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Pathology of Spontaneous Dermatitis in CBy.ALY-aly Mice

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Abstract: CBy.ALY-aly/aly (C-aly) mice develop progressive dermatitis in areas of the face and dorsal neck from around three months of age. *Staphylococcus aureus* was detected in the skin lesions of C-aly mice. However, even when the mice were raised under *S. aureus* free conditions, a similar, though less severe, dermatitis was still observed. The mice showed a marked increase in the number of eosinophils in the peripheral blood and skin lesions, with no changes in plasma IgE levels. These findings suggest that the dermatitis of these mice may be an atypical allergic condition with little or no involvement of IgE. C-aly mice may be a useful animal model of chronic dermatitis or pruritus without elevated IgE levels.

Key words: CBy.ALY-aly mouse, dermatitis, eosinophil

In the course of breeding the CBy.ALY-aly mouse as a congenic strain of ALY/Nsc-aly/aly (ALY), which is characterized by a lack of lymph nodes and Peyer's patches [9], dermatitis developed in areas of the face and dorsal neck from around three months of age. Dermatitis was observed only in the homozygote CBy.ALY-aly/aly (C-aly) and its severity increased with age, while ALY mice, which are homozygous for the *aly* mutation, do not develop such dermatitis. This paper describes the clinical and pathological status of C-aly mice in respect of their development of dermatitis. (In the CBy.ALY-aly mouse, the *aly* gene of the ALY mouse has been transferred to the BALB/cBy genetic background by repeated backcrossing for 12 generations.)

C-aly mice were bred under a barrier system. The animal room was kept at 20–26°C and a relative humidity of 35–75%, with a 12-h light-dark cycle (8:00–20:00). Mice were housed 1 to 3 per cage with paper bedding (Paper Clean; Japan SLC, Hamamatsu, Japan). They were fed commercial food pellets (F-2; Funabashi Farms,

Funabashi, Japan) and provided with tap water *ad libitum*. All experimental procedures were carried out according to a protocol approved by the Animal Care and Use Committee of Nippon Shinyaku Co.

The identification of *aly* homozygotes was based on the demonstration of low IgA levels in the feces. Fecal IgA levels were measured with a mouse IgA ELISA quantitation kit (Bethyl Laboratories, Montgomery, TX, USA) in supernatant obtained from a PBS suspension of feces collected at 7–10 weeks of age (15 µl PBS/mg feces). Individuals showing low IgA levels were judged to be homozygotes (*aly/aly*). Phenotypically normal mice of unknown genotype (*+aly* or *+/+*) are represented as *+/-* in this paper.

Sixty-one female and sixty-one male C-aly mice were observed for dermatitis up to 26 weeks of age. Measurement of plasma immunoglobulin levels, hematological examination and histological examination were carried out on *Staphylococcus aureus* free female mice (*aly/aly* and *+/-*; 5 mice each) and *S. aureus* positive female mice

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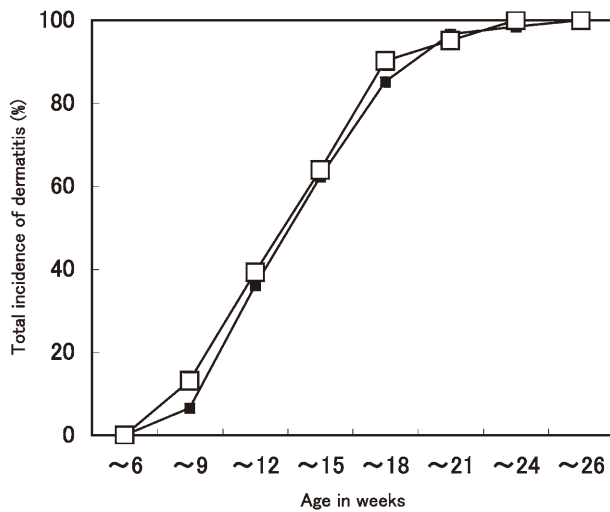


Fig. 1. Incidence of dermatitis in CBy.ALX-aly/aly mice (■, male, n=61; □, female, n=61).

(aly/aly and +/-; 5 mice each) at 22–25 weeks of age. Plasma IgA levels were measured with a mouse IgA ELISA quantitation kit (Bethyl Laboratories), plasma IgG levels with a mouse IgG ELISA quantitation kit (Bethyl Laboratories), and plasma IgE levels with a mouse IgE enzyme immunoassay kit (Yamasa, Chiba, Japan). Hematology was performed with a Sysmex NE-6000 automated blood cell counter and differential white blood cell counts were performed with a Microx HEG-120A automated blood cell analyzer. The histological examination was carried out on the skin of the neck and dorsal regions by staining with hematoxylin and eosin, Congo red, or toluidine blue. *S. aureus* in the skin lesions of C-aly mice was identified by the coagulase test after culture in egg yolk agar.

Experimental data are presented as the mean $\pm$ SD. Statistical analysis was carried out with Student's *t*-test (for samples with equal variance) or Welch's *t*-test (for samples with unequal variance). All tests were two-tailed and differences were considered significant at the $P < 0.05$ level. Statistical analyses were performed with the SAS system (ver. 8.2; SAS Institute Inc., Cary, NC, USA).

Dermatitis in C-aly mice was observed in both sexes (n=61 for each group) as early as 9 weeks of age, and the incidence increased with age, reaching 90% at 18 weeks and 100% at 26 weeks (Fig. 1). In 77.8% of the

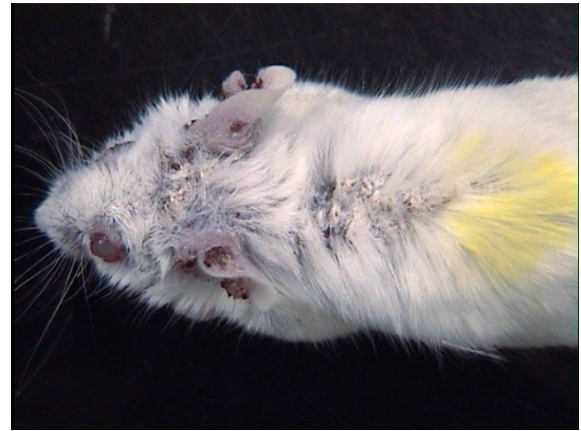


Fig. 2. Spontaneous development of dermatitis in a CBy.ALX-aly/aly mouse at 23 weeks of age.

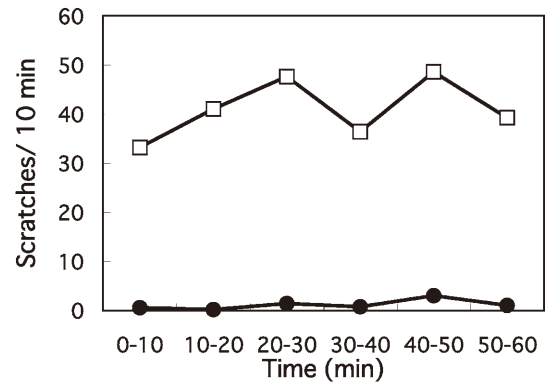


Fig. 3. Scratching behavior in CBy.ALX-aly/aly female mice. The points show the mean number of scratching events counted in 10-min intervals (●, +/-; □, aly/aly; n=5).

mice, dermatitis developed between 10 and 18 weeks of age. The first skin lesions to appear were erythema around the eyelids, and, with progression of the lesions, the eyes became permanently closed with accompanying exudates and scabs. In the full-blown stage of the dermatitis, the skin lesions extended to the entire face, both ears with hemorrhage and thickening, and the dorsal neck area with crust formation (Fig. 2). Dermatitis was occasionally seen also in the mandibular area, while the skin of the central and lower parts of the back was almost normal. The severity of the dermatitis increased with age, and no difference was observed in the appearance of the dermatitis between mice housed individually and mice housed in groups.

Table 1. Plasma immunoglobulin levels in +/- and *aly/aly* female mice at 22–25 weeks of age

	<i>S. aureus</i> (–)		<i>S. aureus</i> (+)	
	+/-	<i>aly/aly</i>	+/-	<i>aly/aly</i>
IgG (mg/dl)	173 ± 51	16 ± 7**	519 ± 391	79 ± 19*
IgA (mg/dl)	106 ± 29	7 ± 2**	174 ± 72	6 ± 1**
IgE (ng/ml)	ND ^a	ND ^a	307 ± 314	101 ± 142

^a Not detected. Each value represents the mean ± SD (n=5). Significantly different from +/- mice (**P*<0.05, ***P*<0.01).

Table 2. Numbers of red and white blood cells in the peripheral blood of +/- and *aly/aly* female mice at 22–25 weeks of age

	<i>S. aureus</i> (–)		<i>S. aureus</i> (+)	
	+/-	<i>aly/aly</i>	+/-	<i>aly/aly</i>
RBC (×10 ⁴ /μl)	960 ± 25	965 ± 69	955 ± 20	1017 ± 10**
WBC (×10 ² /μl)	28 ± 13	81 ± 10**	45 ± 20	107 ± 50*
Basophils (/μl)	0	0	0	0
Eosinophils (/μl)	46 ± 37	2049 ± 474**	109 ± 71	2140 ± 985*
Neutrophils (/μl)	303 ± 158	958 ± 478*	1040 ± 945	1229 ± 569
Lymphocytes (/μl)	2449 ± 1198	5032 ± 1164*	3318 ± 1060	7222 ± 3685
Monocytes (/μl)	20 ± 17	101 ± 57*	35 ± 67	150 ± 165

Each value represents the mean ± SD (n=5). Significantly different from +/- mice (**P*<0.05, ***P*<0.01).

C-*aly* mice with dermatitis frequently scratched the face, ear, jaw or dorsal neck with the hind limb. When scratching events were counted in 10-min intervals, a relatively even distribution of the events over time was observed, in the range of 30–50 events/10 min in female mice at 20 weeks of age (n=5; Fig. 3). More than 300 scratching events in 60 min were occasionally observed in some mice.

Microbiological examination of the affected skin from four female C-*aly* mice (22–25 weeks of age) revealed a mass of *S. aureus* in all of the mice. To investigate the pathological relevance of *S. aureus* in the development of the dermatitis, a C-*aly* mouse colony free from this pathogen was established. Even in these *S. aureus* free C-*aly* mice (n=5), dermatitis was observed from around three months of age on the face and dorsal neck, though the clinical and histological features were less severe than those of *S. aureus* positive animals. These results suggest that infection with *S. aureus* is not a direct cause of the development of dermatitis in C-*aly* mice, though it could be an exacerbating factor.

IgA and IgG levels in C-*aly* mice were significantly lower than those observed in +/- mice, while IgE levels were similar (Table 1). C-*aly* mice showed an increase in the numbers of white blood cells, especially eosinophils, in the peripheral blood compared with +/- mice (45-fold more in *S. aureus* negative and 20-fold more in *S. aureus* positive mice; Table 2). Histological examination of the skin lesions showed hyperkeratosis, acanthosis, parakeratosis, and ulcers, with infiltration of inflammatory cells, predominantly eosinophils, granulated mast cells and macrophages (Fig. 4). These findings are similar to those observed in mouse models of atopic dermatitis (AD) such as NC/Nga [7] and DS-Nh [12]. The numbers of these cells in the dermis of the neck and dorsal regions tended to be greater in C-*aly* mice than in +/- mice (Tables 3 and 4).

HogenEsch *et al.* [5] have observed chronic dermatitis in *cpdm/cpdm* immunodeficient mice. The *cpdm/cpdm* mouse is similar to the C-*aly* mouse in that immunoglobulin production levels are very low and IgA is undetectable in the feces. The levels of secretory IgA

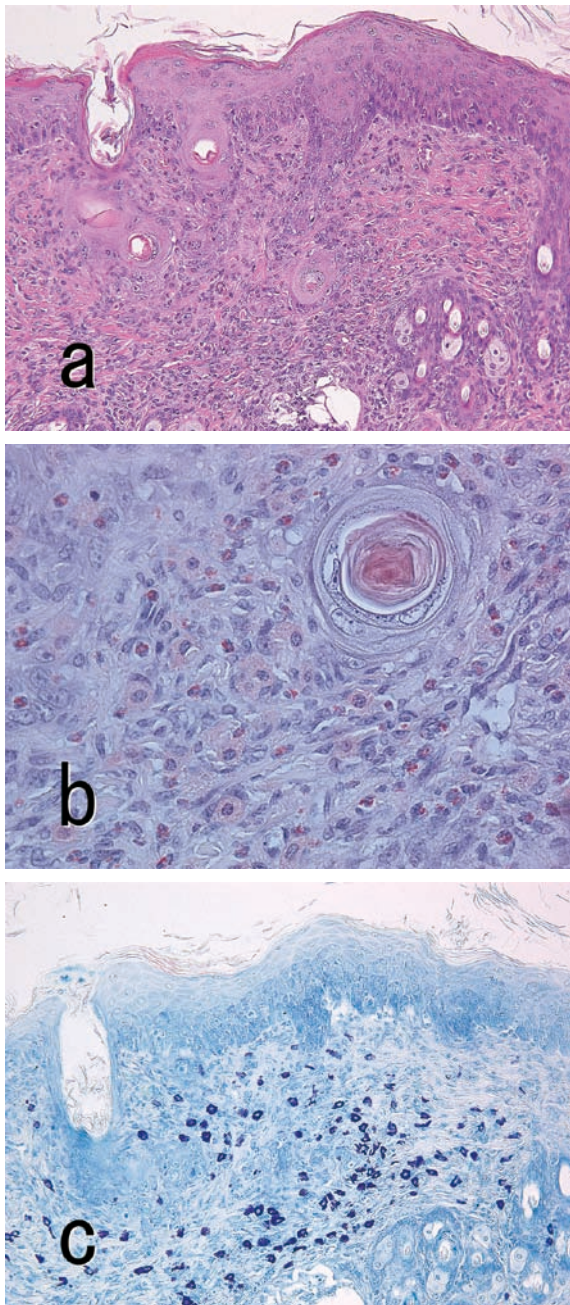


Fig. 4. Section of a dorsal neck skin lesion from a CBy.ALY-*aly/aly* mouse bred under *S. aureus* free conditions (23 weeks of age). (a) Staining with hematoxylin and eosin, original magnification $\times 100$, (b) staining with Congo red, $\times 280$, (c) staining with toluidine blue, $\times 100$.

in the skin of patients with AD are also known to be low [6]. Because *C-aly* mice lack humoral secretory IgA in the skin, their skin barrier might be easily destroyed by scratching, leading to further scratching and further de-

struction of the skin barrier. The destruction of the skin barrier might facilitate the invasion of foreign antigens and increase the susceptibility to infection. However, the original ALY strain with IgA deficiency does not develop dermatitis, so IgA deficiency may not be a direct cause of the development of dermatitis in *C-aly* mice. These observations suggest the involvement of a genetic factor in the development of the dermatitis. A preliminary mating experiment between the *C-aly* and the ALY strains suggests that the incidence of dermatitis increases with increasing contribution of the BALB/c genome (data not shown).

IgE has been proposed to play a key role in AD, because most AD patients have high levels of IgE, mainly directed to environmental antigens [10]. Spontaneous mouse models of dermatitis such as NC/Nga [7], DS-Nh [12] and NOA [3] also show high levels of IgE in the blood. Other studies, however, have shown that IgE levels are not correlated with the course of clinical development [2] and that some AD patients do not show elevated IgE levels [13]. Furthermore, there are transgenic mice lacking IgE altogether that nevertheless develop dermatitis [4]. Taken together, these observations suggest that despite the apparent link between high IgE levels and AD, IgE in fact has little relevance to the development of the disease [2, 8, 11]. An increase in the number of peripheral blood eosinophils is seen in human AD, and NC/Nga mice show infiltration of inflammatory cells, mainly eosinophils, at the site of dermatitis [7]. Eosinophils are the major effector cells in allergic inflammation. They migrate toward chemoattractants released by mast cells and play a critical role in the development of allergic dermatitis by secreting various cytotoxic and inflammatory mediators [1]. It has not been established, however, whether the increased numbers of eosinophils in the blood and dermis of *C-aly* mice is the cause or the result of the dermatitis and accompanying scratching behavior.

CBy.ALY-*aly/aly* mice tended to have greater numbers of eosinophils and mast cells in the dermis and blood compared with their phenotypically normal counterparts. However, in addition to the lack of elevated IgE levels, there was also a lack of degranulated mast cells in the lesions, suggesting the presence of an allergic condition with little or no involvement of IgE. Even though ele-

Table 3. Numbers of infiltrating eosinophils in the skin (neck and dorsal region) of +/- and *aly/aly* female mice at 22–25 weeks of age

	<i>S. aureus</i> (–)		<i>S. aureus</i> (+)	
	+/-	<i>aly/aly</i>	+/-	<i>aly/aly</i>
Dorsal (0.43/mm ²)	1.6 ± 0.9	136 ± 117*	1.2 ± 2.2	124 ± 95**
Neck (0.43/mm ²)	1.2 ± 1.3	230 ± 139**	0.8 ± 1.8	185 ± 65**

Each value represents the mean ± SD (n=5). Significantly different from +/- mice (**P*<0.05, ***P*<0.01).

Table 4. Numbers of infiltrating mast cells in the skin (neck and dorsal region) of +/- and *aly/aly* female mice at 22–25 weeks of age

	<i>S. aureus</i> (–)		<i>S. aureus</i> (+)	
	+/-	<i>aly/aly</i>	+/-	<i>aly/aly</i>
Dorsal (0.43/mm ²)	17 ± 8	59 ± 40	19 ± 10	146 ± 60**
Neck (0.43/mm ²)	27 ± 5	160 ± 42**	19 ± 6	150 ± 30**

Each value represents the mean ± SD (n=5). Significantly different from +/- mice (***P*<0.01).

vated IgE levels were not observed in CBy.ALY-*aly/aly* mice, the dermatitis of these mice still resembled AD in that it showed a chronic course of development and was accompanied by frequent scratching. As an animal model of chronic dermatitis or pruritus without elevated IgE levels, CBy.ALY-*aly/aly* mice have the potential to contribute to the elucidation of the mechanisms of these ailments and the development of appropriate therapeutic agents.

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