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WBN/Kob-Ht Rats Spontaneously Develop Dermatitis under Conventional Conditions: Another Possible Model for Atopic Dermatitis

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Abstract: WBN/Kob-Ht rats (Ht-rats) raised under conventional conditions spontaneously developed dermatitis. In this study, we carried out histopathological analysis to elucidate the pathological features of the dermatitis in Ht-rats. We then tried to detect *Staphylococcus* species recovered from the skin lesions of Ht-rats. We also measured the serum levels of total IgE, IL-4 and IFN- γ in these rats. The histopathological data indicated that inflammatory cells had infiltrated the skin lesions. *Staphylococcus aureus* was recovered from the skin lesions, and the serum levels of total IgE and IL-4 were elevated in Ht-rats with dermatitis. These results suggest that dermatitis in Ht-rats is similar to that in the DS-Nh mice, which has recently been proposed as an animal model for human atopic dermatitis.

Key words: atopic dermatitis, DS-Nh mice, WBN/Kob-Ht rats

The WBN/Kob-Ht rat (Ht-rat) is a spontaneous hairless mutant of the Wistar strain that was established at Ishikawa Laboratory Animal Company, Saitama, Japan. The inheritance mode of the *Ht* mutation is autosomal dominant, and the *Ht* locus is mapped on chromosome 10 [1]. Since the inheritance mode of the *Ht* gene is dominant and *Ht* homozygote rats have high morbidity, the *Ht*-rat used for this study was of the *Ht* heterozygote type. In a previous study, these rats spontaneously developed dermatitis under conventional

conditions, with an incidence of approximately 4% at 20 weeks of age [17]. However, the pathogenic mechanism and characteristics of the dermatitis were not examined from the viewpoint of studying allergy.

In human atopic dermatitis (AD), an increase in the number of infiltrating CD4⁺-bearing T cells, mast cells, and eosinophils has been detected in acute skin lesions as compared with the normal skin [8, 9, 14]. The lesions show a significant increase in the number of cells expressing IL-4, IL-5 and IL-13 mRNA, this suggest-

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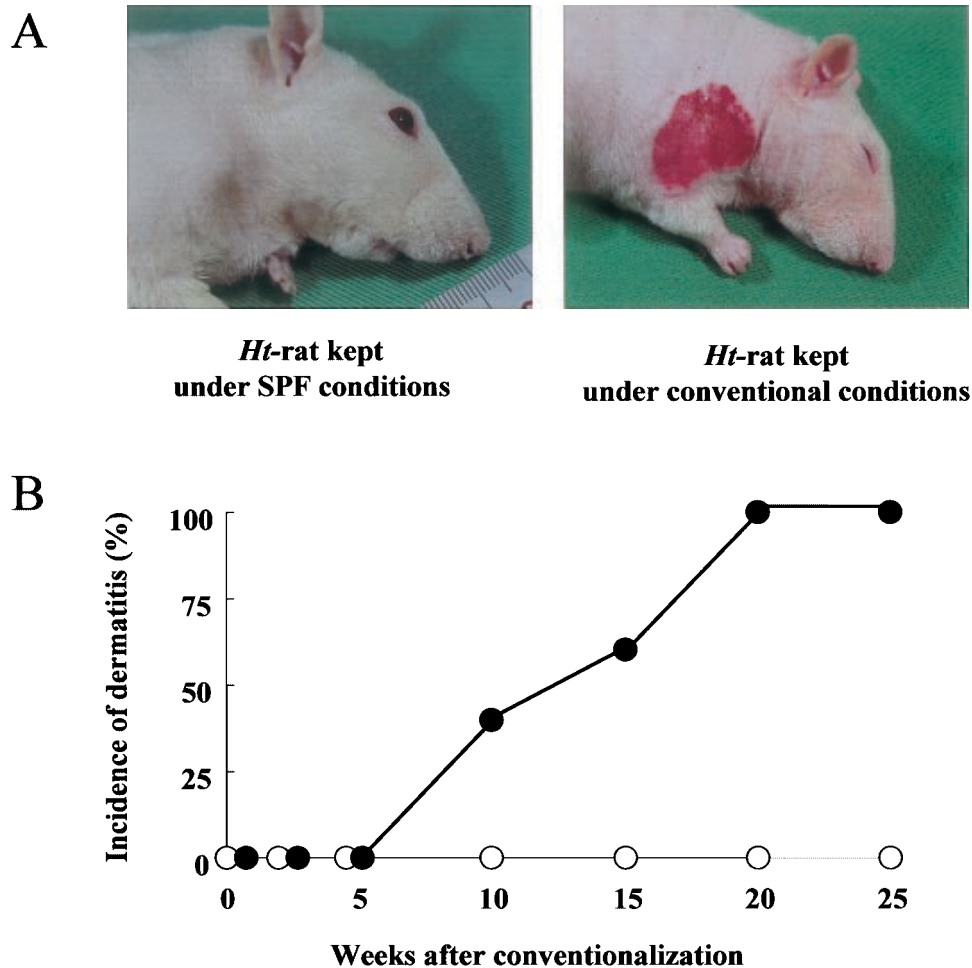


Fig. 1. (A) Gross lesions of dermatitis of *Ht*-rats maintained under conventional or SPF conditions at 25 weeks of age. (B) Incidence of dermatitis in male *Ht*-rats after conventionalization. Rats were conventionalized at 5 weeks of age. ○: Rats maintained under conventional conditions (n=5), ●: Rats maintained under SPF conditions (n=5).

ing preferential accumulation of Th2-type cells. In addition, an increase in the expression of IL-4 and of IL-5 has been detected in CD4- and CD8-bearing T cells in patients with AD [12, 18]. The Th2-type cytokines have a critical role in the initiation of the allergic response in *Staphylococcus aureus*-associated dermatitis [10]. IL-4 plays an important role in the induction of IgE production [13] and as an inhibitor of bacterial clearance [5, 16]. Kasamatsu *et al.* demonstrated an increased amount of serum IL-4 in acute AD compared with the normal control [7]. Furthermore, up to 10^7 colony-forming units of *S. aureus* can be isolated from skin lesions of more than 90% of AD patients [11], while only 5% of normal subjects carry *S. aureus* on

their skin.

In this study, we analyzed dermatitis in the *Ht*-rat to pursue its potential as an animal model for investigating the pathogenesis of atopic dermatitis (AD) and to develop new therapeutic approaches or drugs to treat AD. We discuss the characteristics of *Ht*-rats in comparison with *Nh*-mice, which have recently been proposed as an animal model for human AD [3, 4, 19].

Ht-rats kept under a conventional condition until 25 weeks of age displayed prominent symptoms of dermatitis including edema and erythema at the neck (Fig. 1A). Dermatitis was observed in all *Ht*-rats that were maintained under conventional conditions for 20 weeks, but not in those maintained under SPF conditions (Fig.

Table 1. Comparison of the % frequency of detectable *S. aureus* and the number of mast cells, eosinophils, and CD4- or CD8-bearing cells in the skin lesions from hairless rats and mice maintained under SPF or conventional conditions for 24 weeks

	<i>Nh</i> -mouse (<i>Nh</i> /+) ^b		<i>Ht</i> -rat (<i>Ht</i> /+)	
	SPF	Conventional	SPF	Conventional
% Frequency of <i>S. aureus</i>	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0 ^c
Number of cells ^a				
Mast cells	40.0 ± 11.6	101.7 ± 28.2 ^c	5.9 ± 1.8	16.1 ± 3.8 ^c
Eosinophils	0.0 ± 0.0	7.2 ± 4.2	3.0 ± 2.6	35.6 ± 8.1 ^c
CD4-bearing cells	16.0 ± 10.1	81.0 ± 68.6 ^c	0.0 ± 0.0	54.1 ± 37.1 ^c
CD8-bearing cells	5.4 ± 8.2	23.5 ± 15.3	0.0 ± 0.0	27.8 ± 26.1

a: Number of cells per mm² of skin area. b: With reference to previous reports [4] and [19]. c: Significant increase ($P < 0.05$) in comparison with SPF.

1B). The rats could develop dermatitis only under conventional conditions. To evaluate the involvement of bacteria in the formation of the lesions, bacterial cultures were obtained from the facial skin surface of each *Ht*-rat kept under conventional or SPF conditions. Samples were inoculated onto a salt, egg yolk, agar plate (Nissui, Tokyo, Japan), and incubated at 37°C. Ten colonies per rat were randomly picked up, and the sub-species of *Staphylococci* were identified with an AN-ID Test-SP18 kit (Nissui, Tokyo, Japan).

The animals were humanely euthanized in accordance with the guidelines for animal experimentation at Shionogi Research Laboratories. Skin samples were fixed in formalin or frozen in a freezer. Four-micrometer thick paraffin sections of skin lesions of the *Ht*-rat with or without dermatitis were stained with hematoxylin-eosin (HE) for histopathological observation, acidic toluidine blue to detect mast cells, and by the Luna method for eosinophils. Frozen sections of the *Ht*-rat skin with or without dermatitis were immunostained using mouse anti-rat CD4 (OX-35, PharMingen, San Diego, CA, USA) and mouse anti-rat CD8a (OX-8, PharMingen) antibodies. Positive reactions on the sections were visualized with peroxidase-diaminobenzidine, and the number of positive cells was counted.

Staphylococcus aureus was not isolated from 25-week-old *Ht*-rats maintained under the SPF condition (Table 1), and approximately 70% of the isolated bacteria from the animals housed conventionally were common *Staphylococcus* species (*S. cohnii*B and *S. xylosus*); the rest could not be identified. These bacterial strains were completely replaced by *S. aureus* in

conventionally housed *Ht*-rats with dermatitis (Table 1). In view of these results, *S. aureus* appears to play an important role in the development of dermatitis in *Ht*-rats. Significant histopathological changes observed in the skin lesion of all *Ht*-rats included hyperkeratosis, acanthosis, slight intracellular edema of the epidermis, slight swelling of the epidermal cells, and the presence of inflammatory cells (Fig. 2A). Higher levels of eosinophils and whole and degranulated mast cells were observed in the skin lesions of *Ht*-rats with dermatitis as compared with skin samples from the rats without dermatitis (Table 1, Figs. 2Ac and 2Ad). In rats maintained under the conventional condition, the number of CD4-bearing T cells increased, while CD8-bearing T cells appeared to show a slight increase in number. The ratio of CD4 to CD8 cells was constantly high. Both CD4- and CD8-bearing T cells were rarely found in *Ht*-rats maintained under the SPF condition (Table 1, Figs. 2Ae and 2Af).

We measured the serum IgE and IL-4 levels, since IgE and IL-4 play important roles in the pathogenesis of human AD [2, 12]. Serum samples from rats maintained under the SPF or conventional conditions were collected. Total IgE and IL-4 levels in the serum were measured using sandwich ELISA kits (Bethyl, Montgomery, TX, USA; and Biosource, Camarillo, CA, USA). The level of serum IgE increased only in the *Ht*-rats that were maintained under the conventional condition, but not in those maintained under the SPF conditions. Serum IL-4 was detected only in *Ht*-rats with dermatitis (Fig. 2B). Serum IFN- γ was not detectable in any of the rats (data not shown). Considering

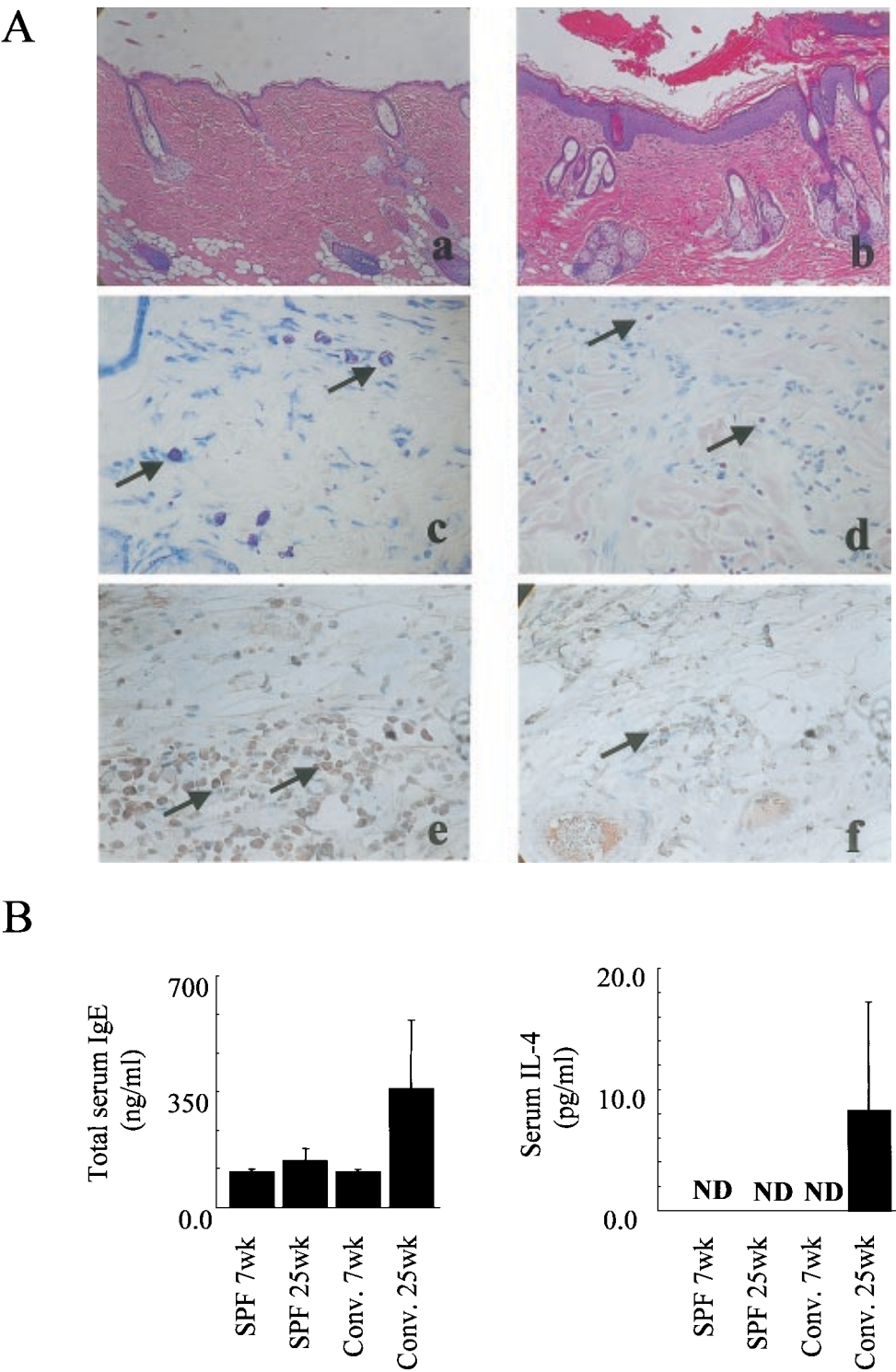


Fig. 2. (A) Histological and immunohistochemical features of the skin lesion of a conventionally housed *Ht*-rat at 25 weeks of age. Paraffin sections stained with HE, of SPF (a) and conventionally (b) housed rats at 24 weeks of age. Paraffin sections of the skin of an *Ht*-rat at 24 weeks of age with dermatitis stained with acidic TB (c) or by the Luna method (d). A frozen section immunostained for rat CD4 (e) and rat CD8a (f). Arrows indicate mast cells, eosinophils, and T cells, respectively. (B) Serum IgE and IL-4 levels in each group of *Ht*-rats. ND: not detected.

the results, we propose that the *Ht*-rat can serve as a new animal model for human AD.

The present rat model was compared with the DS-*Nh* mouse, which was recently introduced by Hikita *et al.* [4] and Yoshioka *et al.* [19]. The rat and mouse models may be similar to human AD with respect to the following features [4, 8, 11, 14, 15]: (1) *S. aureus* can be isolated from skin lesions, (2) serum levels of IgE and IL-4 increase significantly, and (3) the number of whole mast cells and CD4-bearing T cells significantly increase. Although both rodent models have much in common, there are some differences. We found that all the *S. aureus* from the DS-*Nh* mice with dermatitis secrete staphylococcal enterotoxin (SE) C, while *S. aureus* from the *Ht*-rats did not secrete superantigens (SEA, SEB, SEC, SED, SEE, and toxic shock syndrome toxin) (data not shown). In addition, the number of eosinophils significantly increased only in rats that were maintained under the conventional condition, but no increase was observed in the mice.

As described above, there are some genetic similarities between *Ht* and *Nh* mutations. The inheritance mode of both mutations is autosomal dominant [1, 3]. The *Nh* locus is mapped on mouse chromosome 11 and the *Ht* locus on rat chromosome 10 [1, 3], and both the genes are located in contiguity with the linkage site (unpublished data). Furthermore, there are some phenotypical similarities between both mutated rodents. Higher levels of mast cells were observed in the skin lesions of *Ht*- or *Nh*-mutated rodents compared with skin samples of age-matched rodents without these mutations (data not shown). In *Ht*-rats and *Nh*-mice, we considered that the increasing number of mast cells caused by these mutations and *S. aureus* colonization may play an important role in developing AD-like dermatitis. We are currently confirming whether these genes are the same by using the positional cloning technique.

In conclusion, *Ht*-rats develop dermatitis similar to that in DS-*Nh* mice and should be useful as an additional model for human atopic dermatitis.

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