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-
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. 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 subspecies 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). 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

### 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

<table>
<thead>
<tr>
<th></th>
<th>Nb-mouse (Nh/+)b</th>
<th>Ht-rat (Ht/+)c</th>
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<tr>
<td></td>
<td>SPF</td>
<td>Conventional</td>
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<tr>
<td>% Frequency of <em>S. aureus</em></td>
<td>0.0 ± 0.0</td>
<td>100.0 ± 0.0</td>
</tr>
<tr>
<td>Number of cellsa</td>
<td></td>
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<tr>
<td>Mast cells</td>
<td>40.0 ± 11.6</td>
<td>101.7 ± 28.2c</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.0 ± 0.0</td>
<td>7.2 ± 4.2</td>
</tr>
<tr>
<td>CD4-bearing cells</td>
<td>16.0 ± 10.1</td>
<td>81.0 ± 68.6c</td>
</tr>
<tr>
<td>CD8-bearing cells</td>
<td>5.4 ± 8.2</td>
<td>23.5 ± 15.3</td>
</tr>
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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.
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 Hi-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 *S. aureus* from the DS-Nh mice with dermatitis secrete staphylococcal enterotoxin (SE) C, while *S. aureus* from the Hi-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 Hi 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 Hi 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 Hi- or Nh-mutated rodents compared with skin samples of age-matched rodents without these mutations (data not shown). In Hi-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, Hi-rats develop dermatitis similar to that in DS-Nh mice and should be useful as an additional model for human atopic dermatitis.

References