

Association of T-cell receptor V β haplotypes with dry skin in DS-*Nh* mice

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Summary

Background. Although dry skin and T cell-dependent disease exacerbation are characteristic features of atopic dermatitis (AD), the involvement of T cells in the development of dry skin remains unclear.

Aims. We aimed to elucidate the role of T cells in the development of dry skin in DS-*Nh* mice as a model for AD, and to evaluate this skin condition pharmacologically.

Methods. We prepared DS-*Nh* mice harbouring a T-cell receptor (TCR)V β^a haplotype with a central deletion in the *TCRBV* gene segments, and mice harbouring a TCRV β^b haplotype without any deletion. We analysed the TCRV β chain usage and cytokine response to antimouse CD3 monoclonal antibodies in the splenocytes from the two mouse substrains. Transepidermal water loss (TEWL) was measured, and histochemical examination of these mice was carried out. Finally, a pharmacological analysis using loratadine was also performed to evaluate the features of spontaneous dry skin in DS-*Nh* mice as a model of AD.

Results. Although the deletion of *TCRBV* gene segments in the TCRV β^a haplotype yielded different representations of each TCRV β mRNA, this deletion did not evoke distinct cytokine profiles in the splenocytes compared with those of mice with the TCRV β^b haplotype. Furthermore, our results indicated that the onset of dry skin occurred earlier in mice with TCRV β^b than in those with TCRV β^a . Pharmacologically, AD-like dry skin in DS-*Nh* with TCRV β^b mice is susceptible to an H1 blocker.

Conclusions. A specific lymphocyte subpopulation bearing T-cell receptors may be responsible for loratadine-responsive dermatitis in DS-*Nh* mice.

Introduction

Human atopic dermatitis (AD) is a chronic inflammatory skin disease affecting > 10% of children, and is also a major cause of skin disease-related occupational disabilities.¹ It is pathogenically characterized by an increased number of a certain type of T cells in skin

lesions. Dry skin is also a common finding in patients with AD, apart from dermatitis, and about 80% of patients with AD regard their skin as being dry.² Considering these facts, abnormal dysfunctions of T cells and epidermal skin are thought to be closely associated with AD. However, only a few reports have attempted to elucidate the possible functional correlations between T cells and dry skin in AD.

We recently reported that the Gly573Ser substitution in transient receptor potential V3 (TRPV3) caused a hairless phenotype in DS-*Nh* mice.^{3–5} DS-*Nh* mice are considered to be a suitable model of AD because they have marked increases in: (i) values of transepidermal water loss (TEWL) on the lateral face skin; (ii) serum

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levels of IgE, interleukin (IL)-4, and IL-13; (iii) numbers of CD4-bearing T cells; (iv) itching behaviour; and (v) tissue levels of nerve growth factor.^{6–9}

To investigate the role of the T-cell receptor variable beta chain (TCRV β) haplotype in the development of dermatitis, DS-*Nh* mice and their congenic strain harbouring the TCRV β^a haplotype with a central deletion in the *TCRBV* gene segments were prepared.⁹ Interestingly, we previously showed that there was a delayed onset of dermatitis in DS-*Nh* mice with TCRV β^a compared with those with TCRV β^b . Furthermore, another strain harbouring the TCRV β^a haplotype has been characterized as a 'low-responder' strain in terms of IgE-dependent immediate-type hypersensitivity associated with Th2 cytokine in an experimental asthma model.¹⁰

We investigated the roles of T cells in the development of dry skin in DS-*Nh* mice as a model for AD, and evaluated this skin condition pharmacologically using loratadine.

Methods

Animals

All mice used in this study were male. They were maintained in cages, exposed to a 12 h light/dark cycle. They were provided with standard food and water *ad libitum*. Animals were kept under specific pathogen-free conditions for 5 weeks, and then moved to conventional conditions. This study was conducted according to the guidelines for animal experimentation at Shionogi & Co. Ltd.

Analysis of mRNA for T-cell receptor usage and cytokine profile in splenocytes

Crude cellular RNA was extracted from splenocytes using a commercial reagent (TRIzol™ LS Reagent; Invitrogen, CA, USA), according to the manufacturer's instructions. Adaptor-ligation PCR and microplate hybridization assay were carried out as previously described.^{7,11–14}

Murine spleen cells (5×10^5) were incubated in control microplates and in microplates coated with anti-CD3 monoclonal antibody (clone 2C11; BD Bioscience, MA, USA) in RPMI 1640 medium with 10% fetal calf serum, 100 U/mL penicillin, and 100 μ g/mL streptomycin at 37 °C for 2 days. Cell-free supernatants were collected, and the profile of 18 cytokines in these supernatants was determined semiquantitatively (Bio-Plex Multiplex System; Bio-Rad Laboratories, CA, USA) according to the manufacturer's instructions. We

estimated the values below the detection limits of the kit as the threshold values of each standard.

Histochemical analysis

Each mouse was killed after being anaesthetised with ether, and the skin on the right cheek dissected. Because DS-*Nh* mice develop skin symptoms on the forehead but not on the back,³ paraffin wax-embedded sections of facial skin from mice at 6, 9, and 20 weeks of age were stained with haematoxylin and eosin for histopathological analysis and with toluidine blue for analysis of mast cells.

Measurement of transepidermal water loss

We measured TEWL of mouse skin using a commercial meter (Tewameter TM-210; Courage and Khazaka Electronic GmbH, Köln, Germany). The room conditions were kept constant during the experiments (24 ± 2 °C and $45 \pm 5\%$ relative humidity). Two different skin areas were selected for the assessment of TEWL: the lateral facial skin and dorsal skin.

Results

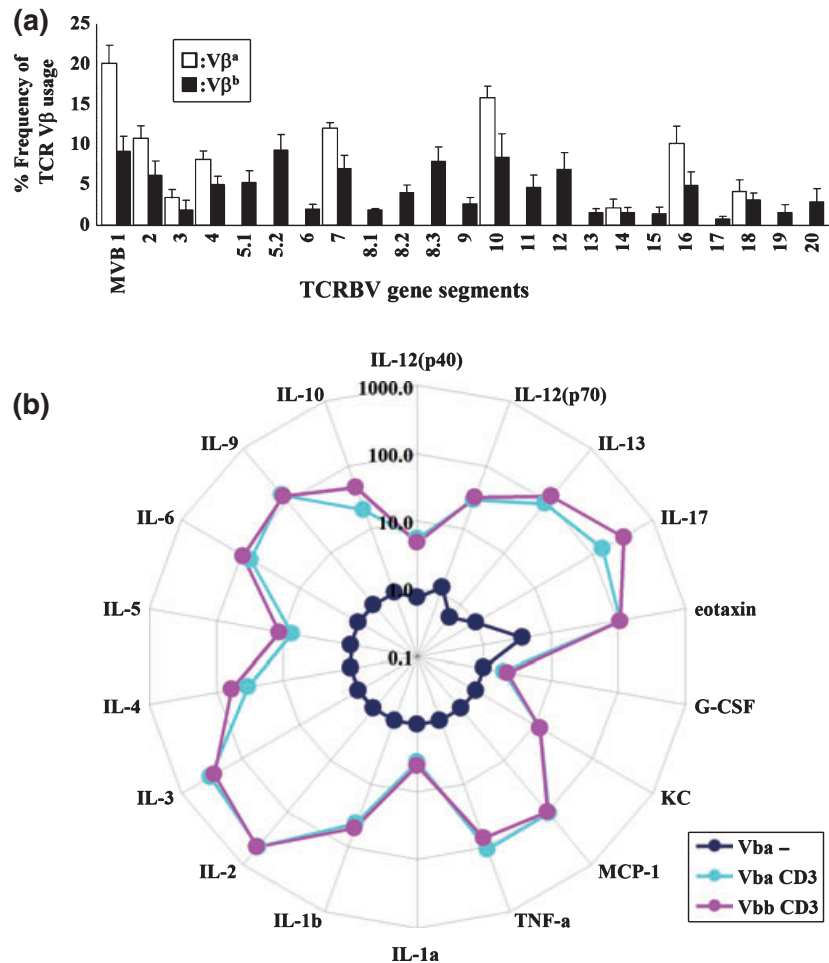
Analysis of the usage of the *TCRBV* gene

TCRV β^b is the most common TCRV β haplotype, which is found in the majority of laboratory mouse strains including DS-*Nh*.⁹ One of the major variant TCRV β loci is the TCRV β^a haplotype, which is characterized by the genomic deletion of several *TCRBV* gene segments. Hence, the TCRV β repertoire in spleen cells from DS-*Nh* mice and their substrains with TCRV β^a haplotypes at 5 weeks old was analysed to investigate the effects of deletion of the *TCRBV* gene segments on the representation of each TCRV β mRNA in DS-*Nh* mice with the TCRV β^a haplotype. The results of the relative TCRV β usages in spleen cells from both strains indicated that TCRV β 5, 6, 8, 9, 11, 12, 13, 15, 17, 19, and 20 were not detectable in mice with the TCRV β^a haplotype (Fig. 1a).

Cytokine response to antimouse CD3 monoclonal antibody in splenocytes

We compared cytokine responses to antimouse CD3 monoclonal antibody in splenocytes from both substrains. Splenocytes were cultured with and without antimouse CD3 for 2 days and pooled culture supernatants were assessed for 18 types of cytokines (Fig. 1b). Although our data indicated that about half

Figure 1 Effects of TCRV β deletion on the expressions of each TCRV β (a) mRNA and (b) cytokine profile in splenocytes. (a) Relative TCRBV usages in spleen cells from TCR V β^a and V β^b mice at 5 weeks of age under specific pathogen-free conditions. Data are mean (blocks) and SD (bars) of TCRV β repertoires in splenocytes from five mice. (b) The profiles of cytokines in cultured spleen cells from V β^a and V β^b mice stimulated with and without anti-mouse CD3 mAb. We used pooled culture supernatants of spleen cells from mice with both TCRV β haplotypes to identify the expression profiles of the following cytokines: interleukin (IL)-1 α , -1 β , -2, -3, -4, -5, -6, -9, -10, -12(p40), -12(p70), -13 and -17, eotaxin, granulocyte colony-stimulating factor (G-CSF), keratinocyte-derived chemokine (KC), monocyte chemoattractant protein-1 (MCP-1), and tumour necrosis factor (TNF)- α . Relative cytokine expression was calculated by the following formula: (level of cytokine in each group of mice/level of cytokine for V β^b mice without stimulation). The logarithmic axial figures between the range of 0.1 and 1000 represent the value of the relative cytokine expression by this calculation, hence each coloured line on the pie chart represents the cytokine profile of the mice with or without CD3 stimulation. Two independent experiments were carried out, and comparable results were obtained.



of the TCRBV gene segments were deleted in mice with the TCRV β^a haplotype, these deletions did not create a large difference in the profile of cytokine production between these substrains (Fig. 1).

Histochemical assays

Marked histological changes including hyperkeratosis, acanthosis and slight intercellular oedema of the epidermis, and infiltration by inflammatory cells to just below the keratinocytes were observed in the skin lesions of all mice with TCRV β^a and TCRV β^b from 15 weeks onwards (Fig. 2). Earlier onset of hyperkeratosis was observed in mice with TCRV β^b at 4 weeks onwards compared with those of age-matched TCRV β^a . Although the numbers of total and degranulated mast cells were significantly increased at 20 weeks of age compared with those at 6 weeks of age in both mouse strains, there was no significant difference in the number of mast cells between these age-matched strains.

Measurement of transepidermal water loss

Because defective barrier function of the skin is a typical feature of patients with AD and can be shown by an increase in TEWL,¹⁵ we also measured TEWL in the facial and back skin of these mice. Our previous study⁹ indicated that earlier onset of erythema was observed in mice with TCRV β^b compared with those with TCRV β^a . However, erythema did not appear in all mice with TCRV β^b until 1 week postexposure. Surprisingly, the values of TEWL in the facial skin of mice with TCRV β^b significantly increased before the skin symptoms began to develop (Fig. 3). In contrast, TEWL values on facial skin of mice with TCRV β^a increased after 4 weeks of age, when dermatitis began to develop. Furthermore, it is particularly notable that the TEWL values of the back skin from both strains significantly increased in a similar fashion to those of the facial skin, although there was no marked lesion on the back of these mice. Finally, no increase in

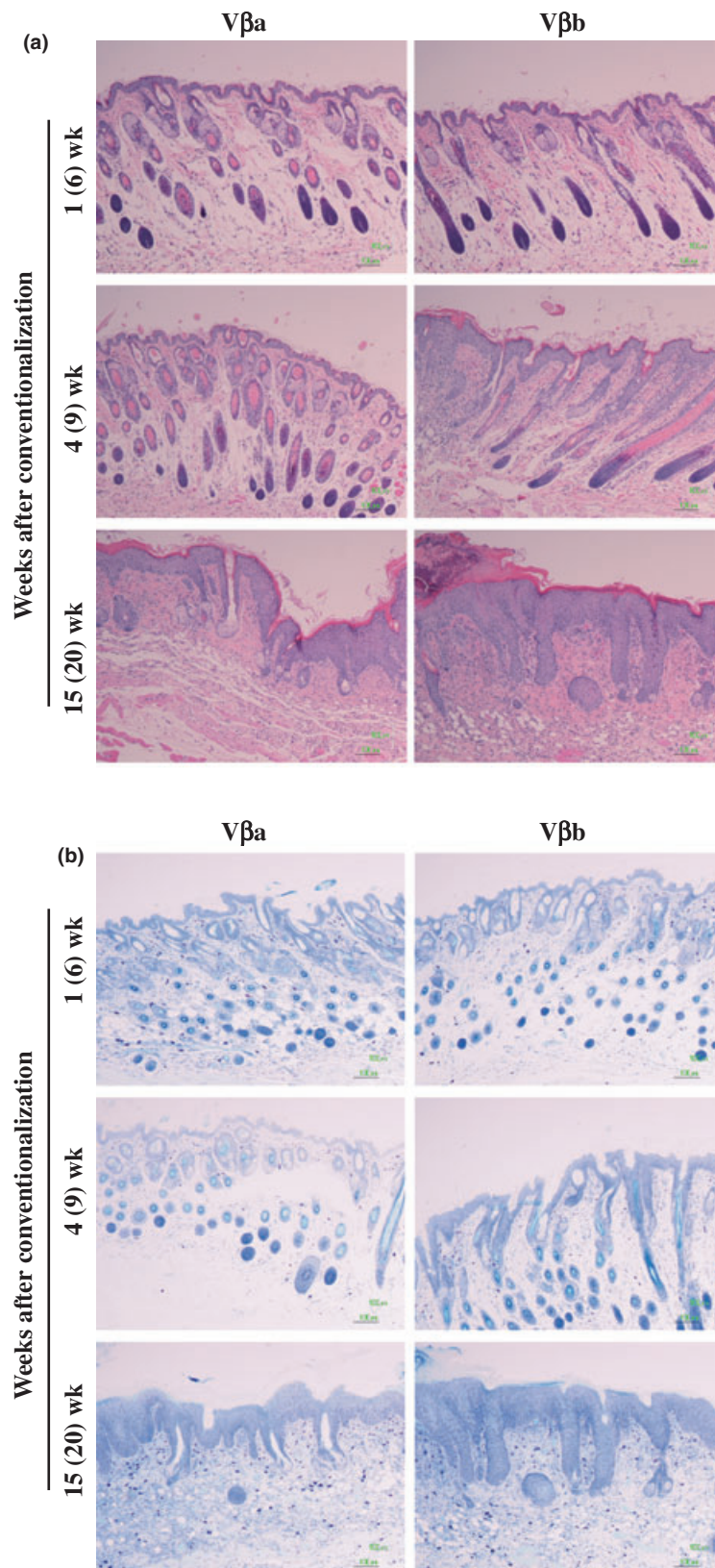


Figure 2 Histological and immunohistological features of facial skin tissues. Paraffin wax-embedded sections from conventionally housed mice at 6, 9, and 20 weeks of age (1, 4, and 15 weeks onwards) were stained with (a) haematoxylin and eosin or (b) toluidine blue. Each panel shows a representative image taken with a $\times 10$ objective to illustrate a wide area. In these experiments, we used ≥ 3 mice per age group, and observed ≥ 4 slides per mouse.

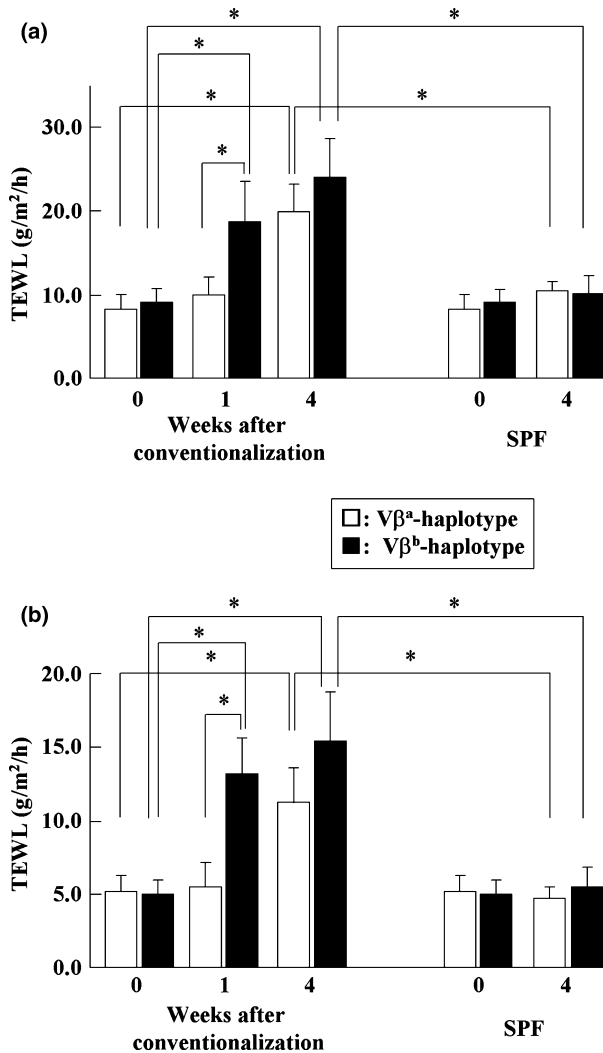


Figure 3 Effects of TCRV β haplotypes on the skin barrier functions in mice after conventionalization. As alterations in the skin barrier *in vivo* produce an increase in transepidermal water loss (TEWL), we measured TEWL on (a) facial and (b) back skin of the mice at 5, 6 and 9 weeks of age (0, 1, and 4 weeks onwards) to assess the barrier function of uninvolved skin in DS-*Nh* mice with the TCRV β^i or TCRV β^b haplotype. White bar, V β^i haplotype; black bar, V β^b haplotype. Values represent means $\pm$ SDs of 6–8D. S-*Nh* mice with either of the TCRV β haplotypes, which were housed in conventional or specific pathogen-free conditions.

TEWL was observed in mice while they were being raised under SPF conditions.

Effects of loratadine

We measured the TEWL of mouse skin after loratadine treatment to investigate the effects of loratadine on the AD-like dry skin in DS-*Nh* mice. As shown in Fig. 4, TEWL values on the facial and back skin were

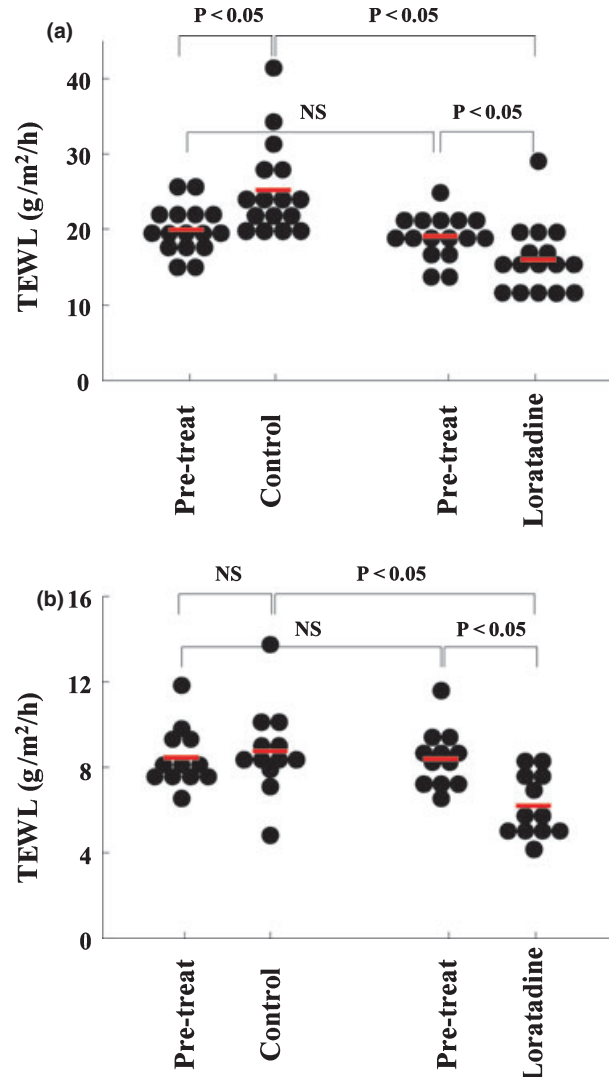


Figure 4 Effects of loratadine in DS-*Nh* mice with atopic dermatitis-like dry skin at 15 weeks of age (10 weeks onwards). Pumps (Alzet Osmotic Pumps, Cupertino, CA USA) filled with loratadine were implanted subcutaneously, and the given loratadine dosage was 3 mg/kg/day for 7 days in each DS-*Nh* mouse. We showed that the levels of transepidermal water loss (TEWL) in (a) facial and (b) back skin of DS-*Nh* mice were significantly reduced after loratadine treatment compared with the levels before loratadine treatment and after treatment with control vehicle. Each dot represents the mean value of TEWL for each mouse ($n = 12$ –16), and the red bars represent the mean values of the groups of mice. NS, not significant.

significantly decreased compared with those in mice that received vehicle. Interestingly, the vehicle worsened the TEWL values on facial skin.

Discussion

We reported previously that *Staphylococcus aureus* producing staphylococcal enterotoxin C (SEC) plays an

important role in the development of AD in this mouse strain.^{7,9} Here, these mice were used to investigate the role of the TCRV β -haplotype in the development of dry skin. Earlier onset of dry skin was observed in DS-*Nh* mice with TCRV β^b compared with those with TCRV β^a when they were moved to conventional conditions. Neither strain spontaneously developed dry skin when they were kept under *S. aureus*-free conditions, indicating that these bacteria contribute to the development of dry skin. However, only small numbers of *S. aureus* were recovered from the skin of 6-week-old mice with dry skin kept in conventional conditions for 1 week.⁷ This might be partially due to the change in the ratio of different types of bacteria in the skin lesions, which is believed to be important for the development of AD-like dry skin. Indeed, changing the DS-*Nh* mice to conventional conditions resulted in a change in the ratio of *S. sciuri* and *S. cohnii* B in the skin lesions after this shorter elapsed time.⁷

Epidermal barrier functions are important for dermatophylaxis associated with increased allergen sensitization, TEWL, and viral and bacterial skin infections. In AD, the skin barrier is significantly compromised, and this is partly due to loss-of-function mutations of filaggrin.¹⁶ Previously, we also reported that the ligands of TLR2 synergistically produce IL-13 with SEC in splenocytes of mice with TCRV β^b but not in mice with TCRV β^a , and much higher serum levels of IL-13 were found in mice with TCRV β^b than in those with TCRV β^a .⁹ Interestingly, the expression of filaggrin is decreased in the skin of patients with AD, and this is caused by the overexpression of Th2 cytokines such as IL-13.¹⁷ However, we did not find any significant difference in the tissue and/or serum level of IL-13⁷ and the tissue level of filaggrin between the mouse strains at 1–2 weeks onwards (data not shown).

Because the TCRV β^b haplotype contributes to the earlier onset of AD⁶ and dry skin in our mice, we hypothesize that some cytokines produced by particular T cells, which are deleted in V β^a mice, play an important role in the development of AD-like dry skin. We analysed the cytokine response to antimouse CD3 in splenocytes in both mouse strains to test our hypothesis. Contrary to our expectations, the cytokine response was not affected by the genome deletion, although the differences in TCR haplotypes significantly affected the expression of TCRV β mRNA. Next, we analysed in the skin lesions the numbers of mast cells, which were thought to be reciprocally affected by their quality and quantity¹⁸ because there might be some crosstalk between the mast cells and T cells, and mast cells might be associated with dry skin via the effect of histamine.

However, there were no significant differences in the number of mast cells and the amount of total histamine in the skin lesions (data not shown) between the two mouse strains.

Recently, some investigators studied the correlation between AD and TCR haplotypes. They indicated that natural killer (NK) T cells were decreased in mice with TCRV β^a haplotype compared with those with the TCRV β^b haplotype.¹⁹ These NKT cells were thought to contribute to the development and maintenance of the skin disorders in the hapten-treated mouse model. Interestingly, cutaneous immunization rapidly activates liver NKT cells or causes the migration of NKT cells to the liver.²⁰ In our study, the movement of the mice into conventional conditions meant that the bacterial density in the skin lesions increased and acted as an irritant. These bacterial irritants may cause dermal disorders via the activation or migration of liver NKT cells. Better understanding of the association between NKT cells and AD in these AD models should be further investigated in the future.

We previously reported that clinical severity including the scratching behaviour in DS-*Nh* mice was alleviated by loratadine treatment, and that levels of histamine in the skin tissues of DS-*Nh* mice at 5–6 weeks of age significantly increased compared with those of DS mice.⁴ There was no difference in the histamine level in other tissues between the two mouse strains (data not shown). Furthermore, it is clear that mast cells possess a higher histamine potency in the skin. These results indicate that the scratching behaviour is partially due to a larger amount of histamine in the skin mast cells. To validate this experimental dry skin model in DS-*Nh* mice, we carried out a pharmacological study using loratadine. Because the scratched areas of DS-*Nh* mice were localized to the forehead, and the scratching behaviour caused a barrier disruption at the skin lesions, we considered that improvement in the TEWL values in facial skin caused by loratadine had an antipruritic effect. On the other hand, the improvement of dry skin on the back was partly due to the prevention of scratching and to other unknown mechanisms. This loratadine-reactive dry skin model may become a tool for the development of new agents for the treatment of AD.

These results provide for the first time an experimental support for direct involvement of T cells in the development of dry skin.

Acknowledgement

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