic dermatitis (AD) is a chronic and relapsing skin disorder with inflammatory or pruritic skin symptoms, and is one of the most common skin diseases. It often occurs in families with other atopic diseases such as bronchial asthma, allergic rhinitis, and conjunctivitis. The etiology of AD is multifactorial; the relations of genetic, environmental, immunological, and psychological factors, as well as physical ones such as skin barrier to the disease, have been reported. Among these, loss-of-function mutations within the profilaggrin gene (FLG gene) is one genetic factor that correlates to the exacerbation of AD, and has been found in approximately 20% of AD patients. Because filaggrin, the cleaved form of profilaggrin, is essential for the regulation of epidermal homeostasis, it indicates the importance of factors that impact skin barrier function, including those which are not oriented from genetic backgrounds. In fact, for example, abnormal microbial colonization such as Staphylococcus aureus is reported to accelerate the exacerbation of the symptoms.

The skin barrier can be mechanically damaged by scratching behavior, derived from the itch sensation, which is one of the most problematic symptoms of AD. The control of itch sensation is difficult because the itch sensation evokes the scratching behaviors, which worsen the disease by triggering the release of pro-inflammatory cytokines, including tumor necrosis factor α (TNF-α) and interleukin-2 (IL-2), from keratinocytes, as well as itch sensation-enhancing neuropeptides such as substance P or calcitonin gene related peptide (CGRP) from C fiber. These factors further stimulate the vascular endothelial cells or mast cells to produce chemical mediators such as histamine, consequently producing a “vicious circle” of disease exacerbation. Recently, while the mechanisms of itch sensation have become clear, controversy remains regarding how to distinguish between itch and pain sensation. In this review, we introduce discoveries regarding the pain/itch sensation, as well as skin barrier function, which severely affect the itch sensation, using NC/Tnd mice, a useful animal model for human AD (Fig. 1).

2. CONTROVERSIES REGARDING ITCH/PAIN SENSATION

There have been two theories regarding the itch/pain sensation. One is the concept that itch and pain are perceived by...
distinct nerves that are specialized in processing each sensation. This theory is supported by several reports, such as the discovery of nerve fibers that specifically transduce the itch sensation. Namer et al.\textsuperscript{13} identified distinct populations of C-fibers that react to the corresponding itch sensations in human. In addition, Sun et al.\textsuperscript{14} demonstrated that gastrin releasing peptide receptor-positive neuronal cells in the spinal cord were specific for the itch sensation in mice. Another theory describes the same afferent neurons being capable of triggering both itch and pain sensations, depending on the pattern or intensity of the stimulation. It is supported by the findings that a voltage-dependent cation channel, transient receptor potential (TRP) V1 and TRPA1 are responsible for evoking itch sensation, though these receptors had previously been recognized as pain-sensing receptors.\textsuperscript{15,16} Mechanisms of itch sensation are still complicated; however, recent findings, including ours, indicate that some neuronal signals may exert an influence on both itch and pain sensation. The next chapter describes what we have found regarding the itch sensation by using NC/Tnd mice.

3. OBSERVATIONS REGARDING ITCH SENSATION IN THE MOUSE MODEL OF AD

Recently, we found that the neural response to capsaicin, which is one of the major stimuli that evokes pain, is much lower in NC/Tnd mice than in other strains (article in preparation, Table 1). Interestingly, scratching behavior caused by histamine, which shares the TRPV1 signaling with capsaicin,\textsuperscript{15,16} was not different between NC/Tnd mice and two other standard strains (Table 2). On the other hand, responses to other pruritogens, including serotonin (phospholipase Cβ3-dependent),\textsuperscript{17} chloroquine and SLIGRL-NH\textsubscript{2} (TRPA1-dependent),\textsuperscript{18} were significantly lower in NC/Tnd mice (Table 2). These results indicate an alteration in the skin sensitivity of NC/Tnd mice, probably resulting in an increase of itch sensation via TRPV1 receptors. It also raised the possibility that disruption of the itch/pain sensation balance may drive the itch sensation in AD. A TRPV1 antagonist has been reported to have potential as a therapeutic agent for itching in both pruritogen-induced allergic dermatitis models and spontaneous NC/Nga mice.\textsuperscript{19,20} On the other hand, a TRPV1 agonist, capsaicin, has been reported to exert a moderate suppressive effect on histamine-, substance P-, and proteinase-activated receptor-2 (PAR-2) agonist-induced itch responses.\textsuperscript{21} By using conventional NC/Tnd mice, we have already confirmed that serious scratching behavior was decreased after the application of capsaicin onto the affected skin (data in preparation). The different information indicates that modulating the itch/pain balance through the TRP channel family may be a novel strategy for controlling itch in AD (Fig. 2).

4. OBSERVATIONS OF SKIN BARRIER FUNCTION IN THE MOUSE MODEL OF ATOPIC DERMATITIS

As mentioned above, scratching behavior damages skin barrier function. Cutaneous abnormalities such as dry skin may initiate the itch sensation, thus there is a close relationship between itch and skin barrier function. We have already revealed...
that the ceramide content in skin is decreased in conventional NC/Tnd mice with AD, which impairs the water retention of the skin.\textsuperscript{22)} In NC/Tnd mice, the generation and supplementation of ceramide in the stratum corneum during development of AD is insufficient due to the low activity of sphingomyelinase.\textsuperscript{22)} However, factors which trigger the itch sensation after barrier disruption of the skin has been unclear. Recently, we found that ingredients taken from drinking water altered the clinical severity of dermatitis in the mouse model.\textsuperscript{23)} In the study, soft ground water from Jeju Island, which contains little magnesium and calcium, was superior to hard water for the prevention of scratching behavior in NC/Tnd mice with AD (Fig. 3), indicating that the intake of mineral ions exhibits certain effects on modulating allergic features.\textsuperscript{22)}

5. SCLABA-Real\textsuperscript{®}, A NOVEL SCRATCH ANALYZING SYSTEM

Itch is one of the most serious sensations for the exacerbation of AD. For the alleviation of AD symptoms, controlling both itching and scratching is the most important issue. Measurement of scratching behavior of laboratory mice gives valuable information for us to develop new treatments for atopic itch; however, quantitative analysis of scratching behavior of mice was quite difficult. SCLABA-Real\textsuperscript{®} is a novel system for the real-time quantification of scratching behavior in laboratory mice without performing any kinds of stressful operations on the mouse\textsuperscript{24,25)} (Fig. 4). Conventionally, experimenters had to count the number of the scratching incidents, which required a lot of tasks and time. Instead, SCLABA-Real\textsuperscript{®} enabled us to analyze the scratching behavior of four mice, by simultaneously recording the motion of each mouse

Table 2. Scratching Behavior of NC/Tnd Mice Caused by Several Pruritogens

<table>
<thead>
<tr>
<th>Strain</th>
<th>Control</th>
<th>Histamine</th>
<th>Serotonin</th>
<th>Chloroquine</th>
<th>SLIGRL-NH\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>−</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>−</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NC/Tnd (SPF)</td>
<td>−</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Scratching frequency level of NC/Tnd mice after topical application of 4 different pruritogens on dorsal skin was indicated. −, < 20 times; +, 20–50 times; ++, 51–100 times; +++ > 100 times of scratching behavior/1h.

Fig. 3. Changes in Scratching Frequency after Supplementation of Various Kinds of Bottled Water

The graph indicates the number of scratching behaviors of NC/Tnd mice that had been supplied tap water or very soft Jeju ground water for the indicated period. Each value represents mean±S.E. of 10 mice in each group. *\textit{p}<0.05, when compared with the control group supplied tap water for 8 weeks.

Fig. 4. A Novel Scratch Analyzing System, SCLABA-Real\textsuperscript{®}

The high-speed camera containing an image processing system and the recording platform with a built-in near-infrared light panel for real-time quantification of scratching behavior of mice (A). Real time record and analysis window (B).
with a high-speed digital camera from the top of an exclusive cage placed on near-infrared light. SCLABA-Real software accurately detects the algorithm that is specific for scratching behavior in mice and records data in real-time. Basically, it captures animal behavior at the rate of 240 frames per second using a high-speed camera containing an image processing system, making it possible to quantify the number and duration of scratching behavior automatically by time-course analysis of “frame-to-frame difference.” This analysis method is advantageous in the successive measurement of itch/scratch behavior under normal conditions. Therefore, this system can detect alterations of scratching frequency, it also provides useful information of therapeutic targets. Recent findings, including TRPV1 antagonist as a role of pain-sensitizing TRPA1 in itch.}

6. REMARKS

Compared to the artificially induced model, the spontaneous AD model provides valuable information regarding the pathogenesis, sensitivity and barrier function in the skin, and aids in the selection of effective new drugs and supplements. Because AD is a multifactorial disease, the utilization of proper experimental models is necessary for the discovery of therapeutic targets. Recent findings, including TRPV1 may be an appropriate target to control unbearable itch sensation in AD. Besides evaluating scratching frequency, it also provides useful information for the screening of compounds because itch is one of the outcomes resulting from the mixture of multiple pathogenic alterations. Thus, applications of an accurate scratch analyzer on animal models of AD will provide a useful platform for further research.

REFERENCES

24) Ishii I, Kurozumi K, Orito K, Matsuda H. Automatic scratching