Naturally Occurring Dermatitis Associated with Staphylococcus aureus in DS-Nh Mice

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Abstract: A naturally occurring epizootic of dermatitis involved all the mice, provisionally designated as DS-Nh, housed under conventional conditions, regardless of age or sex. The disease primarily attacked the lateral aspect of the face, neck and shoulders. The histopathologic features of the dermatitis varied in severity, but all affected regions showed signs of chronic dermatitis, including infiltration of inflammatory cells, parakeratosis and amyloidosis, and contained Gram-positive cocci clusters. Bacteriologically, coagulase-positive Staphylococcus aureus (S. aureus) was recovered in pure culture from the skin lesions. The disease experimentally induced with the S. aureus isolates was indistinguishable from those observed in naturally occurring cases. The results suggested that S. aureus may be causally associated with the disease.

Key words: dermatitis, DS-Nh mouse, S. aureus

We recently encountered some cases of moist dermatitis in euthymic DS-Nh mice which had been kept for a long period under conventional conditions. Some investigators have associated S. aureus with mouse dermatitis [1, 3, 5]. Since 1974, DS-Nh mice have been free of dermal illness in the breeding colony sustained under a barrier system, so that we considered the possibility that, etiologically, some microbial agent(s) was associated with the epizootic dermatitis and studied it.

Athymic nude (nude) mice, BALB/c- nu/nu, were purchased from CLEA Japan Inc., Tokyo, Japan. DS-Nh mice from a colony of inbred DS strain developed in 1954 from an outbred dd stock of the Central Institute for Experimental Animals, Tokyo, Japan, have been maintained at Aburah Laboratory, Shionogi & Co., Ltd., Shiga, Japan. As the Nh phenotype, non-hair, is inherited in an autosomal dominant fashion, DS-Nh mice used for this study were obtained as F1 (Nh/+), and the animals were used as recipients of the skin lesions. The animal facility for this study has been maintained under conventional conditions and has been receiving mice and rats from several sources for a long times. We first tried to determine whether age and/or sex might be a factor(s) contributing to the morbidity of the disease. DS-Nh, DS and nude mice of both sexes at 4, 7 and 10 weeks of age were brought immediately into the room where various strains of mice, including DS-Nh, BALB/c, C3H, ICR and C57BL/6, were retained for other experimental purposes. The dermatitis was noticed only in DS-Nh mice. At first, in most animals, the cheek skin showed discoloration with a white, powdery rough surface lasting 1 to 3 weeks.

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Thereafter the affected regions increased in size, becoming light red and subsequently became moist and ulcerated usually within a few weeks after the initial appearance of redness. The initial hyperemic foci of the syndrome were preferentially found unilaterally on the skin of the snout, and then gradually spread out in all directions on the anterior region of the animal, including the cheek, ears, jaw, neck, chest and forelegs, as shown in Fig. 1. Data regarding the incidence of the disease for each group of DS-Nh mice are presented cumulatively in Fig. 2. In all age groups of female mice, the first symptoms were observed in 5 to 10% of the animals by 8 weeks postexposure (this word is hereafter used as the meaning of the experiment under conventional conditions), and persisted at a constant level of morbidity for about 7 weeks. After that the morbidity increased in a time-dependent manner until it reached 100% by 24 weeks postexposure in all age groups. In male mice, although the first signs were detected in 10% of 7- and 10-week-old animals at 15 weeks postexposure, the morbidity showed similar progression in all three age groups. Ultimately the morbidity reached 100% in all age groups of both sexes at 24 weeks postexposure. On the other hand, both nude and DS mice housed under the same conventional conditions as well as the other 4 strains of mice described above showed no signs of the disease.

We next examined the correlation between the occurrence of dermatitis and the density of S. aureus on the skin of 4-week-old animals (Figs. 3 and 4). Nutrient agar for the titration of total bacteria, salt egg yolk agar base “Nissui” (egg yolk with mannitol salt agar; EY agar) for the titration of S. aureus, and an N-1D Test-SP18 (ID test) kit used for differentiation into subspecies of Staphylococci, were purchased from Nissui Pharmaceutical Co., Ltd., Tokyo, Japan. Each sample was obtained with a sterile cotton swab-stick according to the method of Williamson and Kligman [6]. As shown in Fig. 3, the lesion was first observed in one of 5 DS-Nh mice housed under conventional conditions at 2 weeks postexposure (B), and all the animals displayed clinical signs of dermatitis within 2 weeks after the first occurrence of the disease, but isolator-sustained DS-Nh mice (A) and nude mice housed under the same conventional conditions (C) did not exhibit any sign of dermatitis. Newly arrived DS-Nh mice usually carried relatively small populations, approximately $7.0 \times 10^2$ to $6.0 \times 10^3$ CFU/cm², of yolk-reaction-negative Sta-
Fig. 3. Kinetics of total aerobes and staphylococcal density on the skin of mice, and occurrence of dermatitis. Four-week-old DS-Nh (B) and nude (C) mice were housed, 5 animals per cage, in a given room under conventional conditions. DS-Nh mice of the same age (A) were also housed with a vinyl isolator in the same room. For bacterial isolation, each sample was obtained continuously from the same site on the animal in a time-dependent manner at 2-week intervals over 12 weeks. During this experiment period, about 400 to 500 mice, including the strains of DS, BALB/c, C3H, ICR, C57BL/6, and DS-Nh, were housed with the mice in the experimental groups in the room described above where the epizootic occurred only in DS-Nh mice. Each symbol represents the bacterial density in each animal, △: total bacteria isolated; ○: yolk-reaction-negative Staphylococci; ●: yolk-reaction-positive Staphylococci. Mice with dermatitis (□) are plotted at the bottom.

phylococci at 0 week in Fig. 3. The density of total staphylococcal organisms remained constant in the isolator-sustained animals during the experiment period (Fig. 3A). In contrast, the total bacterial density of DS-Nh mice housed under conventional conditions increased to approximately $1.0 \times 10^6$ to $1.0 \times 10^7$ CFU/cm² in a time-dependent manner and reached the maximum level at 10 weeks postexposure, together with the incidence of dermatitis (Fig. 3B). We also examined alterations in staphylococcal species on the skin of DS-Nh mice housed under conventional conditions and compared them with those of DS-Nh mice sustained with a vinyl isolator. The kinetic percentage of each species of Staphylococci is shown in Fig. 4. S. aureus was not detectable in the samples obtained prior to exposure. At that time, the common staphylococcal species cultured from the skin of the healthy animals, both DS-Nh and nude mice, were S. cohnii, S. epidermidis, S. gallinarum and S. capitis, as presented at the time of 0 week postexposure. Among them, S. cohnii and S. epidermidis were the dominant organisms on the skin of DS-Nh and nude mice, respectively. After that, S. aureus accounted for almost all the bacteria cultured from the skin of DS-Nh mice housed under conventional conditions 4 to 12 weeks postexposure (Fig. 4B), and S. cohnii was the dominant organism on the skin of DS-Nh mice sustained with a vinyl isolator throughout the experiment, as well as on that of DS-Nh mice prior to exposure (Fig. 4A). Thus, under the conventional conditions, it was confirmed that the occupation of normal bacterial flora by S. aureus occurred on the skin of DS-Nh mice with the incidence of
Fig. 4. Frequency of occurrence and relative percentage of staphylococcal species isolated from the skin of mice postexposure. (A) presents the transitions of staphylococcal flora on the skin of DS-Nh mice sustained with a vinyl isolator. (B) and (C) show the transitions of staphylococcal flora on the skin of DS-Nh and nude mice housed under conventional conditions, respectively. During the study, samples were collected from individuals and the levels of staphylococcal species were measured. The percentage of species of total Staphylococci are expressed as episodes of each species per 50–100 Staphylococci isolated.

dermatitis. In contrast, nude mice also carried similar levels of yolk-reaction-negative Staphylococci on arrival as DS-Nh mice, but the total amount of staphylococcal organisms somewhat decreased at 4 to 12 weeks postexposure in all animals housed under conventional conditions (Fig. 3C). At that time, although temporary occupation of normal bacterial flora by S. aureus was observed on the skin of nude mice at 4 weeks postexposure, S. cohnii became the dominant organism with small amounts of S. aureus at 8 to 12 weeks postexposure (Fig. 4C).

Macroscopically, the surface margins of the dermatitis were sharply demarcated from the healthy area of the skin (Fig. 1). There was total destruction of the skin in a certain area, and the presence of exudative fluid on the surface was noted. In the course of time, the affected animals developed severe clinical lesions, including swelling of the liver, spleen and regional lymph nodes (Fig. 5). Such animals were killed and necropsied when moribund at 36 weeks postexposure. The magnitude of liver, spleen and lymph nodes had increased almost twofold when compared to those of control animals sustained with a vinyl isolator. In the microscopic study, the skin lesions were confined almost exclusively to the epidermis and were occasionally ulcerated. Furthermore, parakeratosis and hyperkeratosis in the epidermis to the corium and thickening of the stratum spinosum were observed, and leukocytic infiltration varied with the age of the lesions and was either composed predominantly of neutrophils or of lymphocytes and macrophages or a mixture of all three (Fig. 6). Sections stained with toluidine blue indicated marked mast cell infiltration (data not shown). Gram staining revealed Gram positive coccoid clusters in the lesions with parakeratosis (Fig. 7). Microscopic study of other organs, including the kidney, liver and spleen, revealed varying degrees of amyloidosis (data not shown).
To examine the susceptibility of DS-Nh mice to infection with *S. aureus* isolates and the virulence for the mice following intranasal (i.n.) inoculation with the isolates, DS-Nh mice used in the test were divided into 2 groups of 15 each. The animals in one group were inoculated i.n. with *S. aureus* isolates and those in the other were used as the uninfected control. The same number of nude mice was used as an infected control. As a result, all DS-Nh mice infected with *S. aureus* isolates had developed the representative dermatitis. Samples taken from the lesions were inoculated onto EY agars and incubated at 37°C. All samples yielded a heavy growth of the *S. aureus* identified with an ID test kit. In contrast, none of the uninfected DS-Nh mice or nude mice inoculated with *S. aureus* isolates showed any sign of illness during the course of the experiment, although infected nude mice had *S. aureus* on their skin.

Our results therefore indicated that *S. aureus* infection is an essential factor in inducing the dermatitis in DS-Nh mice, but we could not detect the dermatitis in nude mice subjected to experimental infection with *S. aureus* isolates or in those housed under the conventional conditions described above. Some investigators have reported a significant difference in the susceptibility of various strains of mice to infection with *S. aureus* [1, 2, 4]. A genetic factor or factors therefore predispose mice to develop the disease in addition to the *S. aureus* infection in a unique dermatitis in DS-Nh mice. Further studies are now in progress to clarify the actual role of *S. aureus* in this disease.

**References**