Mini Review

Atopic dermatitis model of itching behavior in hairless mice

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In atopic dermatitis (AD), severe itching and scratching not only impacts the patient's quality of life, but also exacerbates the disease; however, the precise underlying mechanisms of itching in AD remain elusive. We have previously reported a special diet-induced, mouse model of AD. This article reviews the characteristics of the model, and the chronological and pharmacological analyses of the pathology. HR-1 hairless mice fed a commercial special diet, HR-AD, but not a normal diet, showed scaly dry red skin resembling AD. To evaluate the degree of itching, spontaneous scratching was analyzed. As a result, scratching with long duration was observed in HR-AD-fed mice, as confirmed by prolongation of duration of one scratching bout. Chronological analysis of the pathologies in HR-AD-fed mice revealed that the prolonged scratching was coincident with the development of skin barrier dysfunction, while skin inflammation with recruitment of inflammatory cells, such as CD4+ T cells, eosinophils, and mast cells, and serum IgE elevations apparently followed the prolonged scratching. Epidermal nerve sprouting was detected almost coincidently with the prolonged scratching. Pharmacological analysis of the scratching behavior indicated that the prolonged scratching was suppressed by a \( \mu \)-opioid receptor antagonist, but not by antihistamine, similar to AD patients. Although treatment with corticosteroid suppressed the skin inflammation, it was not effective against the prolonged scratching, the dry skin symptoms, and the epidermal nerve sprouting. Petrolatum ointment inhibited the prolonged scratching during the amelioration of skin barrier dysfunction. Furthermore, the barrier disrupted-dry skin was alleviated by oral administration of essential fatty acid, linoleic acid (LA), suggesting that LA deficiency appears to be responsible for the symptoms. Taken together, these findings suggest that skin barrier dysfunction is closely associated with the development of AD-like pruritus in this model.


Key words  atopic dermatitis, animal model, itching, skin barrier dysfunction

Introduction

Atopic dermatitis (AD) is a common skin disease, which is clinically characterized by pruritus, eczema, and the chronicity of the dermatitis, and most patients have a family history of atopy. Over several decades, the prevalence of AD has been increasing in developed countries, suggesting that environmental factors, as well as genetic factors, play a critical role in determining the expression of this disease.

In the inflamed skin of AD, the infiltration of eosinophils, the number of mast cells, and Th2-type immune responses are gen-
erally increased. In addition, most AD patients show elevated serum levels of IgE against various allergens. Thus, the pathogenesis of AD had been attributed largely to immunological abnormalities, including Th1/Th2 cell dysregulation, IgE production, increased function of dendritic cells, and mast cell hyperactivity. Accordingly, therapy has been focused on ameliorating the Th2-mediated inflammation and pruritus. On the other hand, it is clinically known that dry skin accompanied by reduced function of the skin barrier is observed in patients with AD, not only in eczematous lesions but also in clinically unaffected skin. It is considered that the defective barrier increases the risk of allergen penetration and the succeeding inflammatory response, thus contributing to development and exacerbation of dermatitis. However, whether the skin barrier abnormality is a primary defect or an event secondary to the inflammation remains unanswered. As well, the interaction between the immunological responses and skin barrier dysfunction is not well understood.

Pruritus is the most important diagnostic criterion of AD, and it significantly impacts the patient’s quality of life. In addition, scratching caused by the itching worsens the skin inflammation, which in turn triggers further itching. Thus, reducing the itching and scratching is crucial for the treatment of AD. Histamine is well known to be a potent itch mediator in humans, but antihistamines do not sufficiently inhibit the pruritus of AD. To date, the precise underlying mechanisms of itching in AD remain elusive, and specific antipruritic therapy has not been established.

Experimental animal models are indispensable tools to study pathogenic mechanisms and test novel therapeutic approaches in vivo. Several animal models for AD have been described (see reviews; Shiohara et al.; Jin et al.; Inagaki et al.). They include naturally occurring models, hapten- and protein antigen-induced models, and gene-transgenic and knockout models. On the other hand, we have demonstrated that when HR-1 hairless mice were fed a special diet, HR-AD, AD-like symptoms accompanied by itch-related behavior were induced. This article reviews the characteristics of the special diet-induced model, and the chronological and pharmacological analyses of the pathology.

**Manifestation of AD-like symptoms in hairless mice fed a special diet, HR-AD**

HR-1 hairless mice have been widely used in scientific research, for example, ultraviolet radiation-induced skin tumors, cutaneous sensitivity to chemicals or ultraviolet light, and percutaneous absorption of drugs. On the other hand, it is known that when weanling HR-1 mice are fed a commercial special diet, HR-AD, for several weeks, dry skin accompanied by skin redness is induced (unpublished observation). However, the details of the pathology of HR-AD-fed mice have not been analyzed. Thus, we observed symptoms when the HR-1 mice were fed HR-AD from 4 weeks of age. Four weeks after the start of feeding, although the skin of normal diet-fed mice displayed no visible abnormality (Fig. 1a), that of HR-AD-fed mice showed increased skin creases and slight erythema (Fig. 1b). Interestingly, HR-AD-fed mice at the 11th week showed scaly dry red skin with several excoriations (Fig. 1c). In contrast, no such skin lesions were observed in normal diet-fed mice during the observation period. Since the gross changes in HR-AD-fed mice seem to be similar to manifestations of human AD, we next analyzed whether the HR-AD-fed mice could be a useful model for AD by observing spontaneous scratching behavior.

Prior studies using mice have demonstrated that local pruritogenic stimulation produces increased frequency of scratching, while algesiogenic stimulation does not. Thus, scratching behavior in mice may be a useful indicator of the itching sensation. On the other hand, in our preliminary observational experiments of spontaneous scratching in HR-AD-fed mice, one scratching bout included both long- and short-term scratching. Thus, we...
measured not only the frequency but also the cumulative duration of scratching over 1 h using our originally developed counter, and the duration of one bout was calculated by dividing the cumulative duration by the frequency. Figure 2 shows three representative results when spontaneous scratching was compared in normal diet- and HR-AD-fed mice. There was no consistent tendency in the cumulative duration and frequency, but the duration of one bout was reproducibly prolonged in HR-AD-fed mice. These results imply that the mode rather than the frequency of scratching is altered in this atopic mouse model.

**Chronological analysis of the pathologies in HR-AD-fed mice**

To further characterize the HR-AD-fed mouse model, we chronologically analyzed symptoms related to human AD (summary is shown in Fig.3). First of all, to evaluate the dry skin symptoms, we measured dermal water content (DWC) and transepidermal water loss (TEWL) as parameters of skin dryness and skin barrier function, respectively. In HR-AD-fed mice, although DWC was significantly decreased every 2 weeks from the start of feeding, TEWL was markedly increased from the 4th week; both parameters became exaggerated in the 8th week. Consistent with the result shown in Fig.2, although there was no consistent tendency in the changes in the frequency and cumulative duration of scratching during the measurement period, the duration of one bout was gradually prolonged from 4 weeks after the start of feeding. Histological examination showed that, although epidermal hyperplasia was observed from the 4th week, skin inflammation with recruitment of inflammatory cells, such as CD4+ T cells, eosinophils, and mast cells, was detected on the 8th week but not on the 4th week. Of interest, serum IgE levels were markedly elevated, but this apparently followed the development of the dry skin symptoms and the prolonged scratching. On the other hand, epidermal nerve sprouting was confirmed briefly after the dry skin and the prolonged scratching were seen. After cessation of the HR-AD feeding, the dry skin symptoms readily and completely resolved by 1 week (unpublished observation). The prolonged scratching and the epidermal nerve sprouting were almost completely ameliorated 1 week after cessation. Epidermal hyperplasia and skin inflammation were

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**Fig.2 Three representative results of spontaneous scratching behavior in hairless mice fed a normal diet (Normal) or a special diet (HR-AD)**

All experiments were performed 8-10 weeks after the start of feeding when AD-like symptoms had developed. * *p<0.05, **p<0.01 and ***p<0.001.

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**Fig.3 Summary of chronological analysis of the pathologies in HR-AD-fed mice**
Pharmacological analysis of the scratching behavior in HR-AD-fed mice

To elucidate the mechanism involved in the prolonged scratching, the effects of several drugs were tested (summary is shown in Table 1). Because μ-opioid receptor antagonists are known to suppress a variety of itching types\(^{19}\), we first examined the effect of a μ-opioid receptor antagonist, naloxone, on the prolonged scratching. We found that the scratching response was dose-dependently inhibited by naloxone\(^{19}\), suggesting that the scratching is related to the induction of an itching sensation. We have previously shown that the HR-1 mouse is a high responder for scratching induced by intradermal injection of pruritogens such as histamine and serotonin\(^{20}\). Thus, the effects of mepyramine, an H\(_1\) receptor antagonist, and methysergide, a 5-HT\(_{1\alpha}\) receptor antagonist, on the prolonged scratching were studied. However, these antagonists had no effect on the scratching\(^{19}\), suggesting no involvement of histamine and serotonin in the itch-related response. Topical corticosteroids are most frequently used to treat human AD and are effective in reducing skin inflammation. In HR-AD-fed mice, daily oral treatment with corticosteroid (dexamethasone) during HR-AD feeding suppressed the AD-like skin inflammatory changes, including epidermal hyperplasia and inflammatory cellular infiltration\(^{19}\). In contrast, the dexamethasone treatment did not ameliorate the prolonged scratching, the dry skin symptoms, and epidermal nerve sprouting\(^{18,17}\). Thus, it is further suggested that inflammatory changes are not directly involved in the itch-related behavior. Since the prolonged scratching occurs in parallel with the changes in the dry skin symptoms, we examined the effect of emollient (petrolatum ointment) on the scratching response. The application of petrolatum ointment decreased the increased TEWL within 1 h\(^{19}\) and recovered the decreased DWC until 3 h after application (unpublished observation); the prolonged scratching was inhibited only during the time the increased TEWL was ameliorated\(^{19}\). Collectively, these pharmacological studies further suggest that skin barrier dysfunction rather than skin inflammation is involved in the itch-related scratching. In addition, the epidermal nerve sprouting

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<th>Dry skin symptoms</th>
<th>Skin inflammation</th>
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<td>μ-opioid receptor antagonist</td>
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<td>5-HT(_{1\alpha}) receptor antagonist</td>
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<td>Corticosteroid</td>
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<td>Petrolatum ointment</td>
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μ-opioid receptor antagonist (naloxone, 1 and 10 mg/kg, s.c.) was administered 15 min before observation. H\(_1\) receptor antagonist (mepyramine, 10 mg/kg, i.p.) and 5-HT\(_{1\alpha}\) receptor antagonist (methysergide, 1 mg/kg, i.p.) were administered 30 min before observation. Corticosteroid (dexamethasone, 1 mg/kg, p.o.) was given once daily from the start of feeding. Petrolatum ointment (approx. 200 mg/animal) was applied on the whole skin of the mice. +: effective, −: ineffective, and N.D.: not determined.
could partly contribute to the scratching response.

To examine whether deficiency or excess of some ingredients contributes to the development of dry skin, mice were fed 10% normal diet containing HR-AD after establishment of the symptoms. Ten days after the change of feeding, the dry skin was significantly alleviated\(^5\), indicating that deficiency but not excess of some ingredients in HR-AD is responsible for the dry skin. To discover the deficient ingredients, supplementing effects of deficient minerals or vitamins, such as magnesium, cobalt, choline, folic acid, inositol, and nicotinic acid on the dry skin were investigated. As a result, the deficiency of these minerals and vitamins was not related to the development of the barrier disrupted-dry skin\(^5\). On the other hand, since the amount of crude fat in HR-AD is lower than that in normal diet, the effect of essential fatty acid, linoleic acid (LA), on the dry skin was studied. Although oleic acid used as a vehicle did not affect the dry skin symptoms, LA remarkably relieved them in a dose-dependent manner (Fig.4). Thus, it is suggested that LA deficiency is involved in the development of the barrier disrupted-dry skin.

**Conclusion and perspective**

Our findings obtained from analyses using HR-AD-fed mice show that this mouse model could be used as an experimental model for AD, and that skin barrier dysfunction plays an important role in the development of itch-related behavior and possibly of the AD-like skin inflammation. In other words, HR-AD-fed mice could be considered an AD model based on the skin barrier defect. Interestingly, recent genetic studies have shown that loss-of-function mutations in the gene encoding filaggrin, which result in impaired skin barrier function through an unknown mechanism, are strongly associated with a subset of AD patients\(^{21-23}\). Therefore, it is now increasingly proposed that, in a fraction of patients with AD, the skin barrier defect is not merely a consequence of the dermatitis but rather the driver of dermatitis\(^{24,25}\). However, AD is in fact considered to be a multifactorial and heterogeneous disease entity, so that precise pathomechanisms remain unclear. Further investigations of the etiological mechanisms in this model may improve our knowledge of the nature of AD, and may provide insights into possible therapies for the disease.

**References**