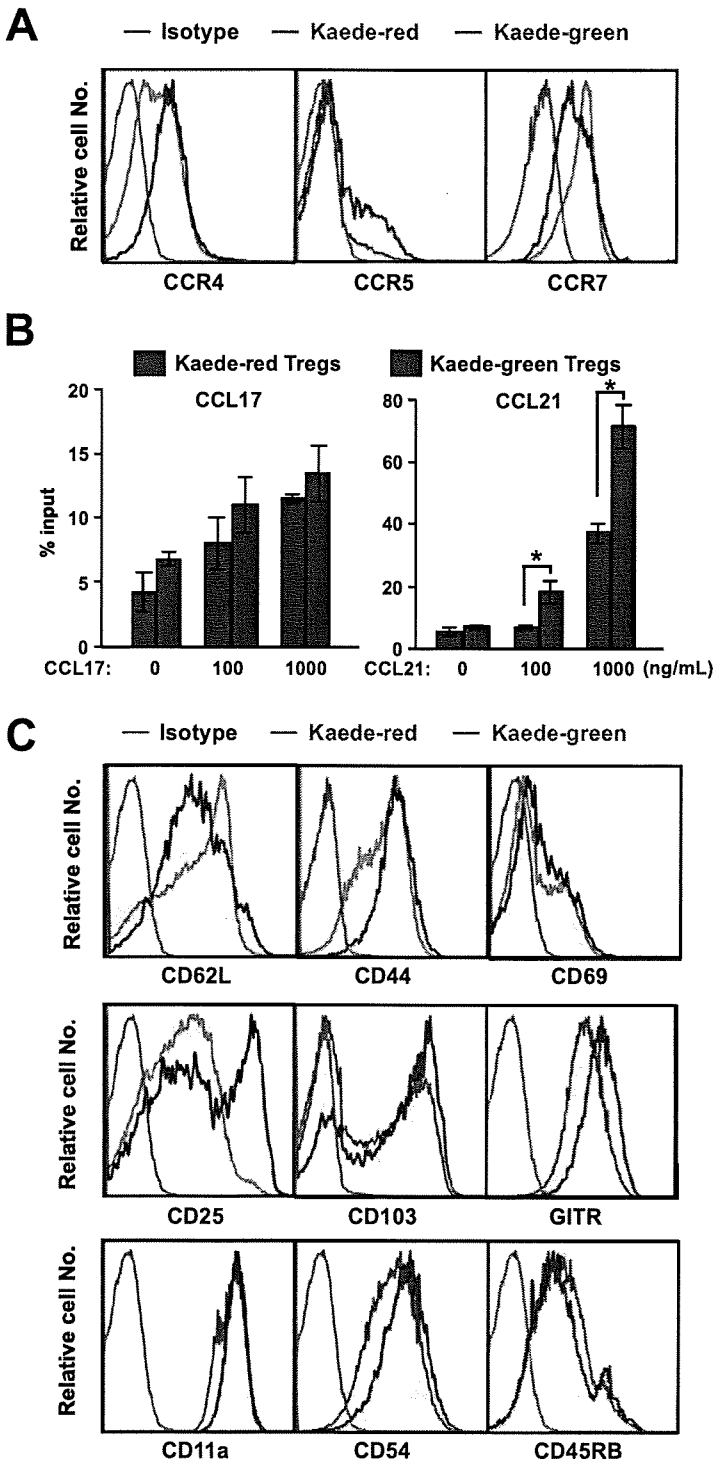
A
— Isotype
— Kaede-red
— Kaede-green
Relative cell No.
CCR4
CCR5
CCR7
B
Kaede-red Tregs
Kaede-green Tregs
CCL17
CCL21
% input
20
15
10
5
0
80
60
40
20
0
*
*
CCL17: 0 100 1000
CCL21: 0 100 1000 (ng/mL)
C
— Isotype
— Kaede-red
— Kaede-green
Relative cell No.
CD62L
CD44
CD69
Relative cell No.
CD25
CD103
GITR
Relative cell No.
CD11a
CD54
CD45RB

Figure 6

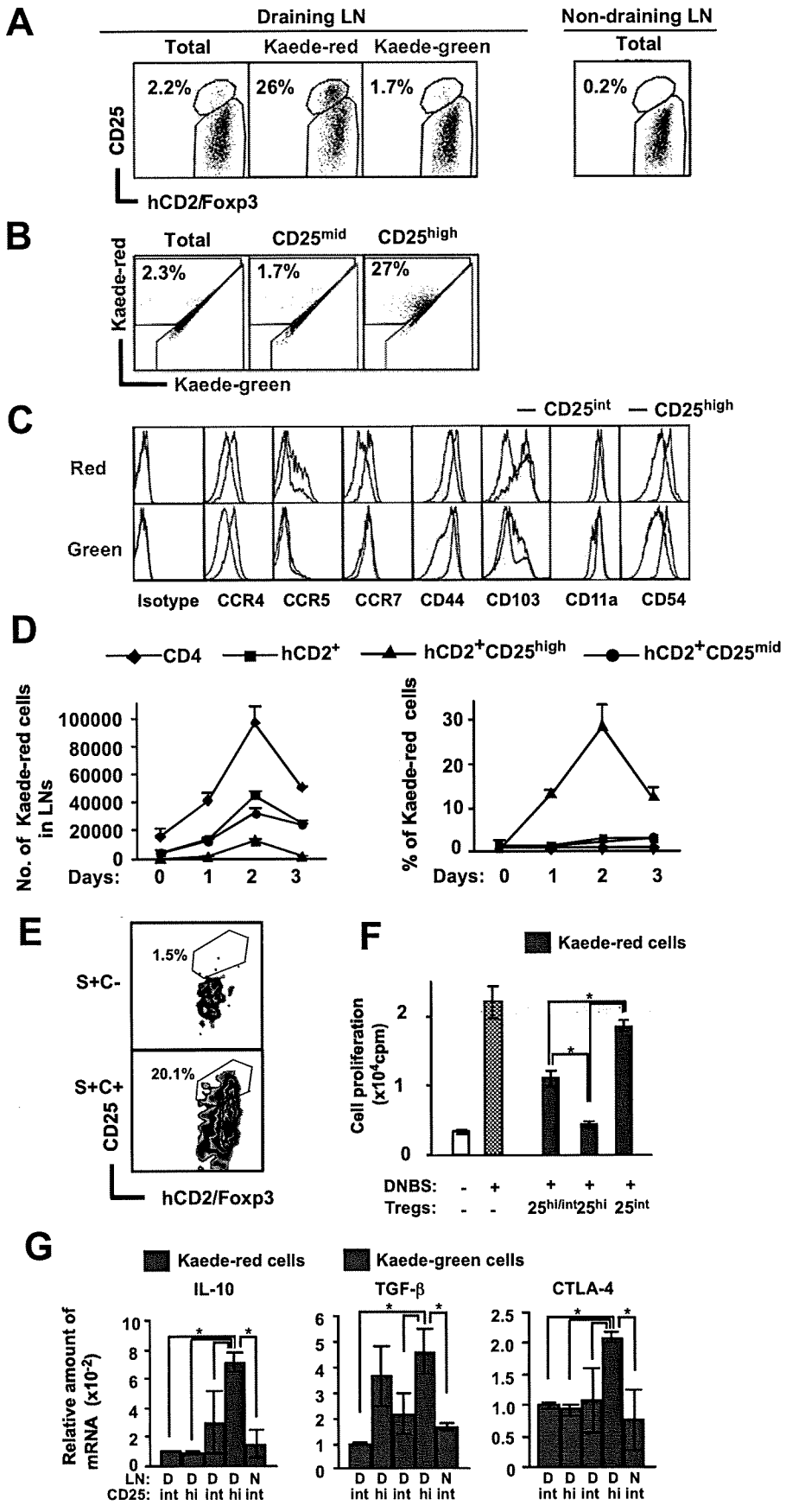
A
Draining LN
Non-draining LN
Total
Kaede-red
Kaede-green
Total
2.2%
26%
1.7%
0.2%
CD25
hCD2/Foxp3
B
Total
CD25mid
CD25high
Kaede-red
2.3%
1.7%
27%
Kaede-green
C
CD25int
CD25high
Red
Green
Isotype
CCR4
CCR5
CCR7
CD44
CD103
CD11a
CD54
D
CD4
hCD2+
hCD2+CD25high
hCD2+CD25mid
No. of Kaede-red cells in LNs
100000
80000
60000
40000
20000
0
Days: 0 1 2 3
% of Kaede-red cells
30
20
10
0
Days: 0 1 2 3
E
S+C-
S+C+
1.5%
20.1%
CD25
hCD2/Foxp3
F
Kaede-red cells
Cell proliferation (x10⁴cpm)
2
1
0
DNBS: - + + + +
Tregs: - - 25hi/int 25hi 25int
G
Kaede-red cells
Kaede-green cells
IL-10
TGF-β
CTLA-4
Relative amount of mRNA (x10-2)
10
8
6
4
2
0
6
5
4
3
2
1
0
2.5
2.0
1.5
1.0
0.5
0
LN: D D D D N
CD25: int hi int hi int

Figure 7

Flaky tail mouse as a possible model of atopic dermatitis

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Total word count: 3694 words

Abstract

Background: The barrier abnormality, derived from a recent link between the incidence of atopic dermatitis (AD) and loss-of-function mutations in the gene encoding filaggrin (*FLG*), is an important factor in the pathogenesis of AD. To further explore the roles of *FLG* mutation on AD, filaggrin-deficient flaky tail (*ft/ft*) mice might be useful.

Objective: We sought to evaluate the immunological and clinical impact of barrier dysfunction from the perspective of AD using *ft/ft* mice.

Methods: Under a specific-pathogen-free (SPF) condition, clinical manifestations, histology of the skin, lymph node cell subsets, integrity of the skin barrier, scratching behavior, and total serum IgE levels were measured. In addition, phorbol myristate acetate-induced irritant contact dermatitis, contact hypersensitivity (CHS), and mite extract-induced dermatitis models were used to predict sequential events.

Results: Even under SPF condition, the majority of *ft/ft* mice showed clinical and histological eczematous skin lesions with scratching, elevated transepidermal water loss, and total serum IgE levels. In addition, irritant contact dermatitis, CHS, and mite extract-induced dermatitis were enhanced in *ft/ft* mice.

Conclusion: These results suggest that *ft/ft* mice can be proposed as an animal model of AD, and provide evidence that the skin barrier defect is as an important component in the pathogenesis of AD.

Key messages:

Flaky tail mice showed spontaneous dermatitis with elevated IgE levels even under a SPF condition.

Filaggrin-deficient flaky tail mice showed outside to inside barrier dysfunction as well as inside to outside barrier abnormality.

Irritant contact dermatitis, CHS, and mite-induced AD-like skin lesion were enhanced in flaky tail mice.

Capsule summary: Flaky tail mice can be a useful animal model of AD due to dysfunction of skin barrier.

Key words: flaky-tail-mice, skin barrier, filaggrin, atopic dermatitis

Abbreviations:

AD (atopic dermatitis); TEWL (transepidermal water loss); *ft/ft* (flaky tail); SPF (specific pathogen free); Dp (*Dermatophagoides pteronyssinus*) ; PAR-2 (protease-activated receptor-2)

Introduction

Atopic dermatitis (AD), which affects at least 15% of children in developed countries, is characterized by eczematous skin lesions, dry skin, and pruritus[1-3]. AD has increased in prevalence over the past few decades[4], and impacts not only on the quality of life of patients but also on medical expenses[5]. Although the precise pathogenic mechanism of AD is as yet unknown, accumulated different lines of evidence suggest that defective skin barrier to environmental stimuli may contribute to the pathogenesis of AD. Dry skin and inside to outside skin barrier dysfunction evaluated by elevated transepidermal water loss (TEWL) have been reported as dermatological features of AD[1,6-8]. It has long been proposed that the barrier abnormality in AD is not merely an epiphenomenon but rather the "driver" of disease activity[9]. The evidence for a primary structural abnormality of stratum corneum is derived from a recent link between the incidence of AD and loss-of-function mutations in the gene encoding filaggrin (*FLG*). Individuals carrying the *FLG* null allele variants tend to develop AD[10-12].

Filaggrin protein is localized in the granular layers of the epidermis, and the keratohyalin granules are mainly composed of the 400-kDa polyprotein, profilaggrin[13-15]. On the differentiation of keratinocytes, profilaggrin is dephosphorylated and cleaved into 10–12 essentially identical 37-kDa filaggrin, which aggregates the keratin cytoskeleton system to form a dense protein-lipid matrix[15]. This structure is thought to prevent epidermal water loss and impede the entry of external stimuli, such as allergens, toxic chemicals, and infectious organisms. Therefore, filaggrin is a key protein in terminal differentiation of the epidermis and skin-barrier function[16].

Since AD is a common disease without satisfactory therapy, understanding the mechanism of AD through animal models is an urgent issue [1,8,17]. Mice that spontaneously develop AD-like skin lesion, such as NC/Nga mice, display many features of human AD[1,7], however, lack of clarification of the intrinsic defect in NC/Nga mice complicates to understand the pathophysiological relevance in this model[1].

Flaky tail (*ft/ft*) mice, first introduced in 1958, are spontaneously mutated mice with smaller ears, tail constrictions, and flaking tail skin appearance, which is more evident between 5 and 14 days of age[18]. The *ft/ft* mice express an abnormal profilaggrin polypeptide that does not form normal keratohyalin F-granules and is not proteolytically processed to filaggrin. Therefore, filaggrin is absent from the cornified layers in the epidermis of the *ft/ft* mice [19,20].

Recently, we have revealed a responsible gene for the *ft/ft* mice as a nonsense mutation of 1-bp deletion analogous to common human *FLG* mutation, and showed that the *ft/ft* mice are predisposed to develop an allergen-specific immune response following sensitization with foreign allergen, ovalbumin (OVA) [20]. Nonetheless, except for the observation of histological inflammatory infiltrates of the *ft/ft* mice in the steady state [20], clinical manifestations of the *ft/ft* mice under specific-pathogen-free (SPF) condition have not been thoroughly studied [18-20]. In addition, other essential components of AD, such as pruritus and skin barrier dysfunction remain to be clarified in the *ft/ft* mice. Further immunological analyses of the *ft/ft* mice are also required to understand the involvement of barrier dysfunction to AD.

Here we sought to examine the relevance of *ft/ft* mice in the perspective of AD. The *ft/ft* mice showed spontaneous dermatitis with elevated IgE levels even in the steady state under SPF condition. In addition, enhancement of irritant contact dermatitis, CHS, and mite-induced AD-like skin lesion was observed in the *ft/ft* mice.

Materials and Methods

Mice

B6 mice were purchased from SLC (Shizuoka, Japan). Flaky tail (STOCK *a/a ma ft/ma ft/J*) mice, outcrossed onto the B6 at Jackson Laboratory (Bar Harbor, ME)[18,19], have double homozygous of flaky tail (*ft*) and matted (*ma*) mutations. Female mice were used otherwise mentioned and were maintained on a 12-hour light/dark cycle at a temperature of 24 °C and at a humidity of 50±10 % under SPF condition at Kyoto University Graduate School of Medicine. All experimental procedures were approved by the institutional animal care and use committee of Kyoto University Faculty of Medicine.

Western blot analysis

For immunoblot studies[21], a 4 mm punch biopsy of dorsal skin was taken from mice. The epidermis was homogenized in a lysis buffer containing 0.1 M Tris hydroxymethyl aminomethane-HCl (pH 9), 6 M urea, 1% 2-mercapto-ethanol, 1% sodium dodecyl sulfate, 1 mM ethylenediamine tetraacetic acid, and 0.1 mM phenylmethanesulfonyl fluoride, and centrifuged (15,000 x g, 1 minute). The supernatants were applied to NuPAGE Novex Bis-Tris Mini Gels (Invitrogen, Carlsbad, CA) then transferred electrophoretically to a polyvinylidene fluoride membrane (Hybond P, Amersham, GE Healthcare, Chalfont St. Giles, United Kingdom). After blocking with 5% skim milk in Tris-Buffered Saline (TBS) containing 0.1% Tween 20, the membrane was incubated with anti-filaggrin polyclonal antibody (Ab) (Covance, San Diego, CA), 1/1000, overnight at 4 °C, followed by washing with TBS containing 0.1% Tween 20. The membrane was incubated with the peroxidase-conjugated anti-rabbit immunoglobulin Ab (Cell Signaling Technology, Danvers, MA), 1:1000, for 1 hour at room temperature. After washing, the membrane was visualized using an enhanced chemiluminescence kit (GE Healthcare).

Clinical observation and histology

The clinical severity of skin lesions was scored according to macroscopic diagnostic criteria for human AD[22]. Briefly, the total clinical score for skin lesions was designated as the sum of individual scores graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe) for the symptoms of pruritus, erythema, edema, erosion and scaling. Scratching behavior was observed for more than 2 minutes before skin manifestations were scored.

For histology, the dorsal skin of mice was stained with hematoxylin and eosin (HE). Toluidine blue staining was used to detect mast cells, and the number of mast cells was calculated as the average from 5 different fields of each sample.

Flow cytometric analysis

The cells of skin draining axillary and inguinal lymph nodes (LNs), and spleen were analyzed by flow cytomery. Fluorescent-labeled anti-CD4 and anti-CD8 Ab were obtained from eBioscience (San Diego, CA) and used for staining of cells. Total number of cells per organ and the number of each subset was examined by flow cytometry, FACS Canto II (Becton Dickinson, San Diego, CA).

Total and mite-specific serum IgE

Total IgE levels of the serum were measured by mouse IgE ELISA quantitation kit (Bethyl Laboratories, Montgomery, TX) according to manufacturer's protocols. For measurement of mite-specific IgE levels, ELISA assay was slightly modified from mouse IgE ELISA quantitation kit (Bethyl Laboratories). Plates were coated and incubated with 10 μg/ml *Dermatophagoides pteronyssinus* (Dp) (Biostir, Hiroshima, Japan) diluted with coating buffer for 60 minutes. After blocking for 30 minutes, 100 μl of 5 time-diluted serum was added into each well and incubated for 2 hours. One hundred μl of anti-mouse IgE-horse radish peroxidase conjugate (1:15,000) was used to conjugate the antigen-Ab complex for 60 minutes at room temperature, and then proceeded according to the manufacturer's protocol. Absorbance was measured at 450 nm. The result was designated as subtraction of the mean of negative control absorbance from the sample absorbance.

160 **Skin barrier function**

161 Mice were shaved on the dorsal skin prior to measurement. To evaluate the inside to outside
162 barrier function, TEWL was measured with a tewameter (Vapo Scan AS-VT100RS, Tokyo,
163 Japan) at 24 °C under 46% relative humidity.

164 To observe the outside to inside barrier function, fluorescein isothiocyanate isomer I
165 (FITC) (Sigma) was used. The shaved dorsal skin of mice was applied with 100 μl of 1%
166 FITC diluted in aceton and dibuthylphalate (1:4), and 3 hours later, this area was tape-
167 stripped (Scotch, St. Paul, MN) for nine times to remove stratum corneum containing the
168 remnant of FITC. The painted area of skin (1.2 cm x 1.2 cm) was taken and subjected to
169 measurement of FITC concentration. The separation between dermis and epidermis was
170 performed with PBS at 60°C for 10 seconds. The epidermis was soaked into 500 μl of PBS,
171 homogenized, and spin down at 2200 g. The supernatant was collected and fluorescent was
172 measured using Wallack 1420 (Arvo SX, Perkin, Waltham, MA). The fluorescence value
173 was compared to standart curve using FITC serial dilutions.

174 **Scratching behavior**

175 The scratching behavior was measured using Sclaba Real (Noveltec, Kobe, Japan). Mice
176 were put into the machine 20 minutes prior to measurement for adaptation. Then the
177 scratching number and duration was counted according to the manufacturer protocol for 15
178 minutes after ointment applications.

179 **Dermatitis models**

180 For irritant contact dermatitis, 20 μL of 0.2 mg/ml phorbol myristate acetate (PMA; Sigma)
181 was applied to both sides of the ears. Ear thickness change was measured at 1, 3, 12, 24
182 hours and 5 days after application. For a CHS response, 25 μl of 0.5% 1-fluoro-2.4-
183 dinitrobenzene (DNFB) (Nacalai tesque, Kyoto, Japan) was painted on the shaved abdomen.
184 Five days later, the ears were challenged with 20 μl of 0.3% DNFB and ear thickness
185 change was measured at 1, 6, 24, and 48 hours after application.

A delayed type hypersensitivity (DTH) model was conducted using OVA (Sigma). Mice were sensitized with 200 μL of 0.5 mg/mL of OVA in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, MI) intraperitoneally, and 5 days later, challenged with 20 μL of 1mg/ml of OVA in incomplete Freund's adjuvant (IFA) (Difco Laboratories) into the hind footpads. Footpad swelling was measured before and 24 hours after challenge. Nonsensitized mice were used as a control.

To develop a murine AD-like skin lesion, 40 mg of 1% *Dermatophagoides pteronyssinus* (Dp) in white petrolatum was applied to ears and upper back skin twice per week for total 8 weeks. Petrolatum without Dp was used as a control. Ear thickness and clinical scores were measured every week. Serum total IgE and mite-specific IgE levels, TEWL, and histology of eczematous skin, were observed 12 hours after final application.

Statistical analysis

Data were analyzed using an unpaired two-tailed *t*-test. *P* value of less than 0.05 was considered to be significant.

Results

Spontaneous dermatitis of *ft/ft* mice in the steady state under SPF condition

As previously described [19,20], the expression of filaggrin monomer was barely detected in the dorsal skin of the *ft/ft* mice compared to B6 mice by Western blotting (fig.1A). The previous reports have not mentioned the precise information on the clinical manifestations on the skin in the steady state under SPF condition. Here we addressed this issue and found that the *ft/ft* mice developed spontaneous dermatitis even under SPF condition (fig.1B). The clinical severities of the skin lesions, including scratch behavior, erythema, edema, erosion, and scaling, were scored. The total clinical scores of the *ft/ft* mice were elevated in line with their age (fig.1C). The manifestations started with erythema and scaling when they were young, and scratching behavior, erosion, and edema consequently followed (fig.1D). On the other hand, no cutaneous manifestation was observed in B6 mice as a control and heterozygous mice intercrossed with *ft/ft* and B6 mice under SPF condition throughout the experiment period (data not shown). In addition, there was no apparent difference in clinical manifestations between male *ft/ft* mice and female *ft/ft* mice throughout the period (data not shown).

Histology of the skin from the *ft/ft* mice showed epidermal acanthosis, increased lymphocyte infiltration and dense fibrous bundle in the dermis, which were not observed in B6 mice (fig.1E). These features were seen both in younger (8 week-old) and older (18 week-old) mice. In addition, toluidine blue staining to detect mast cells showed an increased number of mast cells, especially the degranulated ones in the upper dermis (fig.1F). No mite body was detected in the sections. These data supported the clinical spontaneous dermatitis in *ft/ft* mice in the steady state under SPF condition.

Defect of skin barrier function in *ft/ft* mice

Barrier dysfunction is one of the characteristics of AD[9-12,23]. Therefore, we measured the TEWL, an established indicator to evaluate the barrier function[23]. TEWL was significantly higher in *ft/ft* mice than in B6 mice from an early age (4 week-old) to older age (16 week-

228 old) (fig.2A). Since TEWL reflects the water transportation from the inside to outside of
229 body through the skin, it is of importance to evaluate the outside to inside barrier function
230 in the perspective of invasion of external stimuli. To address this issue, we quantified FITC
231 penetration through the skin from the outside. FITC solution was applied on the shaved
232 dorsal skin of the *ft/ft* and B6 mice. Three hours later, the skin was tape-stripped to remove
233 remnant of FITC in the corneum, and the epidermis was separated and subjected to
234 homogenization to measure the FITC content by a fluorometer. The epidermis of the *ft/ft*
235 mice contained a higher amount of FITC than did B6 control mice (fig.2B). On the other
236 hand, the dermis of both groups of mice did not contain FITC after this procedure (data not
237 shown). These data strongly pointed out the defect of the inside-outside and outside-inside
238 skin barrier in the *ft/ft* mice.
239
240 **Immune status in the steady state**

241 To further elucidate the immune status of *ft/ft* mice in the steady state under SPF condition,
242 we measured the levels of total serum IgE, since AD severity is correlated with elevated
243 serum IgE levels[24]. The IgE levels were significantly higher in *ft/ft* mice than age-matched
244 B6 mice in the steady state of SPF condition (fig 3A). To further analyze the immune status
245 of *ft/ft* mice, single cell suspensions from the skin-draining inguinal and axillary LNs and
246 the spleen were analyzed. The total mononuclear cell number of LNs was significantly
247 higher in *ft/ft* mice than B6 mice, but that of spleen was comparable (fig.3B). In addition,
248 the numbers of $CD4^+$ or $CD8^+$ cells in the skin draining LNs were significantly increased in
249 *ft/ft* mice, but those in spleen did not significantly differ from each other (fig.3C). Thus, an
250 enhanced immune reaction in the *ft/ft* mice seems to be induced by the condition of the skin.

251 **Enhanced dermatitis in the *ft/ft* mice under external stimulus**

252 To characterize feasibility of the cutaneous immune responses, mice were exposed to
253 various external stimuli. Initially we observed irritant contact dermatitis response to PMA
254 as an irritant agent. When we applied PMA to the ears of B6 and *ft/ft* mice, the *ft/ft* mice
255 showed enhanced ear swelling to PMA compared to age-matched B6 mice through the

256 experimental period (fig.4A). We then examined the mice in the magnitude of CHS to
257 DNFB. DNFB was applied on the abdominal skin for sensitization, and 5 days later, the
258 ears were elicited with the same hapten. Consistently, the ear thickness change was more
259 prominent in *ft/ft* mice than B6 mice (fig.4B). To further assess the immune responses of
260 *ft/ft* mice, we applied a DTH response of non-epicutaneous sensitization and challenge.
261 Mice were immunized intraperitoneally with OVA, and elicited subcutaneously with OVA
262 into the footpad. In contrast to the CHS response induced *via* the skin, the footpad swelling
263 response was not high, rather low in *ft/ft* mice, as compared to B6 mice (fig.4C). Thus, the
264 immune and inflammatory response was enhanced in *ft/ft* mice when stimuli operated only
265 through the skin, suggesting that the augmented immune responses of *ft/ft* mice likely
266 depend on the skin barrier dysfunction.

267 It was reported that *ft/ft* mice showed enhanced immune response to OVA[20]. However, the
268 reaction to clinically relevant allergen, such as mites, has not been evaluated in *ft/ft* mice. It
269 was reported that mice exhibit an allergic cutaneous immune response to Dp, as a mite
270 antigen, when applied to the skin after vigorous barrier disruption by tape-stripping or
271 sodium dodecyl sulfate[25,26]. We sought to examine whether ft/ft mice induce skin lesions by
272 applying Dp ointment without additional procedures for skin barrier disruption in order to
273 evaluate the physiological significance of filaggrin. The application of Dp ointment on the
274 shaved back and the ears of B6 mice did not induce any cutaneous manifestation
275 throughout the experiment period (fig.5A, B). On the other hand, Dp application to *ft/ft*
276 mice induced dermatitis, especially on the ear, face, and dorsal skin, which was represented
277 by clinical severity scores. After 16-time applications of Dp ointment over 8 weeks, *ft/ft*
278 mice showed a very severe skin condition compared to the other control groups.
279 Consistently, the ear swelling was most prominent in *ft/ft* mice with Dp ointment (fig.5C).
280 As a control of Dp ointment, petrolatum alone was used, which did not induce any skin
281 manifestations (fig.5 A-C). The histology of HE sections of the involved skin after 16-time
282 applications showed acanthosis, elongation of rete ridges, and dense lymphocyte and
283 neutrophil infiltration in the dermis (fig.5D). In addition, the infiltrate was accompanied
284 with an increased number of mast cells in the dermis (fig.5E). Then, we measured the

285 scratching behavior of these mice. The number and total duration of scratching were
286 significantly higher in *ft/ft* mice than B6 mice irrespective of the treatment with Dp
287 ointment (fig.5F). However, the treatment significantly increased the number and total
288 duration of scratching. We further evaluated the barrier function of each group by
289 measuring TEWL. The TEWL of *ft/ft* mice was higher than that of B6 mice, and even
290 markedly increased in *ft/ft* mice by the application of Dp ointment (fig.5G). Finally, we
291 examined the mite-specific IgE level in the sera of the mice after the last application, and
292 found that the Dp-specific IgE of *ft/ft* mice was much higher than that of B6 mice (fig. H).
293 Thus, the clinical manifestations together with laboratory findings, that corresponded to
294 human AD, were prominently enhanced by the application of Dp ointment even without
295 further barrier disruption.

296

Discussion

Herein we demonstrated that *ft/ft* mice exhibited spontaneous dermatitis with lymphadenopathy and elevated IgE levels as well as disrupted skin barrier even in the steady state under SPF condition. These outcomes are compatible with the features of human AD which include chronic eczema, pruritus, dry skin with elevated TEWL, and increased serum IgE levels [1,6-8 27,28]. In addition, the enhancement of irritant contact dermatitis, CHS, and mite-induced AD-like dermatitis was observed in *ft/ft* mice in a comparison with B6 mice. These results suggest that the barrier defect in this strain of mice can induce spontaneous dermatitis even in the steady state and enhance cutaneous immune responses and inflammation.

Of note, the previous reports have not mentioned in detail on the skin condition of *ft/ft* mice in the steady state under SPF condition [18-20]. In the first report, Lane P demonstrated that *ft/ft* mice without *ma* mutation showed flaky skin as early as postnatal day 2, but became normal in appearance except for slightly smaller ears by 3 to 4 weeks of age[18]. In another report, the authors used commercially available *ft/ft* mice with both *ft* and *ma* mutations as a model of ichthyosis vulgaris, and therefore, there was no description on the cutaneous inflammatory conditions in the perspective of AD[19]. A recent report documented that there was only histological abnormalities, such as acanthosis and lymphocyte infiltration, without clinical manifestation in the *ft/ft* mice that were eliminated from *ma* mutation by backcrossing to B6 mice 4 more times[20]. Therefore, the discrepancies between their mice and our commercially available mice seem to be caused by the presence or absence of *ma* mutation and/or the genetic background. The effect of *ma* mutation in relation to the *ft* mutation of commercially available *ft/ft* mice in the development of AD needs to be clarified. Furthermore, the study showed that the spontaneous phenotype disappeared in heterozygous mice intercrossed with *ft/ft* mice and B6 mice. This finding makes a contrast with human AD, as most of the patients are seen with *FLG* heterozygous mutation. Not only human but also mouse studies are required for clarification of these relationships.

325 It has been reported that TEWL, as a marker of the inside to outside barrier, is high in AD
326 patients with *FLG* mutation[29] and *ft/ft* mice[20]. However, considering the immunological
327 defense by the skin, assessment of the outside to inside barrier function rather than the
328 inside to outside one is crucial. In fact, the outside to inside barrier dysfunction has recently
329 been considered as a main pathogenesis of AD[9,23]. Here we propose a new method to
330 evaluate the outside to inside barrier function by measuring the penetrance of FITC, a
331 fluorescent agent, through the skin. Using this method, we could detect the outside to inside
332 barrier dysfunction in *ft/ft* mice.

333 The skin condition of AD has been well known as a predisposing factor to the sensitive
334 skin[30,31] and allergic contact dermatitis [32,33], but AD skin produces a similar tuberculin
335 response to healthy controls[34,35]. The sensitive skin in human is defined as reduced
336 tolerance to cutaneous stimulation, with symptoms ranging from visible signs of irritation
337 through to subjective neurosensory discomfort[27,28]. In human, it is still controversial
338 whether AD patients are more prone to allergic contact dermatitis than nonatopic
339 individuals[30]. Therefore, we evaluated the skin responsiveness to PMA as an irritant and
340 found an enhancement of irritant contact dermatitis in *ft/ft* mice. In addition, *ft/ft* mice
341 showed an increased skin-sensitized CHS reaction. In contrast, when mice were sensitized
342 intraperitoneally, no difference was observed between *ft/ft* and B6 mice, in consistent with
343 the human findings that tuberculin test were comparable between AD and healthy
344 subjects[34,35]. Therefore, epicutaneous but not other sensitization enhanced the cutaneous
345 immune response in *ft/ft* mice, suggesting that the skin barrier function regulates the
346 cutaneous immune condition, which hints at possible mechanisms in the human disease.
347 Clinical studies have provided evidence that a house dust mite allergen plays a causative
348 or exacerbating role for human AD[36], and a strong correlation exists between *FLG* null
349 alleles and house dust mite-specific IgE[37]. The induction of AD-like skin lesions by
350 repeated topical application of a mite allergen in NC/Nga mice but not in BALB/c mice
351 have been established[25]. In the present study, we induced AD-like skin lesions clinically
352 and histologically with increased TEWL, scratch behavior, and mite-specific IgE in the *ft/ft*
353 mice by the treatment with Dp ointment. Dp is one of the most frequently encountered

354 aeroallergens associated with AD and has protease activities, specifically from Der p1, Der
355 p3, and Der p9, which may activate protease-activated receptor-2 (PAR-2) in human
356 keratinocytes[38,39]. A recent report has shown that activation of PAR-2 through Dp
357 application significantly delays barrier recovery rate in barrier function-perturbed skin or
358 compromised skin[39]. Therefore, Dp may play dual roles in the onset of AD, as an allergen
359 and proteolytic signaling, as a perturbation factor of the barrier function, resulting in
360 persistence of eczematous lesions in AD[39,40].
361 In this study, we have carefully observed the clinical manifestations of *ft/ft* mice under
362 SPF condition and under external stimulation, suggesting that the *ft/ft* mice can be a
363 possible animal model of human AD.
364
365
366

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